Disentangling multi sensory contribution to navigation in a rat using a novel freely moving virtual reality-enabled path integration task.

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ADN	Anterior Dorsal Nucleus	13
BNC	Bayonet Neill–Concelman	66
BVC	Boundary Vector Cell	13
CA	Cornu Ammonis	7
DAPI	4,6-diamidino-2-phenylindole	60
EC	Entorhinal Cortex	7
FEF	Frontal Eye Field	26
FIJI	Fiji is just image J	60
HD	Head Direction	12
ICE	Image Composition Editor	60
K-S	Kolmogorov-Smirnov	79
LEC	Lateral Entorhinal Cortex	7
LED	Light Emitting Diode	95
LFP	Local Field Potential	59
LIP	lateral Intraparietal Area	26
MEC	Medial Entorhinal Cortex	7
mPFC	medial Prefrontal Cortex	17
MS	Medial Septum	7
MSTd	Medial Superior Temporal Area	26
NMDA	N-methyl-D-Asparat	11
OVC	Object Vector Cell	16
PV	Parvalbumin	13
RSC	Retrosplenial Cortex	13
SC	Subcutaneously	58
SOM	Somatostatin	13
SWR	Sharp-Wave Ripple	39
TTL	Transistor-Transistor Logic	66
UV	Ultra Violet	3
VEVS	Vectorial Selective Cells	34
VR	Virtual Reality	24
VTA	Ventral Tegmental Area	18

ABSTRACT

The ability to assess distance and direction traveled plays an existential role within the animal kingdom. Animals use many kinds of sensory systems in order to be able to achieve this feat. These sensory systems combine information into two frames of reference which animals use to retrieve a previously taken path. Animals are mainly able to use both an allocentric reference frame, which helps to associate objects to each other, and an egocentric reference frame, which ties a connection between ourselves and a given object. The use of the egocentric frame is highlighted when outside cues are limited or absent. Under such conditions, like in the darkness, we rely on our ability to integrate our past path in order to retrieve a starting location. Understanding this process of path integration also means helping to uncover the evolutionary basis for memory; the mechanism for planning and integrating information in our mind has evolved from a physical need to navigate in the environment (György Buzsáki and E. Moser 2013). There is much evidence to support the similarity between physical and mental travel (György Buzsáki and J. Chrobak 2005; Eichenbaum et al. 1999). Researchers have tried to understand this process of integrating egocentric cues using many animal behavioral assays. These assays have been hard to combine with recordings from the brain due to their inability to be repeated over multiple trials within the same animal, or a lack of precision in accurately measuring animal behavior. Many of the assays don't have a full control over visual or vestibular perturbation in order to study their respective contributions.

Here I developed a novel path integration task and used a freely moving virtual reality system (Del Grosso and Sirota 2019) to enable variable homing locations within an arena and implement perturbations of the vestibular input and an optic flow. This task structure can be split into path integration and goal-directed segments within a trial, is suitable for long-term extracellular recordings, and results in a high throughput of trials. I recorded over 50 000 trials and over 100 000 rears which were essential in correctly characterizing the task, quantifying performance, and testing for the use of allocentric information. Flexible homing location and binary response of the rat (rearing) allow for objective measures of the accuracy in the path integration task. With freely moving virtual reality I could create conflicting information to study the contribution of each idiothetic sensory input to the path-integration. I show that transient visual or vestibular perturbations during the task segment associated with path integration strongly deteriorate the performance of the animal on the task. Such perturbations are expected to disrupt the head-direction system, which is necessary for successful path integration, and hence strongly suggests the developed task is indeed purely path integration-dependent. I present a preliminary analysis of the multiple place cells' recordings from the CA1 layer of the hippocampus which provides the first glimpse on the spatial coding of the same cells during different modes of navigation associated with distinct segments of the task. Finally, I discuss how our task can be adapted easily in the future to further investigate this complicated mechanism.

1

INTRODUCTION

1.1 A STORY OF THE BEGINNINGS OF MEMORY

A small scruffy mouse is scurrying around in a lush meadow looking for any piece of seed or a worm it could bring back to its hungry family. A shadow looms above and before it has the time to look up, it is picked up by a bird of prey who rips its head off and enjoys it for lunch.

In an alternative reality this mouse knows to start running as soon as there is a change of luminance due to the bird's shadow above and retreats on the straightest path home to a hole where it is safe from predators and welcomed warmly by its family.

How can this mouse remember the straight shot back to where it started its adventure? How is this path represented in the brain? Which sensory modalities on the way is it using to make its path? How do those help it remember where it was in the past? It must know the approximate distance and direction of its home, otherwise it would not be able to initiate such a brisk and precise movement.

How do we generate a sense of direction? It would be natural to explain this by saying that we take where we have been in the past and our goals for the future and then estimate where we are now. The question of this thesis is: Can we design an improved path integration paradigm with focus on understanding the neural substrate behind this mechanism? Specifically, I am interested in developing a path integration paradigm which is precise, repeatable and allows perturbations of different senses in order to decipher where and how in the brain can a homing vector be retrieved so quickly.

1.2 EVOLUTION

The representation of such a vector is of vital importance to an animal in case a threat looms above; to be able to retreat to safety as quickly and accurately as possible. An innate flight response to a safe house, instead of the middle of the meadow is the difference between death and life. The ability to make such a movement suggests a very automatic memory structure which would provide the scaffold to be able to execute such complicated direction and navigation movement. Evolutionarily this advantage has become a key to survival and one of the first forms of memory in mammals (Murray et al. 2019; György Buzsáki 2019 ; Allen and Fortin 2013). How is this scaffold structured to be retrieved in a split second decision? Could it help in building more types of associative memories? We have evolved from simple input-output brains to being able to associate different parts of memories together. This is largely due to the structure in the brain called the hippocampus (György Buzsáki 2019). We are able to remember memories and even to disassociate from the world; to imagine spaces and make connections which would hardly arise from only observing our environment. All this stems from our existential need to remember a safe place of the origin of our explorations.

1.3 BEHAVIORAL STRATEGIES IN NAVIGATION

To understand the basics of this navigational process we first need to understand how animals navigate in an environment in general. Animals and humans use 4 different strategies: Visible or a cue guided navigation, a stimulus-triggered response, route-based and a map-based navigational strategy (Arleo and Rondi-Reig 2007). These strategies can be used in combination with each other and the preference for a given strategy can vary depending on the familiarity, with route- or map- based strategy navigation used initially when no goal is visible and shifting to more automated strategy which involves habitual behavior, a form of stimulus response strategy (Arleo and Rondi-Reig 2007).

Homing, a form of route-based strategy, has been proven in many other kinds of animals which might use similar mechanisms as in rodents. In a seminal review, (Rogers 1988) describes homing examples in mammals, showing that the larger the animal's body size, the longer distance they are capable of returning. Cats, wolves, deer and bears have all shown an impressive innate ability to to return to their respective homes, even if they were translocated more than 200 km away from their home location (Rogers 1988). These real world example distances

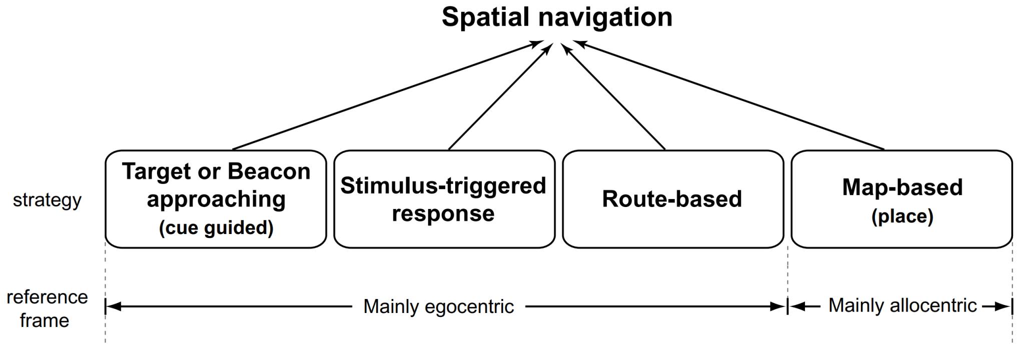

Figure 1: *Schematic of different types of spatial navigation strategies. Map-based strategies are mainly allocentric, whereas beacon approaching, stimulus triggered response and any route based strategies are mainly egocentric. Adapted from (Arleo and Rondi-Reig 2007)*

put in perspective research in controlled laboratories, where many navigational experiments in rodents are done in a very small arena, most within 1 m^2.

One of the most commonly cited examples and most known «navigator» in lay perception is the homing pigeon. Experiments done on pigeons show their ability to use the magnetic poles of the earth to navigate to different locations in order to deliver a message back to a homebase. Magnetoreception is an example of a navigational sensory information which goes beyond vision and can be still enacted in darkness. Evidence on magnetoreception is sparse, but has been found in mole-rats (Burda et al. 1991; Caspar et al. 2020) which lack good visual stimulus. In epigeic rodents there is no proof regarding their use of magnetoreception for navigation (Malkemper et al. 2015, personal communication David Keays). Understanding these processes in the homing-piegeon would be uttmost desireable, however, there have been surprisingly few experiments with pigeons in the context of navigation and pure path integration. In their nocturnal counterparts, the bats, Nachum Ulanovksy and his lab in Israel have been heavily engaged in recording from the bat in a region homologous to the hippocampus. Results showed a vectorial representation of a path in bats and the existence of the same kinds of cells which are found in rodents, namely place cells and grid cells, among others (Sarel, Finkelstein, et al. 2017).

On a smaller scale, bees, ants and flies have also been studied heavily with regard to path integration. Their brains are not as homologous to the human brain as rat brains, but the study of their mechanism revealed a great solution with adaptations to their size and environment (Honkanen et al. 2019) making use of the central complex a part of their brain for a readout of direction of heading.

Ants and bees as well as birds all use the so-called e-vectors or the azimuthal information from the sun in order to determine their positions and where they should be heading. Insects use specific Ultra Violet (UV) receptors in the ventral part of their eyes in order to capture this angular information. (Wehner, Srinivasan, et al. 2003). Besides the use of extra sensory information which we might not think about when an animal explores an environment for the first time, it starts building its own internal map of the environment around itself. This feature is useful in case an animal has to visit the place again, as it already knows where to go. Researchers have studied this feature of the navigation system now in great detail. In 1948 for the first time Edward Tolman, a psychologist, introduced the idea that individuals use map-like features to represent spatial information and navigate in the environment and termed it the "cognitive map" (Tolman 1948). The parts of this metaphysical map whose firing correlates with physical object appearances give its user the ability to orient in a known environment. These parts have been proven to be inside of the brain, even if its full structure and dynamics are still unknown.

1.4 ALLOCENTRIC AND EGOCENTRIC DEFINITIONS

In memory and spatial tasks both of these frames can be understood in parallel and there seems to be a transition from using egocentric to increasingly allocentric navigation depending on the length, complexity or repetition of the task (Burgess 2006).

1.4.1 *Contributions of allocentric and egocentric reference frames*

Theoretical work on the different navigational frames has proven that the use of both strategies is possible to solve an aiming or a guided task. Here researchers simulated an agent solving each of the tasks using a computational architecture similar to the rat brain (Vijayabaskaran and S. Cheng 2022). The agent was able to switch the reference it was using based on the available information during the task. This phenomena is good to understand in computers, but how might it help the society at large? It is only human studies that bring the use of different reference frames into translational terms which help us to underscore the importance of understanding how memory works and how we navigate using the two reference frames. In many cases this basic research improves our treatment of complicated neurodegenerative diseases which decrease the quality of life of those affected.

Some of the more recent examples include description and early diagnosis of Down and Williams syndromes (P. B. Lavenex and P. Lavenex 2021) as well as showing that in elderly population are more reliant on egocentric rather than allocentric navigation with more stereotypical behavior (Stangl et al. 2020), serving as one of the diagnostical hallmarks of early-onset Alzheimer's disease (Ramanoël et al. 2022). Understanding the basics of how the memory originated can help us alleviate some of the societal pain which comes with such diseases.

1.5 LANGUAGE AND NAVIGATION

Similar to memory, an analogy can be made between how humans use language and how they navigate around the environment, because the environment has a large impact on how humans navigate (Majid et al. 2004; Boroditsky and Gaby 2010; Nölle et al. 2020). Language helps to define the way we think about our experiences and how those are saved and recalled in our memories. Linguistics research shows metaphoric structuring as a way to understand time through space (Boroditsky 2000; Casasanto and Boroditsky 2008). Other researchers have also described cultures with languages which do not have an egocentric direction such as left or right but only have allocentric coordinates referring to the south or north arm or leg (Boroditsky and Gaby 2010; Haun et al. 2011). This example introduces a new perspective and a different way language and memory could have evolved from different ways of navigating. In those cultures the allocentric frame of reference could have played a key role in shaping the worldview in contrast to being reliant on an egocentric representation. With different frames of reference giving rise to a variety of memory structures and ways of thinking about self.

1.6 PHYLOGENETIC CONTINUITY.

Similar to allocentric and egocentric navigation, parallel ways of thinking about memory structure exist. We can draw connections from navigation to episodic and semantic memories, since they are not surprisingly thought to be processed in the same brain region. An episodic memory (explicit memory involving personal experience) would be more similar to egocentric navigation like path integration, a homing strategy. We recall the experience of moving in the past from a personal perspective in the same way as using an egocentric reference frame. For semantic memory one needs to draw the connection between the objects themselves but not necessarily with relation to the individual. Hence an allocentric navigation supports the initiation of semantic memory and is more similar to creating a cognitive map (György Buzsáki and E. Moser 2013; M.-B. Moser, Rowland, and E. I. Moser 2015).

As mentioned in the introductory example, our need to create an escape vector has also become one of the first forms of memory (Murray et al. 2019; György Buzsáki 2019; Allen and Fortin 2013). I want to take this a step further and argue that we first used a self-reference frame and only later expanded to disassociating from the self and building memories by referencing objects in relation to each other and not just oneself anymore. If this holds true it would argue against Tulving's evolutionary origin of semantic and episodic memory. The term episodic memory was only coined in 1972 by Tulving himself (Tulving 1972) and the definition has been refined over the years (Tulving 2002). Today's definition argues that episodic memory is explicit. Tulving also argues that egocentric memory evolved from semantic, which evolved from procedural (Tulving 1985). His evolutionary argument for semantic memory coming first is based mainly on autonoesis and sense of subjective time (Tulving 2002). If we assume we have been able to escape from a predator by keeping a memory of a vector, then we are referencing this memory to oneself and this way we would have the ability to travel back in time and to explicitly recall this when needed, making episodic or a self referenced explicit memory the first kind to originate. The study of a self-referenced vector for navigational planning called path integration allows us then to uncover the beginnings of memory.

Where memories reside in the brain is still in question. One hypothesis is that the hippocampus helps in coordinating the sequential recall of items stored in the neocortex (György Buzsáki 2019). This recall needs to be based on an already established structure, a structure of space and time which has been found in the firing of specific cells in the hippocampus. There exist cells which represent the world in our environment in a form of a neural code which when assembled together can create a cognitive map to associate things with each other in an egocentric and allocentric frame, serving as a structure to sequential recall items stored in the neocortex. We target most of our analysis and theory to these kinds of cells which help to tell us the most about how we represent the environment in our brain and how we could create our own mental worlds beginning with referencing things to ourselves and later dissociating those.

1.7 ANATOMY OF THE HIPPOCAMPUS

As mentioned earlier, the hippocampus plays a key role in navigation (György Buzsáki 2019). Its parts make up a large portion of the concept thought of as the cognitive map and these components generally aid our navigation. I will now explain where these parts of the hippocampus are organized within the brain (Figure 2).

The hippocampus is a complex structure which in humans resides within the medial temporal lobe. Thanks to an unfortunate but illuminating surgery on patient Henry Molaisson to treat epilepsy (Dossani, Missios, and Nanda 2015), this area of the brain has been associated as the memory center for humans and rats alike. Anatomically, the rat and the human hippocampal formations are very alike (Figure 2). In both cases they consist of two interlocking C-shaped structures, with 3 distinct sections - the dentate gyrus, the hippocampus proper and the subiculum, the last of which acts as the transition zone between the former two interlocking C's (Figure 2B) (Fogwe, Reddy, and Mesfin 2021). The Cornu Ammonis (CA), named after its shape of a ram's horn (Olry and Haines 1998), is divided into CA1-3 subfields in a rat. The subfields spread on a spectrum from a ventral to dorsal axis. Pyramidal neurons residing in this region are distinguished by their bursty firing accompanied by high amplitude spiking.

There are two main pathways going through this circuit, the perforant and the temporoamonic pathway. The information into both of these pathways comes from various sensory organs some channeling through the Medial Septum (MS) via the septo-hippocampal pathway through different types of cell specific projections (Müller and Remy 2018). Some of these projections are ending in the Entorhinal Cortex (EC), which is divided into lateral and medial (Lateral Entorhinal Cortex (LEC) and Medial Entorhinal Cortex (MEC)) respectively and each of those regions consist of 6 layers with different ratios of neuronal cells (Witter 2010).

The perforant pathway inputs come from the second layer of the EC entering the dentate gyrus with granule cells which then synapse to where the mossy fibers originate their projections. The mossy fibers are synapsing on to the CA3 pyramidal cells with an autoassociative connection to CA3. The Schaffer collateral projection creates the third connection onto the CA1 pyramidal neurons (Figure 2) (Witter, Kleven, and Flatmoen 2017) with an eventual output going through the subiculum. In this pathway there are recurrent connections, which can sustain even a weak electrical stimulation for a long time, allowing replay of connections or memories that leads to improvement of the encoding of memory (Kumar 2011).

The temporoamonic pathway sends projection from the third enthorhinal layer directly into CA1, bypassing the DG and CA3 as which are connected in the perfornat pathway. The relation of memory and hippocampal formation is further underlined by study of neurodegenerative diseases which come with a loss of memory caused by the loss of synapses in this region. Specifically, the CA1

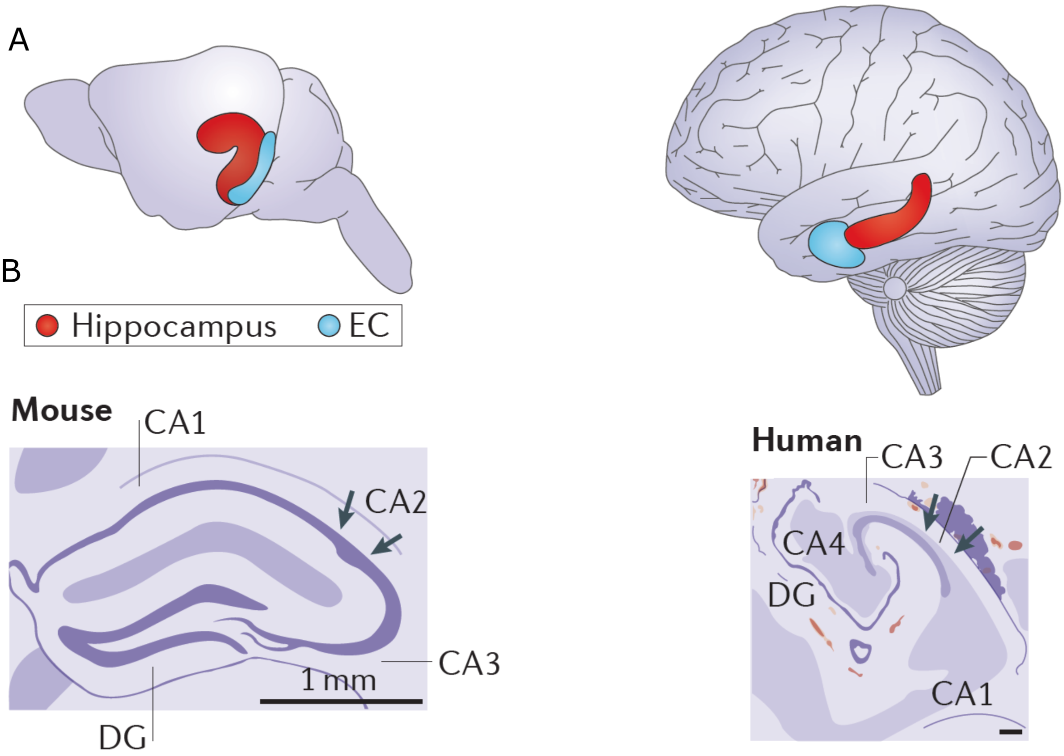

Figure 2: *Anatomical depiction of the hippocampus and entorhinal cortex across rat and human brains. A) Mouse brain (left) and human brain (right) with highlighted hippocampus (red) and the entorhinal cortex (blue). B) Nissl staining from each species highlighting different sections of the hippocampus with different CA layers and dentate gyrus. Adapted with permission from Strange et al. 2014*

region is more vulnerable to global cerebral ischemia, a second leading cause of Alzheimer's (Fogwe, Reddy, and Mesfin 2021).

1.7.1 *The cognitive map*

As mentioned before, Edward Tolman initially described the cognitive map as a theoretical way animals could be navigating (Tolman 1948). Since then the theory has gotten closer to reality as more and more spatially selective cells have been discovered; we now know a whole zoo of them (E. I. Moser, M.-B. Moser, and Bruce L McNaughton 2017). These cells are not only used in the cognitive map, but help us tie the space around us together for navigation.

1.7.2 *Types of spatially selective cells*

What is space? We can understand it as a construction of different features within an environment where we can move around. These features are encoded in the brain via specific subtypes of cells whose rate and frequency of firing differ significantly based on specific attributes of such a space.

1.7.3 *Place cells*

The simplest example of a spatially modulated cell within the brain is a place cell (Figure 3). Place cells were discovered through recording from within a hippocampus by very thin wires and an amplifier which could translate the firing of neurons at the end of the wires into a signal to be recorded. If one places such an implanted rat into an environment, there will be neurons which always respond (fire) in a particular location of the environment, giving or encoding an inherent cue of the animal's location in the environment. This information can later be combined together (firing of multiple place cells) and one can decode the position of the rats just by using the brain signal. After the initial discovery of place cells in 1971 by John O'Keefe and Dostrovsky (John O'Keefe and Dostrovsky 1971), the neuroscience of navigation field has exploded and tried to find different relationships between a particular cell in the brain and a selective feature of space, giving rise to a zoo of "cells" which all code for space in an unique way. Place cells don't require any visual input in order to appear in a new environment, it is an inherent quality of the brain to describe the environment even with limited sensory information. During experiments when only self motion information was available, place cell tuning for that environment emerges and later if light is turned on, the cells stay in the same constellation (Quirk, Muller, and Kubie 1990).

1.7.4 *Grid cells*

Many years later after the discovery of place cells, researchers also recorded from a different brain region to look for spatially selective cells. When they plotted the firing of cells from the MEC on top of the arena where the animal was moving, making a dot for each time one cell fired, they saw a hexagonal or a triangular pattern appear on the map of the environment where dots were situated at each corner of the hexagon (Hafting et al. 2005). This result gave rise to cell types which we now call grid cells (Figure 3). They fire in a given environment with

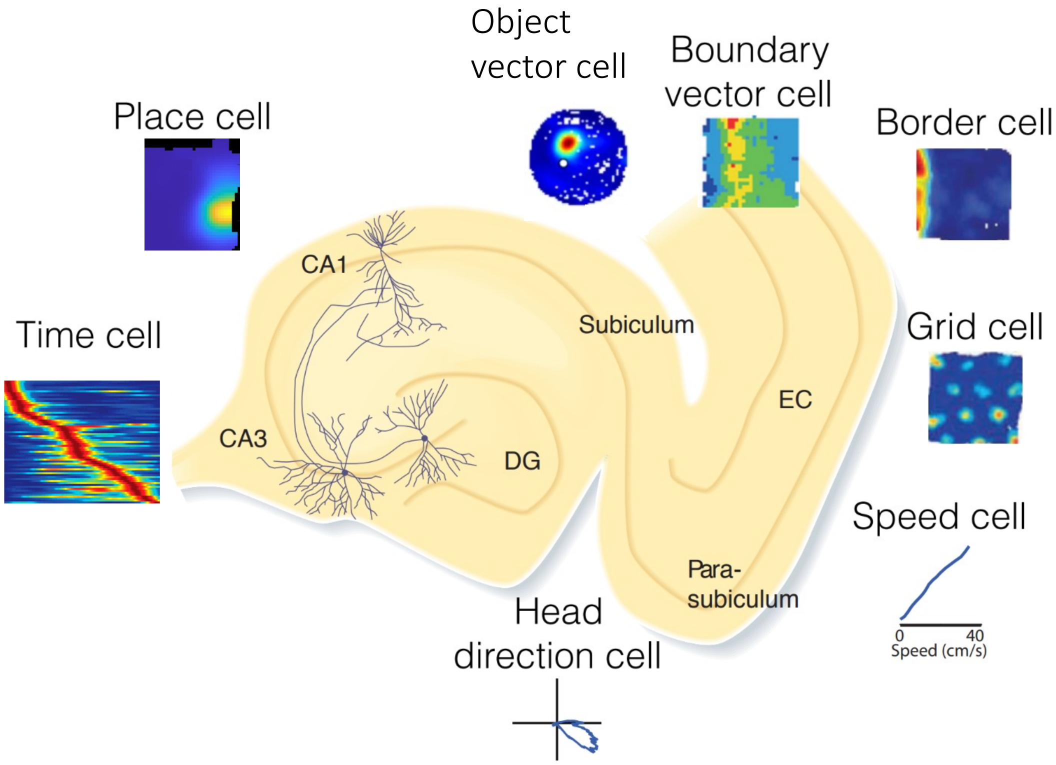

Figure 3: *Spatially selective cells found within the hippocampus. Examples from each publication of how neuronal firing is modulated by a given variable. Left to right: Time cell firing most at given time intervals from the start of a maze even if the animal runs on a treadmill as part of a linear track. Adapted from Salz et al. 2016. Place cell firing is the highest when the animal is in the center of the place field. Head direction cell firing is the highest when the animal turns its head towards a direction preferred by the system (Knierim, Kudrimoti, and Bruce L McNaughton 1998). Object vector cells (Høydal et al. 2019), boundary vector cells (Lever, Burton, Jeewajee, et al. 2009) and border cells (Solstad et al. 2008) function very similarly by having a firing highest at a given distance away from an object or a boundary or when at this location. Grid cells fire in a hexagonal pattern inside of the enclosure and have constant geometrical period between each node (Hafting et al. 2005). Speed cells fire more the faster the animal moves (Kropff, Carmichael, M.-B. Moser, et al. 2015). Background image with hippocampus outline and connection of CA1 and CA3 cells to DG with Enthrorhinal cortex (EC), Subiculum and Parasubiculum adapted with permission from (György Buzsáki and J. Chrobak 2005).*
(Salz et al. 2016) licensed under https://creativecommons.org/licenses/by/4.0/ permission not required. (Lever, Burton, Jeewajee, et al. 2009) (Copyright 2009 Society for Neuroscience). (Solstad et al. 2008). Reprinted with permission from AAAS

a very similar scale between the nodes of the hexagon. These cells have since been thought of as giving the basic metric for distance of movement across the environment. The animal could use the firing of each part of the hexagon to know how far it has traveled within the environment (Fyhn, Molden, et al. 2004). Grid cells also need input from place cells in order to be calibrate and be accurate (Bonnevie et al. 2013, Fernandez-Leon, Uysal, and Ji 2022). To be able to estimate traveled distance on differently sized environments there needs to be different scales of grid cells.

1.7.5 *Size and location of the spatially selective cells in the brain*

As described above in the anatomy section, both grid and place cells have a certain size to which they can be spatially specific similar to resolution of a picture. This resolution is anatomically defined for the dorsal to the ventral part of the hippocampus. In humans this would be posterior to anterior. Grid cells in the more ventral part of the entorhinal cortex usually have a much larger spacing between firing and their field is also much larger (Fyhn, Hafting, et al. 2008); this works similarly for place cells. The more dorsal parts of the anatomy show smaller place fields as well as tighter grid cells (Figure 4). This is an effective way for animals to encode the space on different scales (Brun et al. 2008). The difference between distal and proximal CA1 cells is also highlighted if local and global cues are in conflict where distal CA1 cells can keep a more coherent representation of space than the proximal. This is also caused in part by the MEC sending stronger projections to proximal CA1, and LEC sending stronger projections to distal CA1. In case of a sensory conflict, place cells in proximal CA1 would split but in distal CA1 would rotate with alignment to the global cues (Deshmukh 2021).

Grid cells, could be used as a metric for path integration due to the nature of their firing. Research from Mariana Gil and colleagues has shown that if one disrupts grid cells, this impairs the path integration process in mice. Specifically they showed that there is an impairment on a triangle completion task in mice with blocked N-methyl-D-Asparat (NMDA) receptors in the retro-hippocampal region (Gil et al. 2018). Research from Jeff Taube's lab also showed degeneration of the grid code with disruption of the anterior dorsal nucleus where head direction cells are located (Winter, Clark, and Taube 2015).

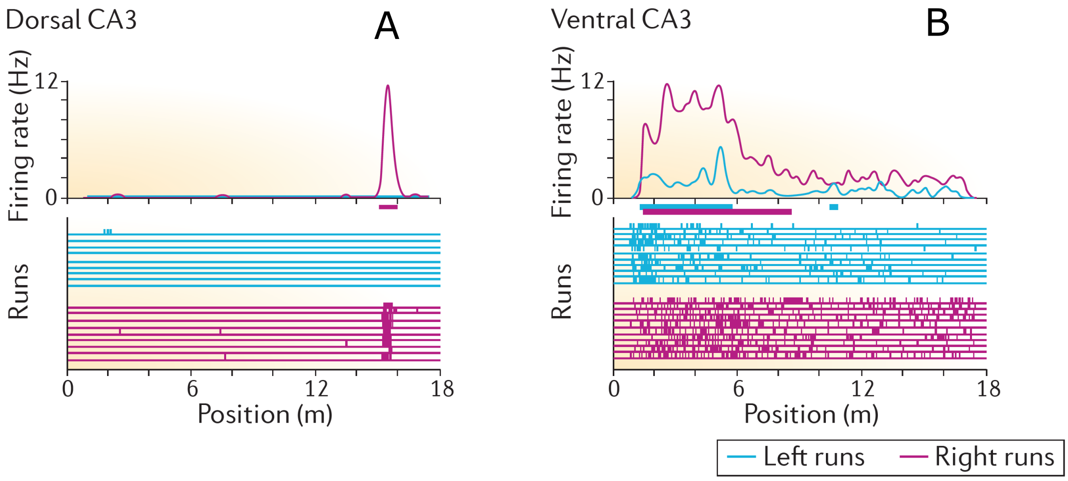

Figure 4: *Differential sizing of place cells depending on the dorsal or ventral CA3 axis of the hippocampus. A) Recordings from an 18m linear track, where each cyan line represents animal running left and magenta represents the animal trajectory on the way back (right). Each tick on the line is a firing of the neuron. The more dorsal recordings from CA3 show very localized firing creating a sharp and small place field (left panel), whereas the ventral recordings B) show more distributed firing along the track creating a broader place field. It is also worth noting that the firing of these neurons is direction dependent as in the dorsal recordings in the cyan color there is no firing of the place cell. Adapted with permission from Strange et al. 2014*

1.7.6 *Head direction cells*

In order to understand the location of oneself with regard to the environment, especially if there is a lack of visual cues, there needs to be a signal about the heading of an animal. The discovery of cells modulated by head direction was first made in 1983 by (Ranck Jr 1984) but fully described in 1990, where researchers recorded from yet another brain region called the anterior dorsal nucleus and found that over a quarter of cells had tuning properties defined by which way the animal was facing (Taube, Muller, and Ranck 1990) (Figure 3).

Similarly to our movement in darkness where we might bump into a corner of a table at night, because we cannot calibrate our precise movement with visual information, an error is also associated with the Head Direction (HD) signal which is collected in darkness if not calibrated over some time. Fortunately, the HD signal is reasonably stable on the order of minutes in the darkness (Yoder, Peck, and Taube 2015), before becoming quite unstable, potentially due to the the influence of other cues introducing errors. There are subtypes of HD cells, namely visually driven and non visually driven. The former are influenced more by visual cues and the latter rely more on the internal orientation (non-visual cells) (Yoder and Taube 2009; Yoder, Clark, et al. 2011). The existence of both types of cells paints a multisensory picture of the HD signal. In general the head direction system is very flexible as in the absence of both visual and olfactory cues the HD network remains intact. However, it drifts if this condition is introduced over an extended period of time (Asumbisa, Peyrache, and Trenholm 2022).

There also exist bidirectional head direction cells which have been shown to have a 180 degree displacement due to orthogonal cues, such as two white cue cards in an arena (Jacob et al. 2017). Further research from the Jeffery lab also highlights the possibility that some HD cells are also driven by visual cells (N. Zhang, Grieves, and Jeffery 2022). HD cells react differently to weakly discriminable cues as compared to black and white cards. Recordings from the Anterior Dorsal Nucleus (ADN) and the postsubiculum also show two types of HD neurons, ones which are pure (driven by vestibular system) and ones from Postsubiculum which can also encode for different environmental stimuli such as if a wall is present on the left or on the right (Peyrache, Schieferstein, and Gyorgy Buzsáki 2017) connected to the visually driven HD cells introduced above.

It seems as if the head direction signal is indeed updated internally via the vestibular formation as well as integration of visual information. This is reflected in the Retrosplenial Cortex (RSC), where research from Sit and Goard has shown that the RSC receives input from ADN and the visual system and is capable of aligning the HD signal that way (Sit and Goard 2022). This is reminiscent of the story of allocentric and egocentrically based memory. Here we can see two subtypes of head direction cells which are separated into ones which are more allocentrically driven and ones which are more egocentrically driven. Head direction cells play a large role in path integration and we will discuss them at a later point again due to their multisensory integration properties.

1.7.7 *Boundary cells*

There is also a subset of cells that fires within a given position or a vector from the boundary itself. They were first thought of in 1996 (Burgess and John O'Keefe 1996). While testing the properties of place cells it became apparent that some of them kept expanding their firing to the corner of an arena which kept being enlarged by the experimenter. Burgess and O'Keefe hypothesized that in order for this remapping to happen there needs to be a way to represent a border of a given arena. This was then proven via the Boundary Vector Cell (BVC) (Figure 3) model which was shown in (Barry et al. 2006) and later physically described in Lever et al. (Lever, Burton, Jeewajee, et al. 2009). These cells fire when the animal is touching a given border of an arena or if it is on a given vector away from a boundary which it can sense.

1.7.8 *Landmark and object vector cells*

Within the space of an arena another signal that is a natural extension of the border vector cells are landmark or object vector cells, These cells encode a certain distance away from a beacon or when an animal is at an object or a beacon itself (Figure 3). The vector cells were discovered in the same lab as the grid cells and originally described to be in the entorhinal cortex (Høydal et al. 2019).These OV cells respond more to plain visual features such as contrast. More research has since also proven them to exist in the CA1 as well (Andersson, E. I. Moser, and M.-B. Moser 2021). These object vector cells are different from border cells, because border cells do not respond to gaps in the environment that the animal can traverse and with many of these 3D objects there seems to be more response if the objects are taller in height, (Høydal et al. 2019) but the cells do not respond to elevated borders. This might serve as a clue to a different encoding mechanism from border vector cells. One hypothesis is that there might be two separate systems - one driven by visual features (putatively dependent on Somatostatin (SOM) interneurons) and a grid cell- based cell system (dependent on Parvalbumin (PV) interneurons to represent an animal's position by updating self-motion. This is also very similar to the firing of HD cells as described above receiving either a visual or a more vestibular driven signal(Andersson, E. I. Moser, and M.-B. Moser 2021) and connected to two streams of information, the allocentric and the egocentric. Building two neuronal populations, one visual and one self reference-based, shows robustness of the system; both populations can intermingle for the correct behavioral readout for an errorless estimation of position, heading or a landmark.

1.7.9 *Conjunctive and complementary cells*

Many of these spatially selective cells not only code for one spatial modality like object vector cells, but rather are likely to be influenced by multiple types of spatial modalities. These cells are called conjuctive cells. Conjuctive cells can combine many modalities; typically one is easier to decode while the others are incrementally less likely to contribute clear information.

Inayat and colleagues (Inayat et al. 2022) recorded from the hippocampus, specifically from CA1, and recorded over 2000 cells using combinations of different stimuli either when the rats were moving or when they were immobile. They found cells coding conjunctively and also complementary, meaning that either they fired and encoded two or more different modalities or the same cell would fire on separate occasions but each time encoding a different sensory stimulus. Complementary cells generally identify novel sensorimotor events and conjunctive encoded familiar ones. Sometimes it may also happen that different populations of cells encode for the same sensory stimulus depending on if the animal is moving or not (Inayat et al. 2022), or if the animal is in a different context (Komorowski, Manns, and Eichenbaum 2009). It is true that during locomotion or immobility different sensory modalities are either heightened or dampened. While vision might be heightened during locomotion (Dipoppa et al. 2018), auditory contribution through its cortex might be dampened (Schneider, Nelson, and Mooney 2014) and the other way around respectively.

An example for usage of conjunctive cells is encoding of time and distance. These kinds of cells could be integral in a path integration mechanism (Kraus et al. 2013). Similarly, in a social context when other animals are present, conjunctive cells encode the identity of another bat as it flies by and then switch to encoding the flying distance (Sarel, Palgi, et al. 2022).

1.7.10 *Time cells*

Besides spatially selective cells, time cells act as yet another natural extension of representing the environment and mirroring it in the mind. Keeping track of time is essential in the generation of memories as well as keeping track of episodes in our own lives. There has been previous effort and initial exploration in the field but it was always very hard to control for location and time separately (Wood, Dudchenko, and Eichenbaum 1999). Later research (Manns, Howard, and Eichenbaum 2007) showed that it is possible to keep the order of odor sampling and indeed that there are sequences of neuronal firing which are created upon this temporal order. The cells with this neuronal firing pattern have since been called time cells (Figure 3) (Eichenbaum 2014; Kraus et al. 2013). These sequences can be of different orders of magnitude, from minutes (Manns, Howard, and Eichenbaum 2007) to hours and days (Mankin et al. 2012) as well as longer periods (Ziv et al. 2013). The time cells have specifically been shown in the CA1 of the hippocampus (Mankin et al. 2012). A possible storage mechanism for such temporal sequences was thought to be through theta phase precession (Dragoi and György Buzsáki 2006; Pastalkova et al. 2008). This mechanism between CA3 and CA1 should be able to bind memories into single episodes (Manns, Howard, and Eichenbaum 2007). Combining time tracking with distance is essential to create a path integration vector as well as to understand speed.

1.7.11 *Speed cells*

Extending the analogy of distance and time, naturally there would also exist a system which is able to decode for the speed of the animal using those two information streams. Initial exposition of such cells was found within the hippocampus (Bruce L McNaughton, Barnes, and Jeff O'Keefe 1983; Wood, Dudchenko, Robitsek, et al. 2000) (Figure 3). Sargolini and colleagues recorded from EC layer II and discovered some grid and head direction cells as well as some which are sensitive to running speed (Sargolini et al. 2006). More recordings from the entorhinal cortex then confirmed this phenomenon (Kropff, Carmichael, M.-B. Moser, et al. 2015). In this case there was less overlap with other spatially selective cells. The advantage of having one's own speed code, independent of the visual stream, is that in the darkness, it still functions and provides information about one's position contributing heavily through the proprioceptive signal to a correct readout for the path integrator (Kropff, Carmichael, M.-B. Moser, et al. 2015). Most of these spatially or time selective cells have been found in the hippocampus, but that is not the only place such cells reside, even if the majority do.

1.7.12 *Spatially selective cells outside of the hippocampus*

Many papers show spatially selective cells outside of the hippocampus (e.g. Long and S.-J. Zhang 2021, Diamanti et al. 2021, Poo et al. 2022). In many of these cases the number of neurons which show the selective modulation within the area of the cortex is very small (such as Object Vector Cell (OVC)). More precisely, the whole population is not working as an integral part of the circuitry like in the example of the hippocampus. Yet this does make us question the hippocampus- centric view of our understanding of how space is represented in the brain (Peyrache and Duszkiewicz 2021). In 2014 researchers focused on the parietal cortex as a possible place where head direction and egocentric distance cells could come together to provide a vector for distant goals (Wilber et al. 2014). Here they taught rats to follow a specific cue and observed their function to keep track of the allocentric direction of a given landmark. These conjunctive cells showed anticipatory firing. This served as a proof that these cells are active in both allocentric and egocentric reference frames. These cells could help an animal remember location of a goal relative to a landmark in the arena to aid in a vector based navigational strategy. These cells could also play an important role in keeping such a vector towards a goal. Similar research from the retrosplenial cortex on the head direction signal showed a similar finding as cited earlier (Sit and Goard 2022).

The cognitive map can also expand beyond the hippocampus. Another example is the medial Prefrontal Cortex (mPFC), where some of the connections from the hippocampus terminate. Researchers have shown a stable topological organization within the mPFC of the mouse while it is doing a virtual reality task (Cholvin, Hainmueller, and Bartos 2021). On the other hand Purandare et al. report a subset of CA1 cells which encode a moving visual stimulus (Purandare et al. 2022). They show a subset of visually evoked cells which can encode information about this visual phenomena in the hippocampus and later be combined with other multi sensory information to generate prospective representations. These cells are similar to already mentioned social cells in the bat (Sarel, Palgi, et al. 2022). This work comes from a larger line of research identifying visual cells modulated by location (Haggerty and Ji 2015). Recently spatial modulation was also described in the V1 area of the cortex (Diamanti et al. 2021 ; Saleem et al. 2018), showing that the hippocampus is not only space-centric but can also project visual information very well. All these kinds of cells were initially identified in the rodent as it is the most established research animal for spatial navigation. But of course this is not just a peculiarity about the rodent brain; many of these cells have since been discovered in bats, monkeys and most importantly humans (Ekstrom et al. 2003). The discovery of more complex cells such as time cells in humans in the entorhinal cortex (Umbach et al. 2020) confirms that speed cell description in humans is just a matter of time.

With a large variety of cell subtypes that contribute to the creation of a cognitive map, it becomes obvious that there is no such thing as one single and stable map. Rather it might be distributed across different brain regions as mentioned above and due to the ever changing environment and an inherently noisy and relatively unstable system like the brain, its stability is in question, especially as it adapts to varied stimuli over time.

1.7.13 *Plasticity of spatially selective cells*

Place cell tuning firing as described above is dependent on the environment that the animal is first introduced into. If this original environment is somehow altered such as by removing a single highly salient cue card, then the firing of some of the place cells changes to reflect this shuffle of the environment. This is similar to HD cells where the visually driven cells might update the position if this cue card moves in an otherwise indistinguishable environment. This observation about place cells was already made by Hetherington and Shapiro (Hetherington and Shapiro 1997). The plasticity of time cells is also very similar to the spatially selective cells (MacDonald et al. 2011). Such a finding puts the plasticity and stability of these cells into perspective. This leads to the questions: what kind of environmental stimuli is necessary to create a given kind of spatially selective cell, and how do different changes in the animal state like hunger influence its cognitive map?

1.7.14 *Degradation of place fields.*

Further studies investigated the role of different sensory inputs in the creation of a cognitive map. In Gothard et al. during a linear track task where an animal had to go from a box to a beacon the distance of the outbound journey was changed while keeping track of place cells (Gothard, W. E. Skaggs, and Bruce L McNaughton 1996). The research showed that during a mismatch the representation of the linear track can completely degrade or simply remap depending on the level of change, keeping the firing either to the start box or the location of the beacon. If animals are transported on a cart through a known environment, their representation changes as well (Terrazas et al. 2005). Researchers have also shown that there can be multiple maps of the same environment, each based upon different internal factors (Sheintuch et al. 2020; Butler, Hardcastle, and Giocomo 2019). Levy et al. 2021 and colleagues show that place cells in mice do not keep stable firing across different days, even though there is rate remapping and most cells in an area larger than 1 m^2 are multimodal (fire in more than one place of the arena). Even given this degradation we can still use the cells to decode which environment the mice are in, by simply taking coactivation of place cells. In the neuroscience navigation field, there is a lot of discussion about the stability of the cognitive map and especially the stability of given subtypes of cells that compose it. A recent study which recorded activity from boutons on incoming connections from the MEC suggests that the stability of these boutons and consistency as well as reliability is lower in the incoming connection rather than in CA1 (Cholvin, Hainmueller, and Bartos 2021). All this points to an adaptable cognitive map which reflects the changing environment, especially when encoding for salient stimuli such as a reward.

1.7.15 *Reward and valence related remapping of place cells*

To state the obvious, reward also modulates the level of engagement in the task. If a rat is not given any reward it is less likely to explore a space on its own. Our own understanding of place is heavily shaped by its valence given our current state. If we are cold we value a sauna; in the summer we value a refreshing pool much more. There must be a way that this representation is updated or provided as a valence layer on top of our cognitive map. It was hypothesized already in 1995 that the reliability of spatial firing might be modulated by a common variable like attention (Markus, Barnes, et al. 1994). Research from Charlotte Boccara and Gauthier et al. shows that the firing of place cells can be heavily influenced by a location of a reward, because the cells integrate much of the signal from the environment as well as our own internal signal like motivation via dopamine (Boccara et al. 2019, Gauthier and Tank 2018). We know that the Ventral Tegmental Area (VTA) sends a lot of dopaminergic projections to the hippocampus and that these projections help to upregulate or downregulate firing of place selective cells and specific memories (Naghavi et al. 2019; Esmaeili et al. 2012; Han et al. 2020).

Gothard and colleagues showed this by recording rats in a circular arena while changing the goal of where the reward location was but nothing else. They reported different subpopulations of neurons, each encoding for either the fixed spatial frame or encoding for the task relevant to mobile objects. They reported that if the mismatch from one of the subpopulations appeared in between internal representation or external cues and it was small, it would get corrected, but if it was large then the subpopulation would "jump" into the new representation (Gothard, W. E. Skaggs, and Bruce L McNaughton 1996, Gothard, W. E. Skaggs, K. M. Moore, et al. 1996). The mechanism of remapping could be hidden in theta oscillations as some of it happens on a very fast timescale (Sanders et al. 2019).

There is also a similar finding for grid cells whose firing shifts over time closer to the reward location in the arena (Boccara et al. 2019). Like valence, our internal state is a reflection of our engagement in the environment. Pettit, Yuan and Harvey showed using a simple paradigm that if a water deprived mouse is running on a treadmill for a water reward, the spatial encoding will be much more specific then if there is no water reward given and the mouse is just supposed to run (Pettit, Yuan, and Harvey 2022). They also showed that this is not a case of having multiple maps as described in other studies (Sheintuch et al. 2020; Butler, Hardcastle, and Giocomo 2019).

There have been many previous studies outlining similar findings as well (Markus, Qin, et al. 1995), bringing into question the stability of the cognitive map and whether it is present at any given moment or if it is recalled only if needed depending on the internal state of the animal and the level of its engagement with the task.

1.7.16 *Population encoding of space.*

Much of this research mentioned in previous pages is concerning single cell examples of how these cells can encode for given space. With the emergence of new technology, specifically the development of Neuropixel probes (Jun et al. 2017), there has been a shift from single cell examples to study of neuronal populations. Population responses are more robust then a firing of an individual neuron. For example, Nagelhus et al. take the representation of an object with its properties in space as an emergent modality of a neural population rather than a single cell (Nagelhus et al. 2022).

One of the stark examples of neuronal population encoding of space is the torus topology discovered within the entorhinal cortex (Gardner et al. 2022), which had been computationally predicted many years before as a model. Some of the earliest examples of neuronal population coding come from Sherington Ensembles (Sherrington 1906) and Lorente chains (De No 1933) where in 1933 the youngest of Santiago Ramón y Cajal disciples showed the ability of the brain to have recurrent synapses specifically within the vestibulo-ocular reflex (Larriva-Sahd 2014). The next ensemble theories were Hebb Assemblies (Hebb 1949), Hopfield attractors (Hopfield 1984) and Abeles Synfire Chains (Abeles 1982). Many theories with different names and increasing complexity over the years, but keeping the basic idea of many neurons which can have recurrent inhibition and work together. The more recent and applicable model for study of navigation is a continuous attractor network (K. Zhang 1996; W. Skaggs et al. 1994; Redish, Elga, and Touretzky 1996; Burak and Fiete 2009). This network has been applied to model localized head direction activity as it moves along a 1D ring, which can be created by excitation between cells with similar tuning direction and the ability to have recurrent inhibition.

One can then repeat this activity on a neural sheet, similar to modeling of the firing of place cells in a 2D arena. But what happens when the animal or the representation gets to the end of the sheet? The solution is to bend this sheet into a tube and connect the ends, creating a toroidal topology (Burak and Fiete 2009), (Figure 5). This was only a theoretical solution until recordings with Neuropixel probes and heavy dimensionality reductions analysis with cohomology helped to discover the underlying structure of the neural landscape (Gardner et al. 2022) (Figure 5). Neuronal population encoding will likely be the path in the future understanding of the brain, yet many of the basic building blocks of the population have not been fully uncovered yet.

After discussing the components of the hippocampus and their spatially selective firing I would like to highlight the sensory information sporadically

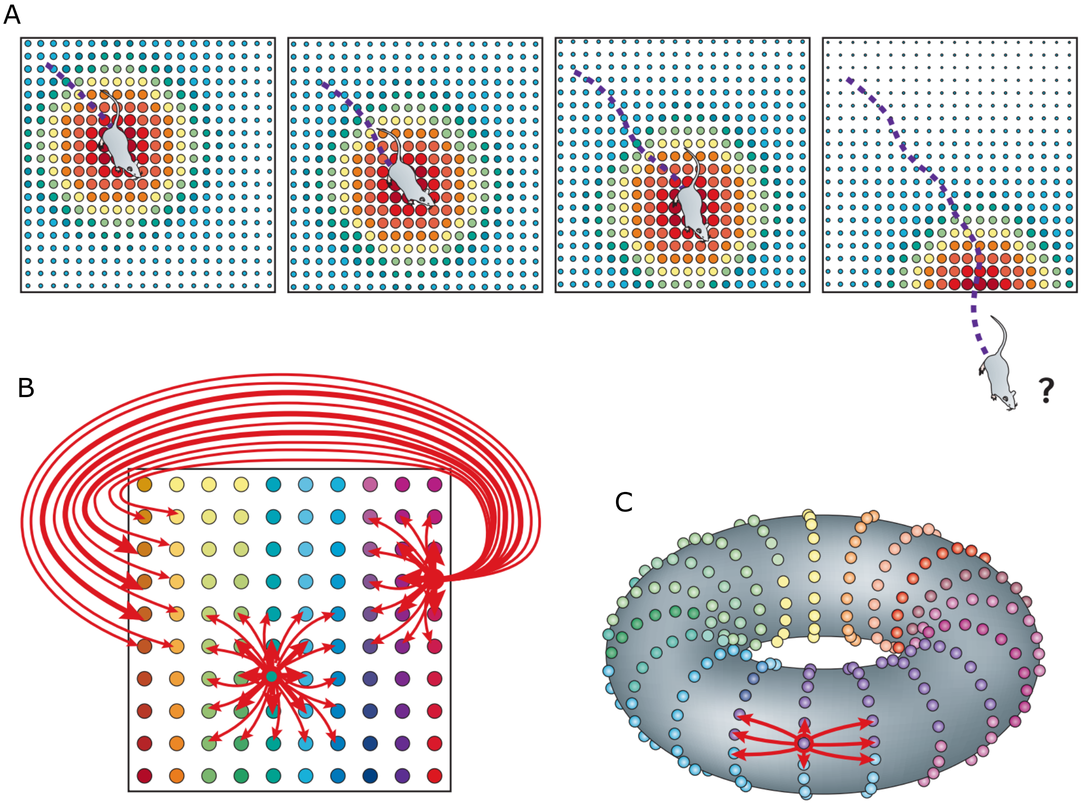

Figure 5: *Place cells on an infinite sheet can be bound to a toroid. A) What happens when place cells reach the end of a neural sheet made up from spatially selective cells? B) Activation of an attractor bump beyond a neural sheet. C) Solution is to wrap the sheet into a toroid. Adapted with permission from Bruce L McNaughton, Battaglia, et al. 2006*

mentioned in the text which informs many of these cells. We have repeatedly discussed the contribution of the visual and vestibular systems in the head direction signal as well as in place cells, and how these signals interact to inform allocentric or egocentric spatial information. These two different streams of information have their own ways of innervating parts of the hippocampus and influence its firing as we have seen. There are also many more streams of information such as proprioception and efference copy, but I will focus the attention mainly on vision and the vestibular or balance system, their contribution to understanding space and how they each aid the allocentric and the egocentric reference frames.

1.8 SENSORY SYSTEMS AND THEIR INTEGRATION

1.8.1 *The visual system*

The visual system provides the hippocampus with predigested spatially modulated and related sensory experiences which enrich the more stable hippocampal place cells (Haggerty and Ji 2015). Once the light enters the retina it is converted into an electrical signal which travels to the back of our brain into the occipital cortex where the whole scene can be taken apart into increasingly complex parts, starting with contrast changes, moving to simple shapes all the way to recognizing moving objects. On its own, the visual system is also modulated by many variables like arousal or attention with many feedback loops going back along the path of light understanding through the brain (Reynolds, Chelazzi, et al. 2004). As we look through our eyes into space we can relate objects with each other, providing a signal where an allocentric reference frame can be anchored to. Vision is not only key for identifying shapes but also depth due to stereoscopic vision, which gives us a sense of visual flow that can be used to estimate not only the speed of a moving object but also our own as we move in an environment (Ludwig et al. 2018). This ability to decode speed and space is highly reminiscent of the cell in hippocampus we mentioned earlier like the HD cells. The signal to these coming from both the visual and the vestibular systems for more robust and redundant understanding of space.

1.8.2 *The role of the vestibular system*

Self motion is clearly one of the essential pieces of information guiding locomotion during path integration. The vestibular system is an evolutionary conserved sense and due to its strong convergence it does not appear as a conscious

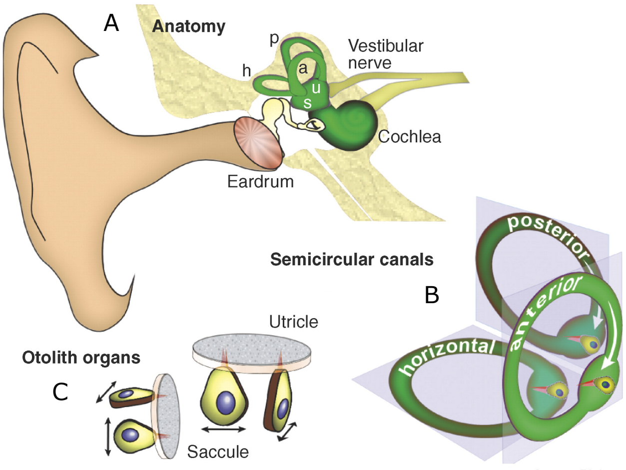

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Figure 6: *The Vestibular system. A) Depiction of the human ear and the vestibular organ with anterior, posterior and horizontal semicircular canals encoding rotational acceleration shown in more detail in B) and the utricle and the saccule as the otoliths organs encoding the linear acceleration enlarged with detail in C). Adapted with permission from Day and Fitzpatrick 2005*

sensation (Straka and Baker 2013; Angelaki and Cullen 2008). It consists of semicircular canals encoding rotational acceleration and the otolith organs (named after calcium carbonate structure in the inner ear) encoding linear acceleration (Lindeman 1969) (Figure 6). Since these organs only detect acceleration, during constant velocity the vestibular signal cannot be used to detect how fast one is going without the use of visual flow. For example, when driving in a car, if we close our eyes it is hard to estimate our speed. A connection between the hippocampus and the vestibular system has also been established. Electrical stimulation of the vestibular sensors induces changes in the field potential of a guinea pig's hippocampus with an approximately 40 ms delay (Cuthbert et al. 2000). Similarly, stimulation of the medial vestibular nucleus increases the firing rates of CA1 complex spiking cells, which likely correspond to place cells (Horii et al. 2004 Russell et al. 2003). These connections indicate a clear role of vestibular information in creating the brain's place code (Smith 1997). Research from Paul Smith and David Bilkey has pointed over and over to this role. In particular, the vestibular system influences theta oscillation in the hippocampus (Russell et al. 2006; Aitken, Zheng, and Smith 2018), largely due to theta being modulated by acceleration of the animal (Kropff, Carmichael, E. I. Moser, et al. 2021; Kennedy et al. 2022). Most recently this link was made clear by specifying connections across different layers of the hippocampus connecting to different part of the vestibular system, pointing to a high degree of specificity and redundancy in the system (Hitier et al. 2021). It is also worth noting human studies where patients who had vestibular deficits were found to have an atrophy of the hippocampus, underlining the use of vestibular information in memory and its function (Brandt et al. 2005; Kremmyda et al. 2016; Smith 2017). The vestibular system and its signal is also influenced by environmental factors and can be modulated by vision and other sensory information (Cullen 2012). In general it provides a reliable egocentric estimation of self motion which can be used together with visual flow to gain understanding of space.

1.8.3 *Other sensory information*

Vestibular and visual information are not the only ones involved in path integration. Other senses such as olfactory, proprioceptive, and auditory play a role in localization. Somatosensory information coming in via whiskers in rats is also an important source of space related information. There is also collateral discharge, more specifically the efference copy and perhaps many others but likely less important than the two mentioned above (Poulter, Hartley, and Lever 2018). How can these two separate sensory information streams integrate to make sense of the environment in an allocentric and an egocentric reference frame? We will now explore this in an example using head direction cells which combine both of these sources.

1.9 MULTISENSORY INTEGRATION

1.9.1 *Example of multisensory integration in HD signal*

An obvious and already well described multisensory signal is the heading direction. As described earlier, this signal is heavily modulated by the vestibular signal, but research shows that it also integrates visual landmark signals and proprioception (Angelaki and Laurens 2020). The HD attractor does not just encode the heading direction in an egocentric reference frame, it also dynamically changes depending on given goals (Angelaki and Laurens 2020). These changes are also connected to differences in active or passive motion of the animal (Laurens and Angelaki 2018). There is also an internal model of the sensors which helps to correct for any error (Figure 7). When presented with differently reliable cues between visual and vestibular signals, the brain will choose the more reliable one (Fetsch et al. 2012).

Dora Angelaki and Jean Laurens proposed a bayesian internal model (Figure 7) predicting how the incoming sensory info should look like (Laurens and Droulez 2007, Angelaki, Shaikh, et al. 2004). If the incoming stimuli are misaligned with this, an error message will arise. In Virtual Reality (VR) with restraint the final

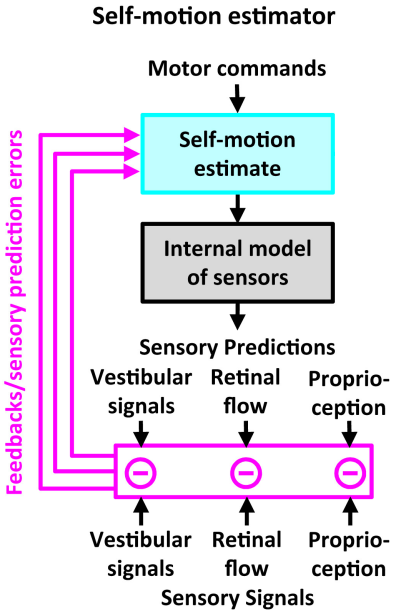

Figure 7: *Sensory signals like the vestibular signal, retinal flow and proprioception meet their predicted signals from efferent copies from the motor commands to create sensory prediction errors (magenta) which are fed into the self motion estimate (cyan). This can be then read out downstream as well as help to update the internal model of the sensors again. Adapted with permission from Laurens and Angelaki 2018.*

self-motion velocity which helps to guide the HD attractor ring can be heavily underestimated during some fast movements. This is due to the restrained animal being affixed on top of a floating ball which drives the VR and creates a mismatch with the internal model. This in turn heavily alters the spatial code produced by the animal (Laurens and Angelaki 2018). Research during restrained VR therefore discounts the use of the vestibular system.

During locomotion the vestibular system might attenuate the amount vision is used in perceiving the environment, specifically the encoding of some conjunctive cells (Dipoppa et al. 2018). If the environment around the animal is rotated the specificity of spatial firing is even worse. Hence the visual stream is not the only contributor in creating spatial maps, but rather plays a role in combination with the vestibular signal for the head direction cells and many others. According to Terrazas et al. 2005 there is an approximately equal contribution coming from ambulation or proprioceptive signal, vestibular and optic flow signals.

Which sensory modality takes the front seat when the animal is locomoting around an arena? Whishaw and colleagues (Maaswinkel and Whishaw 1999) set out to try to answer this question with a path integration paradigm involving blind folding rats and removing all olfactory cues by displacing the arena. Their paradigm was a modified cheeseboard maze task, during which there are food cups in the holes of a table and food deprived rats are tasked to take a large pellet from the middle of the platform and then go back to the original place where they started locomoting from. Rats in general like to go to safety when consuming food. In this case they found that the rats preferred visual information over olfactory over self movement information (Maaswinkel and Whishaw 1999). In human studies this is now being done with the use of virtual reality where research can easily modify the amount of visual feedback a person is receiving (Harootonian, Ekstrom, and R. C. Wilson 2022; Stavropoulos et al. 2022). The same setup has been applied to study monkey's perception as well (Alefantis et al. 2022). These studies show that subjects rely heavily on optic flow when present and bias their expectation even in darkness by building up a model spurring from the optic flow they just experienced. The optic flow cues also emerged as most reliable during a constant velocity where the vestibular system's information becomes less reliable (Zanchi et al. 2022). shows that during an audiovisual task, humans tend to have individual differences in how they process different kinds of sensory cues, but in general they prefer visual over auditory.

Path integration and its multisensory components hence change flexibly weighting and combining different sensory stimuli depending on reliability, environmental constraints and own movement. The way these sensors combine to form our understanding of allocentric and egocentric frames is very dynamic and environment dependent.

Research from Yong Gu's lab in Shanghai discusses the role of the passive and active motion in a monkey perceiving its environment when a passive linear motion is applied (Gu et al. 2006). In this case the research focuses on the parts of the visual (optic flow) and vestibular systems giving rise to the self motion perception. The researchers argue that the signals have different temporal components which need to be taken into account when trying to combine them into one perception of motion. By performing single unit recordings Gu et al. discovered that up to two thirds of neurons in a Medial Superior Temporal Area (MSTd), which is often described as part of the visual pathway, are modulated by visual and vestibular signals (Gu et al. 2006). This result points to MSTd, a multisensory area in the brain, as a possible region to store the HD signal. There are also many other areas which could serve as candidates for having the HD signal. Researchers also recorded from what are originally thought of as more visual areas such as lateral Intraparietal Area (LIP) and Frontal Eye Field (FEF) (Gu et al. 2006). Here they show differential responses in timing of encoding both of the modalities. Integration and its possible readout likely happens in multiple brain regions.

The same study states that the brain processing of optic flow encodes velocity, whereas the vestibular system processing encodes acceleration. It then follows that a more accurate representation of a self motion movement can be done

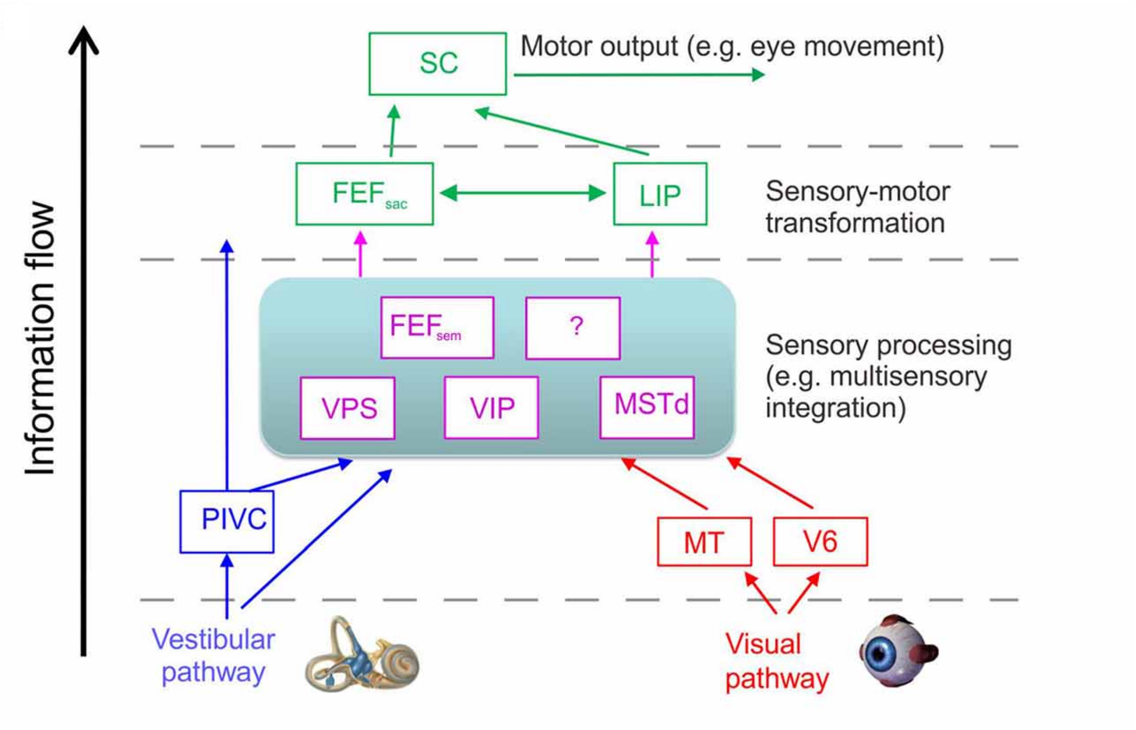

Figure 8: *Multisensory integration for correct motor output (eye movement). Information from vestibular and visual pathways integrates in many possible areas like the VIP or MSTd then for an intentional movement of an eye it is transformed and eventually reaches the superior colliculus (SC) which sends the motor command for an eye movement. The information flows from the sensory pathways to the motor commands. PIVC parieto-insular vestibular cortex; MT, medial temporal area; V6, visual area 6; VPS, visual posterior sylvian area; VIP, ventral intraparietal area; MSTd, the dorsal portion of medial superior temporal area; FEFsem, smooth eye movement region of frontal eye field; FEFsac, saccade region of frontal eye field. LIP, lateral intraparietal area; SC, superior colliculus. Figure from Z. Cheng and Gu 2018 licensed under https://creativecommons.org/licenses/by/4.0/ permission not required.*

for acceleration or deceleration only by the vestibular signal, especially at the beginning when the acceleration signal is much stronger in order to guide the HD signal (Gu et al. 2006). In their review they also suggest that vestibular information is used for heading estimation but that visual flow is used more for distance estimation, together forming the two pillars needed to create a vector for a path integration task (Gu et al. 2006).

Overall, for the integration, it matters how timely and reliable the cues which are presented are (Fetsch et al. 2012), as well as how well they match with an internal model of the world (Figure 7). In general, visual information is preferred over vestibular during path integration. It all depends on the level of exploration and complexity the task requires (Burgess 2006).

1.9.2 *Cue Conflicts*

Path integration is evidently a multisensory process which needs to work flexibly with different kinds of input information. It is essential for path integration to work well in all conditions. It is essential for survival to provide the correct sensory information, especially during conflicting information. In this section I will focus on research pertaining to path integration only.

1.9.3 *Does the winner take all?*

During multisensory integration conflicts can arise where one sensory modality gives a very different readout to the other and the brain must account for this depending on the reliability of the cue. According to some, a cue conflict would not be dealt in a winner take all manner but rather by correcting the new estimation to fit in between the two assumed directions (Knight et al. 2014). Other researchers claim the opposite, where the brain might be influenced by the winner take all competition (Fetsch et al. 2012). When a new polarizing cue is introduced, the HD system in general accounts for the presence of the new cue if it is less than 120 degree away. Once the conflict is over 120 degrees then HD cells learn a weaker influence of these cues. This light cue would have more of a difference for animals which were new to the task rather than experienced animals which would have learned the weaker influence of the light cues and preferred the use of background cues for these longer estimations. The principles for such plastically weighted cue contributions are present in the head direction attractor network (Page et al. 2014), (Figure 8). It is then most likely that small changes should shift the attractor but, if the changes are larger, the whole thing would jump to another attractor state as shown in (Fetterhoff, Sobolev, and Leibold 2021). This holds for other cues as well as there are multiple levels of integration and at each step something else can play a role. For example, at the level of the HD cells, vestibular info might be essential in darkness, but a place cell which receive information from HD system might actually encode space in a Bayesian manner, weighting cues based on a previous model of the world.

1.9.4 *Path integrator resetting*

As we just explored, sensory mismatch can lead to integrating between conflicting cues or by adhering to ones which are deemed more reliable. How long can a human walk with closed eyes before veering off a given path? Can a path integrator work on its own without being reset by the allocentric cues when given? Which frame of reference could take over? How does this affect theta oscillation and which sensory modality dominates the process of constantly updating a vector to a safe space within the arena? Path integration is subject to drift due to accumulated error. A path integrator can be reset on its own through egocentric stimuli or via allocentric cues. The resetting works on the basis of aligning the phases of the dendritic oscillators when the rat is at a known location aligned with allocentric cues (Burgess 2008). When self motion and or visual flow are taken away from animals' sense of orientation the spatial code degrades.

It is important to understand how this resetting is done, since an error can always accumulate due to noisy sensory input or inaccurate information. Sometimes the brain can melt two information streams into one, but sometimes it needs to simply go with one which is deemed more correct. With two kinds of egocentric information coming in, it is important to know which stream to reset to and when especially during path integration. Where could such resetting and the whole path integrator be happening in the brain?

1.10 BRAIN AREAS FOR PATH INTEGRATION

Could there be one region or one area in the brain which is responsible for such a complicated mechanism as path integration? One might suggest, after knowing about all the spatially selective cells, that it might be inside of the hippocampus. Yet some studies with hippocamptomized rats have proven otherwise (Alyan and B. McNaughton 1999). In the Morris water maze task hippocampetomized rats performed with much higher latencies, yet when they were tasked to dig underground after completing an L shaped path they could dig straight to their home port from which they originated the journey. Based on this research perhaps it is possible to think that the hippocampus does not play such a role, but this would also counter the theory of the cognitive map. Maaswinkel and colleagues offer an opposing view. They designed a cheeseboard task to uncover contributions of different sensory modalities to path integration and found that the hippocampetomized rats were not able to complete this if they only had self motion cues available (Maaswinkel, Jarrard, and Whishaw 1999). At the time, research was not clear about how much path integration uses allocentric cues. These are both crude research mechanisms from last century showing opposing views on the role of hippocampus in path integration. Research has since advanced at a lighting speed for example with the ability to excite single neurons with a reversible perturbation.

From Muir and Taube it became clear that the HD signal plays an important role in path integration (Muir and Taube 2002). When animals are able to solve a spatial task using allocentric information they do not need to rely on this signal, but given a pure path integration task they need to rely on the idiothetic cues from which the HD signal arises. Some modeling work has described the egocentric map of space to be in the neocortex (M. Recce and Harris 1996).

Either there is a mechanism which reads out from these cells which direction the rat is heading then computes and represents the vector somewhere else or the whole mechanism might just be stored in the anterior thalamus (Blair and Sharp 1995). Here they described that if a rat was facing and heading in a certain direction towards a goal, the firing of the cells changed in a systematic way such that the tuning curve became taller, narrower and more skewed toward a given side of turning. In this case the brain could be using just the readout from the shape of these firing curves and know its vector only to then decode a similar vector back. Unfortunately they only found about 33 cells within this definition in 10 rats which might not be suggestive of strong results and this finding appears not to have been repeated by other labs. Later, Sharp and colleagues described an attractor model network made with similar kinds of cells, where the suspected HD cells consist of a large network of anatomically diverse cells required to make such a signal which could potentially be later used in path integration (Sharp, Blair, and Cho 2001).

In the same year as Hugh Blair and colleagues published the research above, a paper from McNaughton lab came out regarding *deciphering the hippocampal polyglot*. Here they theorized that the mechanism of navigation on a cognitive map could be thought of as a disc where head direction cells play the biggest role; they are constantly updated with direction and magnitude of travel. Because experiments with head restrained rats or rats with restrained bodies have shown a lack of path integration ability, the vestibular system must play an essential role (Knierim, Kudrimoti, and Bruce L McNaughton 1995). Such information gets updated based on more allocentric cues like vision and other sensory signals which with its flexible connections are supposed to create associative learning connections to the place cells. Thus updates happen only when those are available. The readout from the disc is then the suggested path the animal should take. In this work there is no particular place in the brain where the disc is situated but the signals are described to come from the neocortex, thalamus and the presubiculum.

Can the basic integration metric be hidden in the grid cells whose output is anyways translated into the place cells (Bruce L McNaughton, Battaglia, et al. 2006)? Degradation of grid cells does induce some impairment of a path integrator (Gil et al. 2018; Winter, Clark, and Taube 2015). Recently due to grid cell research advances, this has become a promising candidate brain region to hold the location of the path integrator. In an opposing view, a study in humans with lesioned hippocampus and entorhinal cortex demonstrated their ability to keep a vector traveled on an L shape path (Shrager, Kirwan, and Squire 2008). Some questions still remain unanswered, including: how would the grid cell model of a path integrator be reset if it mainly receives information from head direction cells and no information about the valence of the outside cues in the environment? The entorhinal cortex likely plays an important role due to its possibility to create a metric; as its regular structure allows to keep the same distance between the nodes of the hexagon. (Bruce L McNaughton, Battaglia, et al. 2006; Fukawa et al. 2020). It also feeds a lot of its information into more stable CA1 cells which are modulated by it, but can also fire independently of it (Zutshi et al. 2022; Bowler and Losonczy 2022). Grid cells have been shown to need to the firing of place cells to function properly, perhaps as a way of resetting the grid (Bonnevie et al. 2013, Fernandez-Leon, Uysal, and Ji 2022). There are many connections between grid and place cells as their anatomy suggests (Deadwyler et al. 1975).

Is it possible that the integrator is in the RSC? If we ablate (inactivate) the cells there, the animal is unable to complete an escape trajectory (Vale et al. 2020; Keshavarzi et al. 2022). In RSC is also a place where visual cues can be associated with heading (Sit and Goard 2022). In a similar sense if the HD signal is not receiving inputs from the RSC, which helps to update the orientation with visual landmarks, it must rely only on its own sense of egocentric direction to update the firing of HD cells (Clark et al. 2010), which would over time accumulate large errors. Maybe we should ask the question differently: rather the integrator being only in one small part or subset of the brain, we should think about it as a distributed process. Due to its use of many sensory inputs and cells which can combine parts of the sensory input into a coherent signal like the head direction signal, the convergence of these might not happen only in one region with one kind of cell. It might rather follow a decentralized strategy due to its vital importance in the survival of the species.

1.11 PATH INTEGRATION MECHANISM

After discussing specific pieces of the possible path integration mechanism, starting with place cells and moving all the way to population encoding of space through toroidal topology, I want to shift attention to the mechanism of path integration.

Beginning with Jander (Jander 1963) (behavior) and the Mittelstaedts (H. Mittelstaedt and M.-L. Mittelstaedt 1982) (behavior and model) many original papers only consider behavioral substrates to create a model of a cognitive map and its use for path integration.

How does the path integrator function? Darwin himself said he did not have sufficient data to discuss whether keeping a dead reckoning of a course is better in humans than animals and whether this mechanism is always active (Darwin 1873). This belongs to some of the earliest discussion about a path integrator mechanism, followed by Murphy who elaborates on the mechanism by way of giving an example of a ball suspended by a line in a train cart (Murphy 1873). In this example, anytime the train moves this force is then reflected in the movement of the ball, anytime the train accelerates or moves to a certain direction this is represented as a vector one could read out from the ball using Newton's second law. He then proposes a similar mechanism which might be happening in the brain but refrains from speculating about where or how this integration happens. We can compare this analogy to the vestibular system, where the movement of a suspended fluid in the ear causes a neuronal discharge, giving information about acceleration. It has become clear that the vestibular system plays a key role in creating self movement information. These early models and speculations often fail to consider the inherent error the path integrator can accumulate and how it can read out velocity correctly, as well as steer far away from how this happens in the brain. Some even suggest the existence of two path integrators, one for the inbound and one for the outbound path (H. Mittelstaedt 2000).

Later models split path integration into an encoding and a retrieval of a series of vectors accumulated during the path. As hypothesized in Markus et al., already in 1995 researchers understood that place cell firing can be driven by selectivity of the task either by directedness of their travels or by other inherent structure of the task (Markus, Qin, et al. 1995). Due to prospective firing of place cells, it was hypothesized that place cells can represent vectors to and from specific locations within the maze (Muller and Kubie 1989; Markus, Qin, et al. 1995).

1.12 VECTORIAL REPRESENTATIONS IN THE BRAIN

Since then, much of the research surrounding path integration has been about the ability of the brain to keep track of vectors to represent its own movement in the environment. These vectors could be projected on the cognitive map. But in order to successfully complete a path integration task one should not need the cognitive map. In fact that is one of the hallmarks of the integrator, that one can explore new spaces and always find a way back to the original environment just by summation of the previous vectors taken from an initial point (Bruce L McNaughton, Knierim, and Matthew A Wilson 1995).

Research from Jercog et al. 2019 and Sarel, Finkelstein, et al. 2017 suggests that head direction can be modulated by the presence of a goal and especially that the head direction signal which fires relative to a reference point in an environment has a large influence on firing of the CA1 cells (Jercog et al. 2019; Sarel, Finkelstein, et al. 2017). Head direction specifically influences CA1 cells to have higher response when the animal moves towards a location of this reference point. A recent collection of high level publications has focused on how the brain could represent such path vectors. Earlier we discussed conjunctive or multiplexed cells, which can simultaneously encode distance along with other variables. A vector is a simple combination of distance and direction. We have now seen this represented individually as well as combined in the case of object vector or landmark vector cells for example found in the CA1 (Høydal et al. 2019, Deshmukh and Knierim 2013). The two following studies I am about to introduce are juxtaposed in their methods. One uses virtual reality with a head fixed rat, yet the other uses a freely moving rat in a real environment, but both record from around the hippocampus and look for how the brain represents distances and angles.

Moore et al. claim that for creating vectors rats use episodic specific representation and that the distance selectivity precedes angular and allothetic spatial selectivity (J. J. Moore et al. 2021). In their description this information comes from upstream structures into CA1 and combines with multi sensory inputs to generate this selectivity. The coding improves the closer the subject is to a given goal and over many trials.

In a second study from Ormond and O'Keefe researchers further explored the finding from Jercog which proved that with uneven sampling the place cells would shift slowly towards the goal location (Ormond and O'Keefe 2022; Jercog et al. 2019). With their highly specialized maze setup Ormond and O'Keefe ensured a more equal sampling and they indeed found given consinks or reference points as mentioned in Jercog which would shift the firing of a given place field (Ormond and O'Keefe 2022; Jercog et al. 2019). The tuning of these cells was stronger than the firing of head direction cells. The consinks would also shift towards a goal in a new place whereas the place cells did not show this significantly, even if previous research has shown this independently (Hetherington and Shapiro 1997). Similar to findings from Moore et al., these consinks were multiplexing, containing the direction, distance and allocentric direction in this order of significance (J. J. Moore et al. 2021). In this case it seems that the relative distance gave the most information in Moore et al., whereas in Ormond and O'Keefe the direction gave the most information (J. J. Moore et al. 2021; Ormond and O'Keefe 2022).

Unfortunately in the latter study they did not show the changes over time due to their maze setup. Both differences could be explained by the use of virtual reality or a real paradigm where the amount of vestibular information is different. The differences between these two studies point to a high level of interest, but a lower level of coherent understanding of how vector addition works in the last few years. Many more studies looking into the representation of vectors in a brain are also upcoming.

Another example are Vectorial Selective Cells (VEVS) which were described in Purandare et al. 2022. VEVS cells are visual cortex-like responding cells that show tuning for direction, distance and velocity of a moving bar stimulus within the hippocampus. These cells fire retrospectively, unlike hippocampal place cells, and do not remap over days. They also serve as place cells in spatially selective tasks but are not modulated by rewards showing possible downstream cells from the visual cortex which could be essential in navigation. In general we can observe that both the vestibular and the visual systems are important in creating a vector representing a path in the brain; how and where the vector is saved and precisely represented in the brain is still unknown.

1.13 THETA - RHYTHM OF NAVIGATION?

As we highlighted earlier there is likely no single place where path integration happens as a whole. To be able to decentralize the process throughout the brain regions, there needs to also be an overarching rhythm which might help to encode and aid the timing across those regions. John O'Keefe suggested already in 1984 that two oscillators which are offset to each other but acting on the same neuron might actually have a specific function. This was later described by O'Keefe who coined the term "phase precession" (John O'Keefe and M. L. Recce 1993). This temporal coding phenomenon, observed first in place cells in 1993 and then also in grid cells, whereby a given cell's action potentials (spikes) occur at progressively earlier phases of the local theta oscillation as the animal traverses the spatial field (Figure 9). When projected across the place fields and the theta sequence one can decode the past and future trajectory of the rat. The precise mechanisms underlying phase precession remain unclear. Phase precession may contribute to coding "distance-through-field" and spatial sequences. Theta precession also shares a mechanism with theta sweeps where an animal, usually at a decision point, might transiently encode a series of certain locations which help it to "lookahead" into possible future paths.

The theta rhythm has been associated with a plethora of animal behaviors (Korotkova et al. 2018). It is for certain that over the last decades of research on

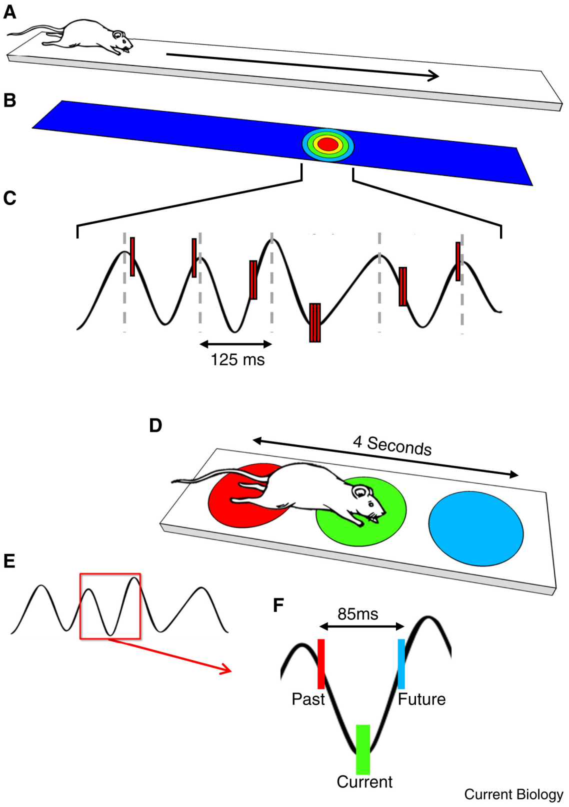

Figure 9: *Theta phase precession. A) Animal running on a linear track. B) A place field recorded from the animal during traversal. C) Theta oscillation with an overlap of the firing of the cell as the animal traverses the place cell field; notice that the center of the oscillation has three spikes and is in the trough of the cycle, showing that those spikes would be from the center of the place field due to their firing density. D) Animal traversing during four seconds across two other adjacent place fields. E) Showing the theta oscillation during this run. F) Close up of one phase of theta and firing of the nearby place cells where the animal is currently. The width corresponds to the amount of firing. Since the animal's head is in the center of the green place field the green spikes are widest. Figure from Poulter, Hartley, and Lever 2018 licensed under https://creativecommons.org/licenses/by/4.0/ permission not required.*

theta rhythm using various animal species that when an animal is immobile very little theta is present, especially if there are no changes in the environment during which an animal could be "thinking" (György Buzsáki 2005).

The cognitive map theory does not explain how a spatial metric needed for distance and angle estimation is created. The spatial metric needs to be created via movement and it needs to be provided during initial exploration when dead reckoning. Moving animals can calculate travel distance from the product of the temporal metric of theta oscillations and the velocity correlated changes of firing rate within the place cells (György Buzsáki 2005; Mehta, A. Lee, and M. Wilson 2002). As mentioned before, research from Paul Smith's lab has pointed over and over to the role of the vestibular signal in theta oscillation (Russell et al. 2006; Aitken, Zheng, and Smith 2018) and the vestibular system encodes very well for changes in speed produced during any movement (Angelaki and Cullen 2008).

Theta cycling is also heavily dependent on the velocity of the animal and is modulated via the vestibular signal (Figure 10) (Terrazas et al. 2005) . Further research from Terrazas et al. points out that locomotion cues are an important aspect in updating location specific firing (Terrazas et al. 2005). If rats are put in a moving object like a toy car and are driven around, their place field widens and more theta cycles (associated with place cells firing) are then required to cross these fields (Figure 10).

Phase precession is when the rate of a place cell firing changes independently of the phase of theta. It is possible to fit 6-9 assemblies within a single theta cycle given a normal speed of a rat being 5-6 cm/s during such theta cycle, representing the past, present and the future within the theta traveling wave. Due to the rules of spike timing-independent plasticity the future location can be bound together in a forward direction in the recurrent CA3 and CA3 to CA1 synapses; this is referred to as the theta "compression" mechanism (W. E. Skaggs et al. 1996).

Theta compression and precession has also become clear from more literature showing prospective and back prospective sweeps of neural activity that are locked within theta oscillations (Johnson and Redish 2007; Pfeiffer and Foster 2013). Research from Pfeiffer et al. suggests that these sweeps exist in the hippocampus of the rat and can be represented in the theta dynamics with the trough of theta representing the future sweeps and the peak of theta representing the present or the past (Pfeiffer and Foster 2013). These have been found in rats who were preparing to jump across a platform (Green et al. 2022).

Researchers have also questioned whether these sweeps of neuronal activity represent possible paths or the path an animal will end up taking towards a goal. In order to study this, rats were given two similar paths to get to a goal;

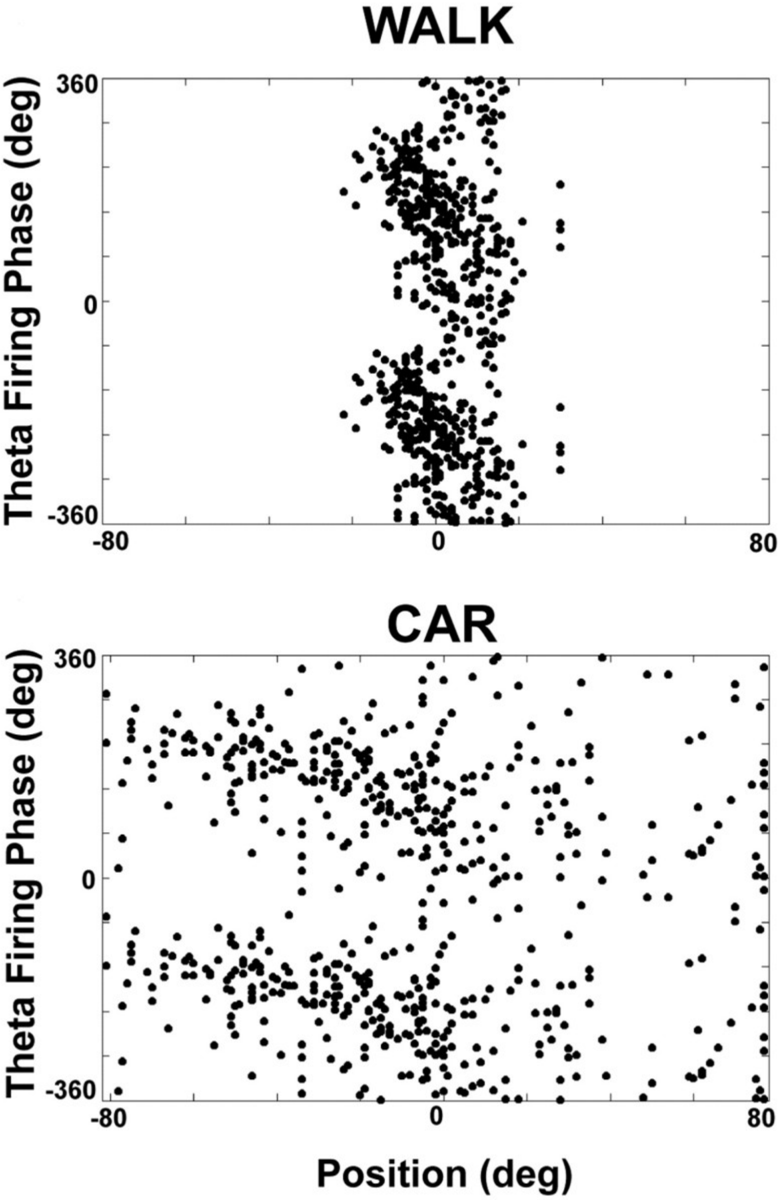

Figure 10: *Difference between self motion and translation in a toy car for preference of theta phase of firing. Each dot represents an animal position during a place cell firing and the given place cells preferred firing rate at the phase of theta. We can clearly see the reduced slope of the theta phase precession as the animal would walk through a given place field with either full sensory information or reduced vestibular flow Terrazas et al. 2005 Copyright 2005 Society for Neuroscience.*

the recordign of the neural activity while the rat was doing the task showed prospective sweeps which represented the route taken and not the goal at the end of the paths (Grieves, Wood, and Dudchenko 2016).

Phase precession creates a link between place cells and time which allows for many further proposals. Based on this phenomenon the creation of a temporal organizing mechanism within the critical time frame for neuronal plasticity was proposed.

1.13.1 *Moser's grid vectors*

Recent research from the lab of May-Britt and Edvard Moser's also suggests that grid cells also use theta sweeps and precession in order to project into future trajectories and help to plan out a given path. This precession alternates every other theta cycle on one or the other side in a given 60 degree; one time to left and one time to the right (Gardner et al. 2022).

This could be a clear mechanism explaining how a homing vector or a goal direction vector is represented in the attractor dynamics of the cognitive map. For the grid cell level these sweeps in 60 degree angles would give the rat a better estimation metric, but might not be able to provide one vector only estimating the correct goal direction, instead multiple to the side of the animal, perhaps feeding the possible vectors towards the hippocampus.

It also prompts speculations that the 2D "cognitive maps" are created by traversing place cells from different angles during initial dead reckoning exploration. This mechanism is also very similar to consolidation of episodic and semantic memory where a given experience or a term needs to be connected from more "angles of thought" in order to be remembered better. The consolidation of this knowledge then takes place during sleep or sharp wave ripples when semantic information and cognitive maps alike are transferred to the neocortex (György Buzsáki 2005). How can this knowledge be recalled if there is no environmental stimuli to help to recall it.

During a lack of environmental stimuli, spontaneously recurring sequences appear in the hippocampus of mice which show an internally hardwired structure giving a metric during movement without external cues (Villette et al. 2015). These hard wired sequences could also be an integral part of the path integrator network, guiding the navigation when external landmarks are nonexistent such as in a path integration task with lack of visual input there might only be these internal sequences to guide navigation by integrating the self motion cues from the vestibular system (Figure

1.14 MEMORY REPLAY

The level of performance on a task is also driven by how well the animal can consolidate the information from the previous run. Sleep plays an important role in cementing the memories. We know that affecting the Sharp-Wave Ripple (SWR) during sleep can cause a selective memory deficit (Gridchyn et al. 2020). More evidence also shows that this replay also happens during sleep and can hence be consolidated (Figure 11) (Diba and György Buzsáki 2007; Benchenane et al. 2010). During sleep many more sequences can be replayed within a sharp wave ripple episode due to the activation of up to 16 percent of pyramidal cells whereas during a wake cycle less than one percent of these cells are active (JJ Chrobak and Buzsáki 1994). Sleep allows us to connect episodes with the firing of the same place cells together. This can also happen over an extended path or experience where an extended ripple is composed of chains of shorter experience (Davidson, Kloosterman, and Matthew A Wilson 2009). Research from Gridchyn et al. shows that if this replay of place cells which encode for reward locations during SWR is disrupted optogenetically, animals exhibit decreased performance during a cheese board maze task where they have to remember a location of a food source (Gridchyn et al. 2020). SWRs also play a role not just in memory replay but help in decision making and planning (Joo and Frank 2018).

The ripples do not only occur during sleep (György Buzsáki, Vanderwolf, et al. 1983), but might play a large role during reward consumption in the performance of the animal on a given path integration task (Singer et al. 2013). They can serve such a “contemplating” function where the animal is thinking about its future trajectories (Carr, Jadhav, and Frank 2011 ;Xu et al. 2019), especially at maze choice points, where sequentially active neuronal assemblies can “look ahead” toward either the right or left arms of a T maze (Papale et al. 2016). During sharp wave ripples, these trajectories, the ones which are progressing towards the goal, are heavily biased (Papale et al. 2016). Moreover, as mentioned above, during sharp wave ripples researchers have found an alignment of HD signal to one particular direction forcing the replay of a given direction of travel (Viejo and Peyrache 2020).

This evidence is mainly gathered from rodent data, but some evidence is emerging that in humans these forward and backwards sweeps might be involved in human navigation as well. A study from Zhu et al. has shown similar dynamics within human eye movements where participants in VR show saccades which

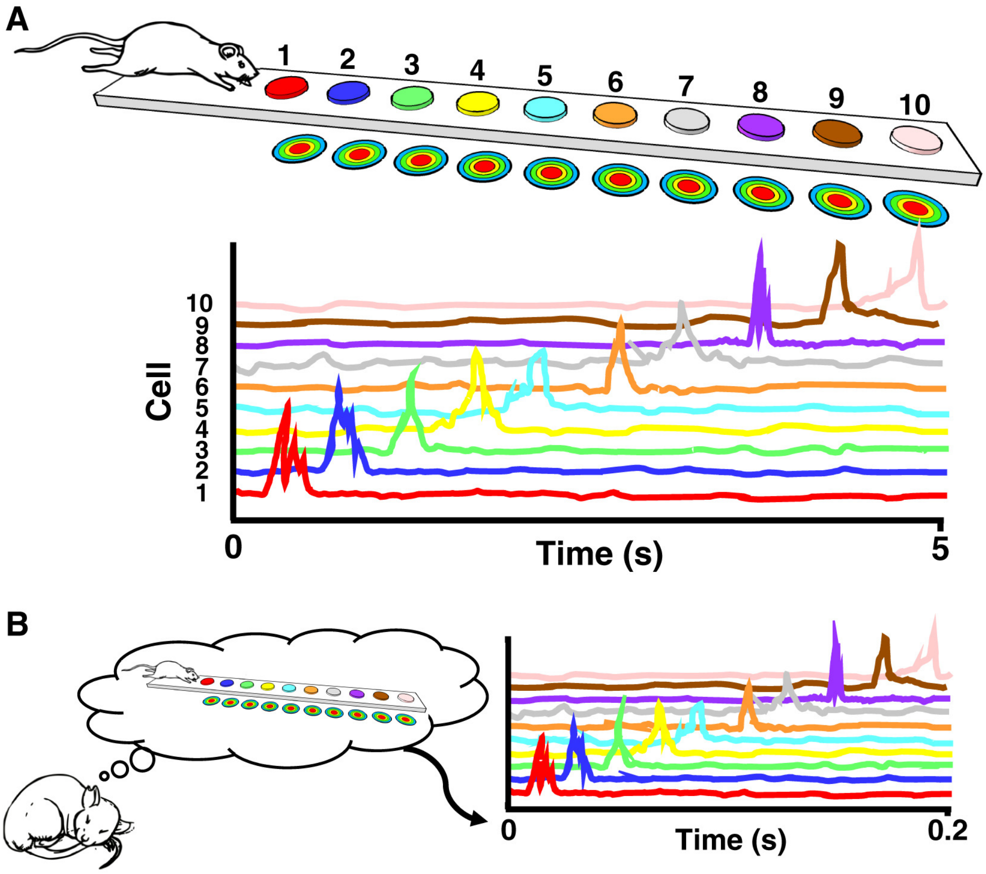

Figure 11: A) Similar to Figure 8 where an animal runs across a set of valent locations like a smell of a predator or food. Below is firing from cells which might represent this given salient stimuli and their respective place field. B) An animal is sleeping and during a sharp wave ripple can experience the same trajectory; note the time axis which is 25 times smaller. Adapted from Poulter, Hartley, and Lever 2018 Figure from Poulter, Hartley, and Lever 2018 licensed under https://creativecommons.org/licenses/by/4.0/ permission not required.

travel forward as well as backwards along the path they have taken to achieve their goal; this is a a physical representation of what might be going on in the brain through theta precession (Zhu et al. 2022).

How can all these mechanisms fit into one one large picture? There has been much work done using simulations of how a path integrator works. I will now explain some of this work.

1.15 PATH INTEGRATION MODELS

Over time there have been a plethora of models describing theoretical ways that this seemingly simple problem of path integration can be solved. A simple example starts with the description of a ball in a train cart above (Murphy 1873). Later, models focused on research on bees and ants around Munich (Jander 1957). Unfortunately many of the earlier models do not consider any error which could occur due to sensory integration and the presence of noise.

2

EXPERIMENTAL DESIGN AND PROCEDURES

In this section I want to briefly describe such a novel path integration task.

2.1 PATH INTEGRATION TASK

In order to execute our path integration task we used a freely moving virtual reality setup as described in (Del Grosso, Graboski, et al. 2017). As mentioned above, head-fixed VR experiments on path integration lack vestibular input, making these kinds of studies less focused on the core mechanism of path integration which requires freely moving vestibular input (see Methods).

The behavioral paradigm was tested on 11 animals, 10 of which successfully learned the task above chance-level accuracy within 15-20 days of training. Training sessions were performed throughout this period once a day for 30 minutes each. There are two types of trials, one visible and one invisible. In an average session rat achieved between 50-100 trials.

In the first visible trial, a pseudorandomly-located beacon is shown in the arena and the rat is tasked to find a 3D virtual green beacon and rear within 7.5 cm of the center of the beacon which is also the size of the beacon. The rest of the arena stays dark to prevent invasive visual cues interfering with the search for the beacon. Once the animal rears (see Methods) within the beacon area, a distinct behavioral output, the arena turns completely dark and a pellet accompanied by a conditioned stimulus sound is simultaneously released (Figure 12A) (see Methods). The pellet location is randomly scattered on the surface of the arena. Each trial can be thought of as having 2 segments, an inbound segment and an outbound segment, referring to direction to or away from the beacon (Figure 12A). By rearing in the arena the animal completes the inbound segment of the visible trial.

In the second trial, now invisible, the animal is tasked to explore the arena in complete darkness and find the pellet reward in the arena. Once found and the rewards is consumed, it concludes the outbound segment of the invisible trial. Next the animal has to go inbound and rear in the original location of the beacon within a given time limit (60 s) (see Methods), otherwise the beacon will become visible and the visible trial restarts again. If the rat reaches the invisible beacon area and rears within 15 cm of the center of the beacon, another pellet is distributed into the arena and the beacon becomes visible again. This outcome also restarts the trial. The increase of the size of the beacon is to prevent frustration during the task. After 10 trials (even the reset ones) the location of the beacon changes to prevent the use of accumulated olfactory cues (Figure 12 B) (see Methods for training description).

Animals go through 3 training stages in order to master the behavioral paradigm, during which they are at 90-95 % of their body weight. (see Methods.)

2.2 BEACON PLACEMENT AND SIZE

All analysis was based on the distance from the beacon center, unless otherwise specified. Our presented beacons were pseudorandomly distributed such that the next beacon would be at least 30 cm away from the last one presented. This distance was chosen after modeling a variety of minimal distances and showing that the median distance from the center of the beacon to the next randomly assigned beacon away would be over 60 cm (Figure 13 A). This way, beacons would also be present in the center of the arena in either segment. If the minimum distance for next beacon would be higher than 30 cm, the simulation showed much larger median distances, presenting the beacons mainly at the ends of the arena rather than occupying the middle. Distances below 30 cm would present the beacon too close to its location in the last appearance. Similar beacon placement between trials creates a confounding factor by increasing the chance that the animals could use an allocentric strategy to navigate to the beacon.

The beacon center was designed to be at least 7.5 cm away from the wall border to avoid supported rear triggers. We focused all analysis on rears which were not supported by the wall.

For the third batch of animals we changed the area size of the invisible beacon segment to a radius of 15 cm (6 % of the arena) (Figure 13B). This increased the rat's success in the trial, leading to more trials and fewer incomplete and unlearned trials.

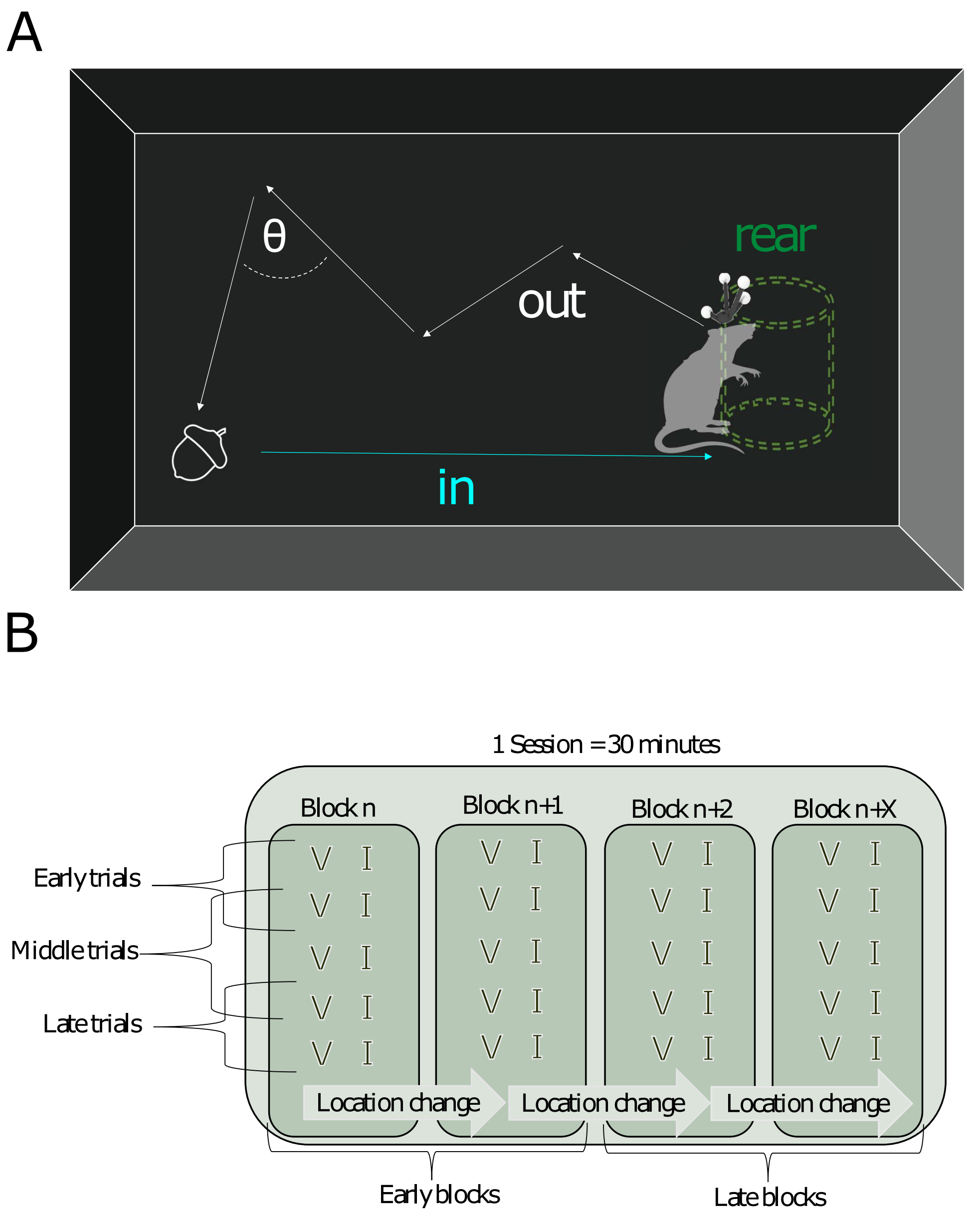

Figure 12: *A) Task schematic highlighting the outbound and inbound portion of the path integration task. Animal is tasked to rear inside of a visible beacon, which upon rearing turns invisible and a pellet is released simultaneously; the animal is then tasked to find the pellet and after consumption go straight back to the invisible location of the beacon. In a visible (beacon visible) and invisible (complete darkness) condition. B) Schematic for blocks of trials in a standard session where after 10 trials the location of the beacon changes. One trial consists of a visible (V) and an invisible (I) part of the trial, each trial can be thought of as having an outbound and an inbound segment. Inbound always concluding when the animal triggers the beacon. On average, the trained animals achieve 50 -100 trials during a 30 minute session. For later analysis we split the trials to early, middle and late trials within a block to prove independence from allocentric cues. We also controlled for early and late blocks for fatigue during performance (For more see Methods).*

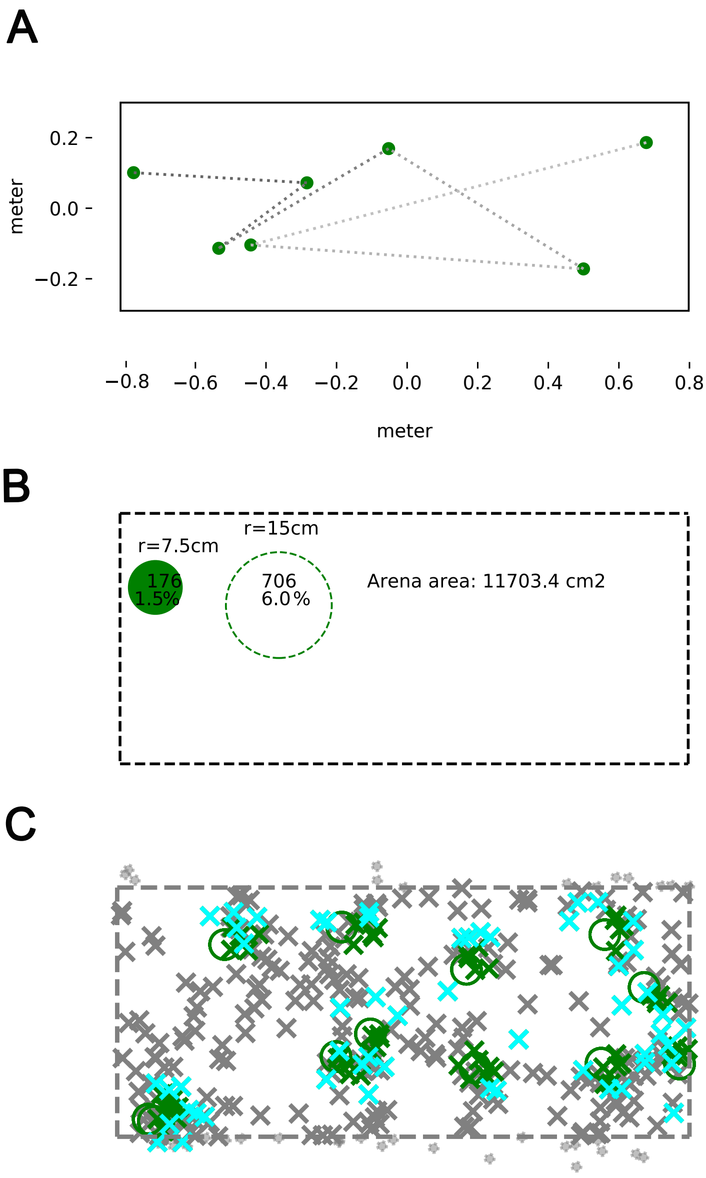

Figure 13: *Beacon and rearing assignment. A) Simulation of 7 pseudo-randomly assigned beacons within our arena space, each beacon is positioned at least 30 cm from the last one and a radius away from the arena border to avoid a similar position in the next set of trials and unreachability. Hue of gray dotted line denotes succession. B) Radius sizes of beacon trigger area within the arena and their respective percentage of the arena floor. For visible beacon, the 1.5% of the arena floor or radius of 7.5 centimeters was used. For invisible condition the radius of the trigger area was set to 15 cm or 6.0% of the arena floor. C) Example session, where* ***green circles*** *denote beacon location both visible and invisible,* ***gray crosses*** *are detected rears within the floor of the arena.* ***Green crosses*** *denote a successful trigger near a visible beacon location* ***cyan crosses*** *for invisible triggered beacon location.* ***Dashed gray lines*** *are the floor of the arena where 70 degree slanted walls start.* ***Gray stars*** *outside of the dashed line denote rears outside of the arena floor, likely wall supported and not counted in our rearing analysis.*

2.3 REARING

After running the behavioral experiment on our initial batch of animals following the observation of the animals interacting with the task the addition of closed loop rearing detection in beacon was added to create a clear behavioral readout of the position. Animals with reinforced rearing increased the amount of rearing in the task over time of training as well as time spent in the rearing zone as compared to non reinforced ones (Figure 14).

2.3.1 *Rearing Detection*

For closed loop rearing detection, we focused on .62 meters above the floor of the experimental room as the threshold. This was based on the ethology of the movements of the animal in the arena. We plotted a histogram of animal heights and observed a distribution where the local minimum between peaks occurs at the transition from high walk to rear (Figure 15 B and 26 A).We have set the threshold there. During our paradigms we have detected over 100 000 rears during over 50 000 trials across 11 subjects.

2.4 PERTURBATIONS

We used vestibular and visual perturbations in order to disrupt the egocentric part of the path integrator. The perturbation was always performed on the outbound segment of the task for 7-10 seconds in the invisible trial. This way the animals had to rely on inaccurate and conflicting sensory accumulating information while they were integrating the parts of the outbound path.

2.4.1 *Vestibular Perturbation*

Translation of the arena (Figure 16A) was done in a sinusoidal manner to stimulate the vestibular system, which is sensitive to acceleration rather than a constant speed (see Methods). The arena moved one way and then back. Movement was initiated after the trigger of the visible beacon, starting the invisible trial.

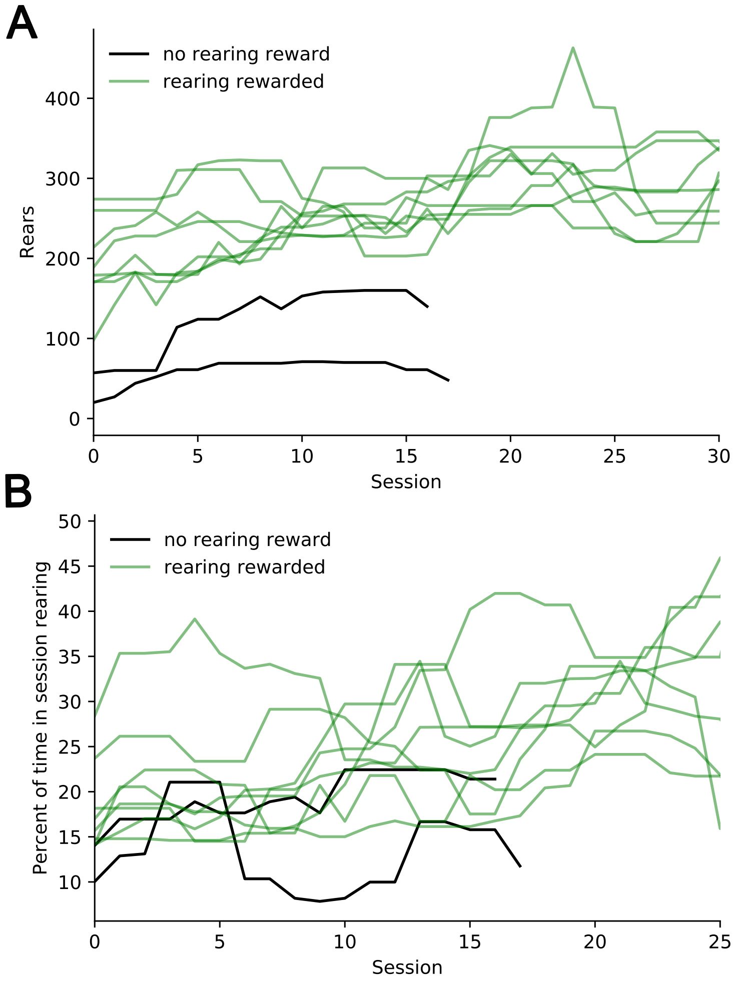

Figure 14: *Behavioral difference between rewarded and unrewarded rearing during a task. A) Rear periods during the first 30 sessions during reinforced rearing in the beacon trigger area (green) and not reinforced during rearing inside of the beacon trigger area (black). B) Same as A), only for percent of time in session spent rearing.*

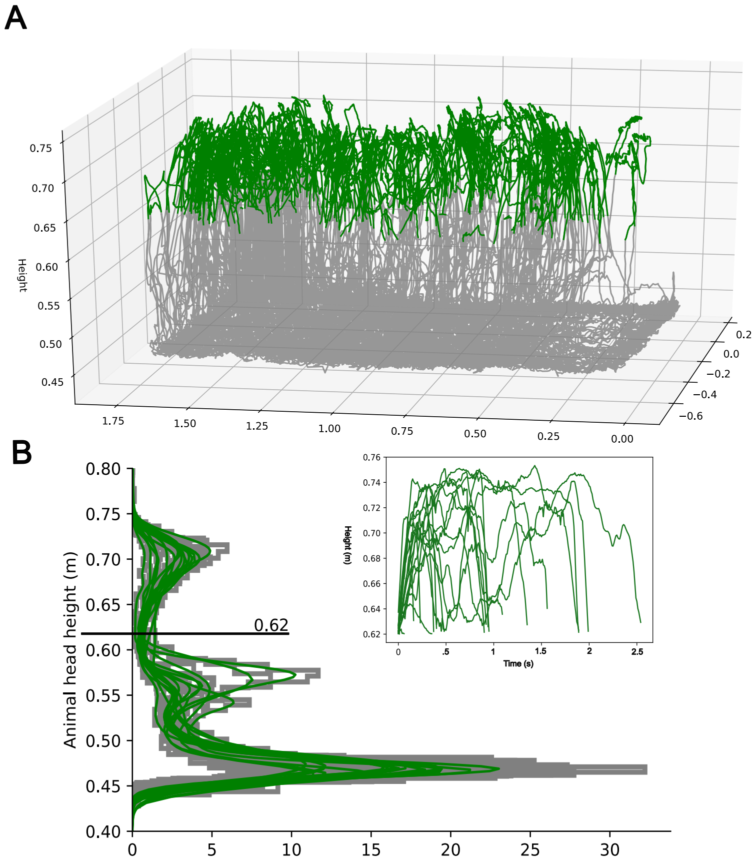

Figure 15: *Rearing detection cutoff based on ethology of the animal in the arena. A) Example session in 3D. In green are any rears highlighted above .62m of the experimental room (one session). B) Height histogram over 17 training sessions from one animal. A cut off at .62m above the floor arena was chosen for online closed loop rear detection corresponding to the minimum on the height histogram. B) (inset) Rears above 62 cm of the room floor from example session and their trajectory in height and time.*

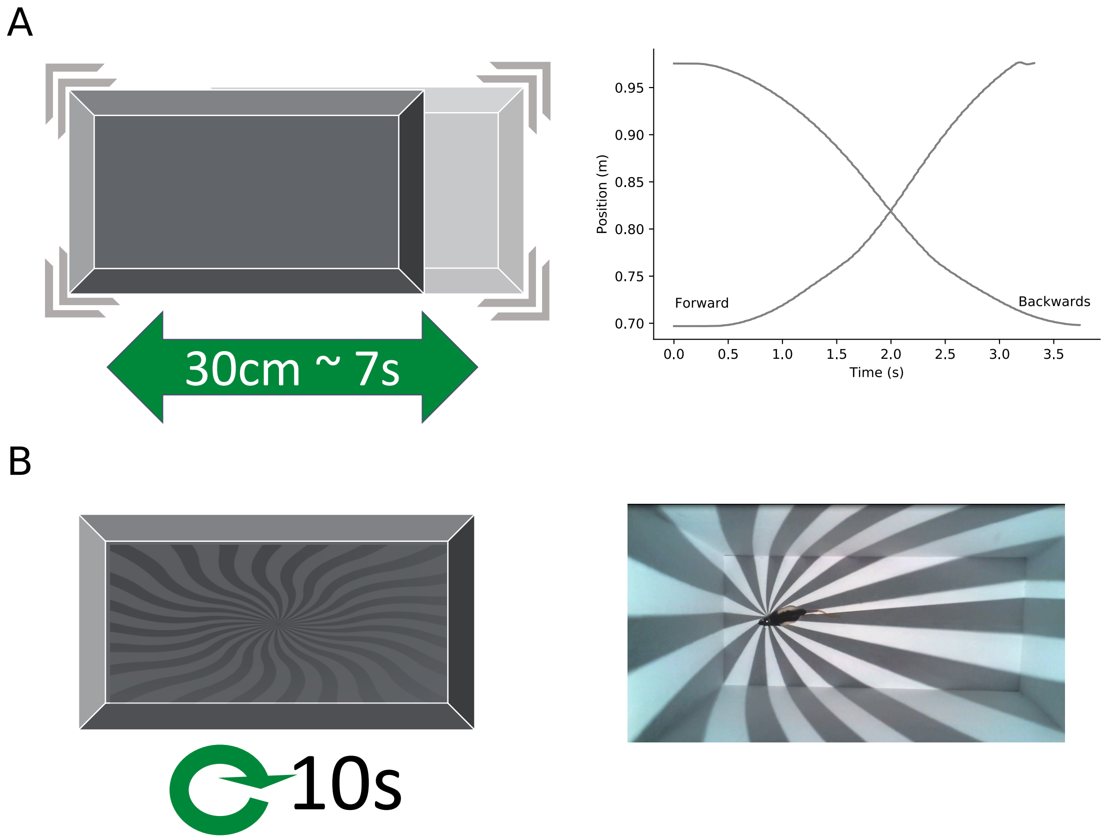

Figure 16: *Perturbation schematics. A) Vestibular perturbation where the arena gets displaced by 30 cm forward and backwards within 7 seconds. On the right is the actual movement of the arena displaced by actuators below the arena as measured by our tracking software. B) Slowly moving visual perturbation consisting of vertical bars aligned with the middle of the rat's eyes at all times as a virtual sphere moving with the rat for 10 seconds. On the right is an example from an overhead video of one of the sessions (for more see Methods).*

2.4.2 *Visual Perturbation*

Visual perturbation (Figure 16 B) was done using a slowly moving vertical bar paradigm where a controlled and varied speed within an effective range was introduced to the animal. The rotation direction was switched randomly as well. The virtual design was a sphere with black and white bars surrounding the rat and anchored in the center of the rat's eye. The stimulation looked similar in all parts of the arena. We did not control for the movement of the rat's head nor try to counter rotate when the animal was moving its head. The intention of the stimulation was to disrupt the visual information the animal was using to calibrate its egocentric reference frame. It is therefore intentional to have no allocentric anchor during the perturbation.

2.5 CONTROL TASKS

During control tasks, animals were subjected to the same perturbation paradigms, but perturbation was uncoupled from animal behavior and was triggered every minute. A pellet release and rewarding stimuli sound also accompanied these control triggers.

2.6 MOVEMENT CONTROL

In order to control for the animal's movement in darkness, we subjected the animals to a completely dark arena without any visual cues or any perturbations, but triggering a pellet reward and sound every minute.

2.7 FULL BODY TRACKING

In order to study the freely moving dynamics of the rats which were trained on our task we also used them for a full ethological analysis using the OptiTrack system, where we could keep track of the spine as well as some of the tail movements by having markers on different spots of the rat's body. Then we could measure the full rearing dynamics and understand how the rearing evolved over the time. We were specifically interested in what the rearing looks like in a full posture given only limited information during the task when the animal had a limited set of markers. This was done mainly in a separate circular arena, but also inside of the VR arena to confirm the findings.

3

MATERIALS AND METHODS

3.1 EXPERIMENTAL PROCEDURE

3.1.1 *Subjects*

Eleven male Long-Evans rats (Charles river) aged 3-18 months were used in our experiment and testing. The use of rats was chosen for their higher visual acuity as compared to mice (1 cycle/degree) and larger body weight to carry different size cranial implants (Prusky, West, and Douglas 2000; Crijns and Op de Beeck 2019). We separated the cohort into three batches, spaced apart by a few months to allow for analysis and improvements of the task or electrophysiological recordings. The first batch of two rats was used for behavioral paradigm testing as well as simple wired electrophysiology. One rat from the second batch was used for testing of the wireless electrophysiology setup. The other four trained on an improved paradigm including rearing reinforcement and wireless electrophysiology. Finally, the last batch of four rats was trained on the same paradigm with even more controlled perturbations.

3.1.2 *Animal housing*

After the initial surgery animals were housed separately with a high ceiling cage top to promote rearing and a maintenance of 12 hour light/dark cycle, switching to dark at 14:00, in order to increase activity of the animals during the afternoon tasks. The choice of this schedule was based on a 12 day activity analysis of the animal behavior using infrared detection of activity (Figure 17) (Mouse E motion , INFRA-E-MOTION GmbH, www.infra-e-motion.de, Germany). Animals had ad libitum access to food and water until after recovery from the initial surgery.

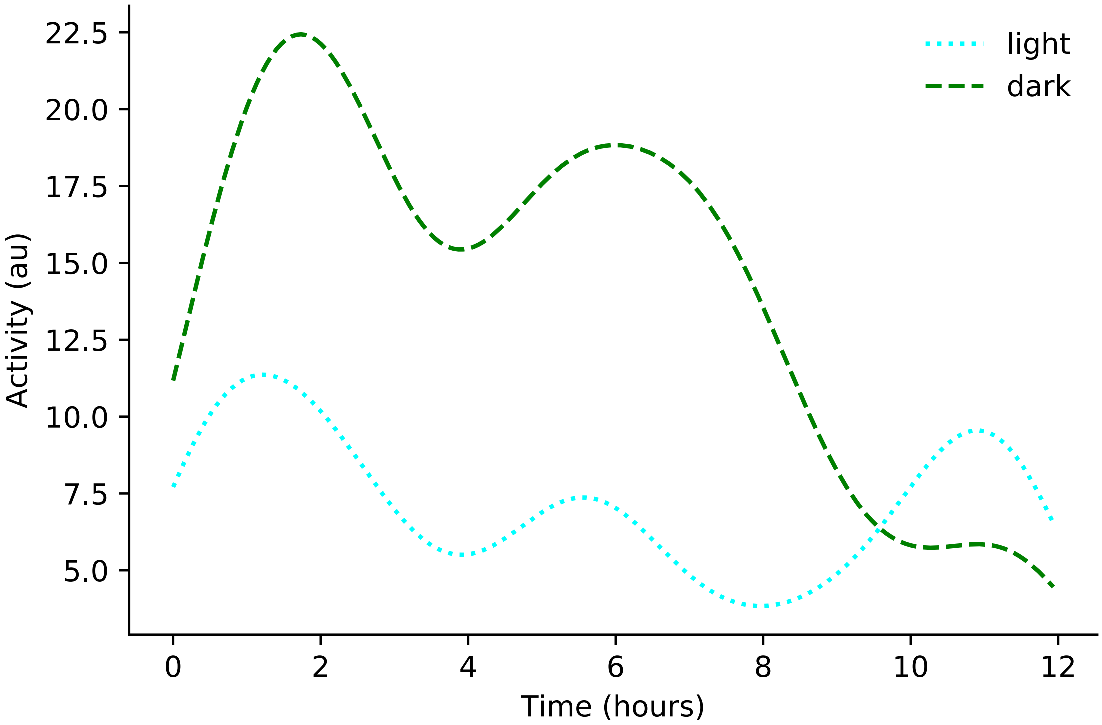

Figure 17: *Analysis of rat activity in the home cage averaged across 12 days. Different lines separate the light (cyan) or dark (green) cycle showing higher activity during the night cycle and peak of activity at 2 hours after switch of the cycle, hence around 16:00 as the time of the day when switch is at 14:00. This time of the day is also when most experiments were recorded.*

During the recordings, animals were kept at 90-95 % of their original ad libitum weight to increase their movement, which was stimulated by foraging for food pellets. All experiments were approved according to national and European guidelines on animal welfare (Reg. von Oberbayern, license number AZ 55.2-1-54-2532-70-2016).

3.2 WIRELESS ELECTROPHYSIOLOGY ACQUISITION

In order to combine smooth virtual reality experience with electrophysiology recordings we used a wireless electrophysiological setup (White matter). Due to the advantage of wireless recordings, animals could be smoothly transitioned to their home cages after the task ended and were recorded during rest, eat and sleep periods in their home cages before starting another task. Wireless recording also allows autonomous closed loop recordings without the presence of an experimenter since cable tangling of a freely moving animal is not a problem anymore. With cable electrophysiology recordings in freely moving VR there is a distracting shadow due to the light source of the projector being in the center of the arena, coinciding with cables. This shadow is not present during wireless recordings. At the same time, wireless recording did not discount channel count or speed. We were still able to implant bilaterally in an animal and record from all 128 channels wirelessly at 25 kHz with battery and memory lasting for over 2 hours of recording before a switch to a new battery was needed (achievable in as little as 1 minute). As previously noted, the cage recordings allowed us to record in the home cage of the animal during rest, before and following the task. This captured precious replay brain dynamics important in remembering the task.

3.2.1 *Implant design*

A standard practice for surgeries in in vivo electrophysiology is to design a rigid copper mesh cemented on top of the craniotomy to protect the probe from being tampered with by the animal. Building such implants is time consuming during surgery and increases the risk of an unsuccessful operation. On top, if an implant is poorly manufactured during a stressful surgery, it doesn't allow for access to the drive to lower silicone probes in order to improve unit yield. The advances and accessibility in 3D printing allowed this to change.

A custom made 3D printed cranial implant was designed in order to record the electrophysiology signal from the animal. We wanted to achieve several characteristics with custom design, namely:

- Long-term stability of the implant on the head of the animal.
- Lightweight design weighing less than 5% of the animal weight
- Replaceable top covers for use during training, in the home cage and with a full electrophysiological setup.
- Removable top to access the probe during recordings for adjustments and cleaning.
- Fully built in-house to prevent missing key pieces and provide per animal customizability
- Shielding from IR radiation from the tracking cameras or other noise sources.

I designed a magnetic cover top intended for wired or wireless electrophysiology with the possibility to remove the wireless logger and to be able to move the microdrive lower after a recording day to improve the cell yield (Figure 18). This ensured a stable flow of the experiment without having to anesthetize the animal to move the microdrive or exchange for a charged logger. After experiments the logger is removed in order to download data and to charge the battery. A removable protective cover is put in place instead and secured with screws over the amplifiers to prevent dust accumulation on the sensitive electronic parts (Figure 18 B3). Such a cranial implant is also built to last in a fully enclosed home cage, withstanding shocks from the cage side, especially when the animal is grooming or during general locomotion.

3.2.2 *Implant roof*

In order to protect the electrophysiological recordings from 120hz noise from the flickering of the infrared light camera, we developed a 3D printed plastic cover (Figure 18 B1) painted over with musou black paint (The Black Market). The cover also protected the implant from dust and hair which sometimes accumulated in the implant.

All pieces of the adaptable base ring were 3D printed in-house. The actual base ring which attaches to the skull and pieces of the microdrive were printed using an Asiga Pico2 3D printer (Asiga). The rest of the larger protective pieces were printed using Ultimaker Pro (Ultimaker) and also painted with musou black paint. (The Black Market). The assembly was done in-house as it requires precise positioning of the magnets, nuts and screws glued to the implant using dental cement or superglue.

3.2.3 *Neural probes*

We used 64-channel silicon probes with 6 shanks (Cambridge Neurotech) in order to increase density of recording sites in the CA1 layer of the hippocampus when compared to tetrode microdrives. In order to flexibly adapt the depth of the recording sites, we designed a movable probe drive which is attached to a cranial implant that is chronically implanted on top of the head of the animal. Now I will describe the different aspects of this cranial implant starting with the microdrive.

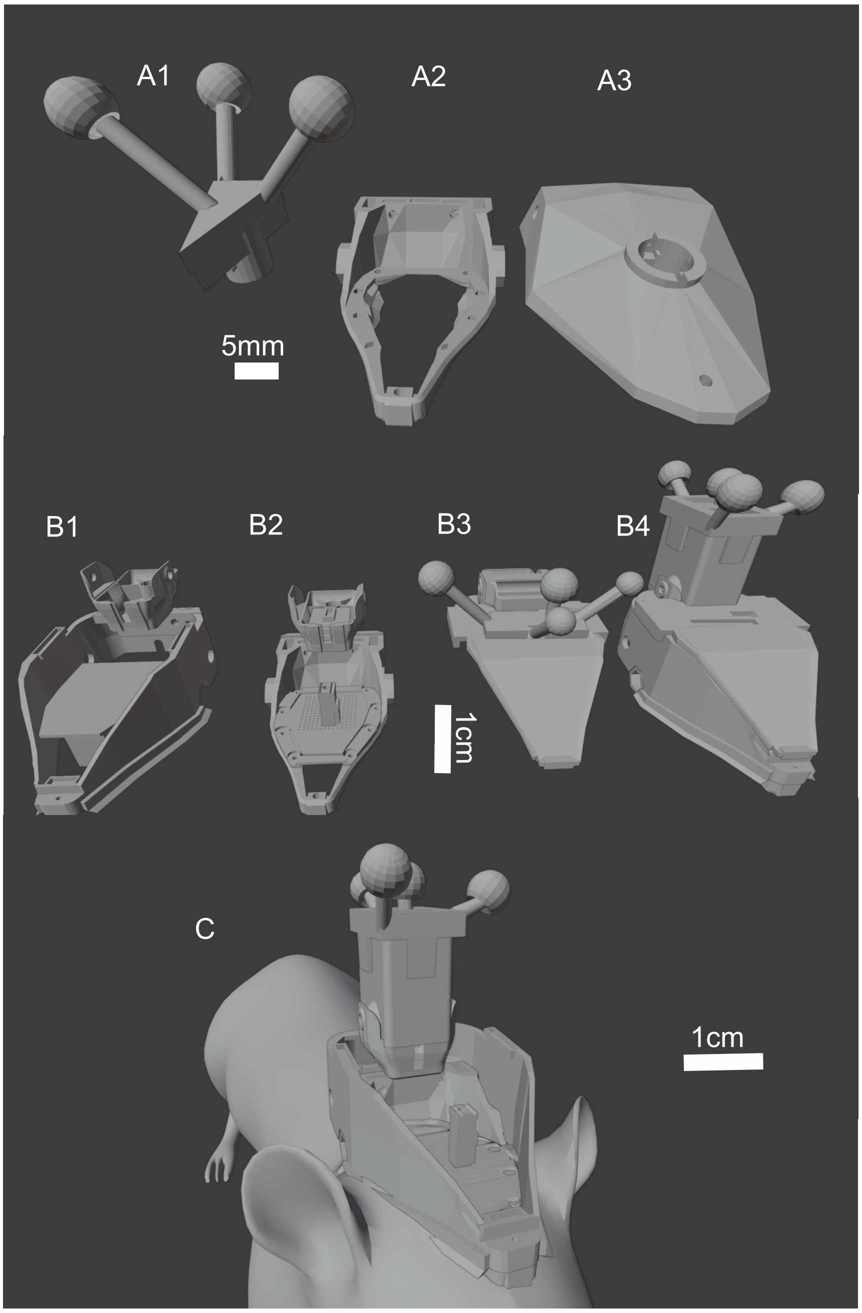

Figure 18: *Cranial implants used for behavioral tracking. A) Setup for behavioral recordings. A1) Behavior tracker used during most training session. A2) Cranial implant used during initial surgery. A3) Implant cover with lower profile for behavioral tracking including a magnet to secure a removable tracker in the same position each time. B) Setup for combined electrophysiology and behavioral recordings. B1) High walled implant cover with an electrode "roof" to prevent light induced artifacts and dust accumulation. B2) Cranial implant with an implanted drive and holder for wireless acquisition system. B3) Cranial cover top with a removable magnetic tracker and cover of the microcircuit boards sitting on the bottom of the holder for recording wireless electrophysiology. B4) Full setup for recording wireless electrophysiology with a tracker on top of the wireless logger. C) Exposed electrophysiology setup showing details on top of a 3D model of a rat.*

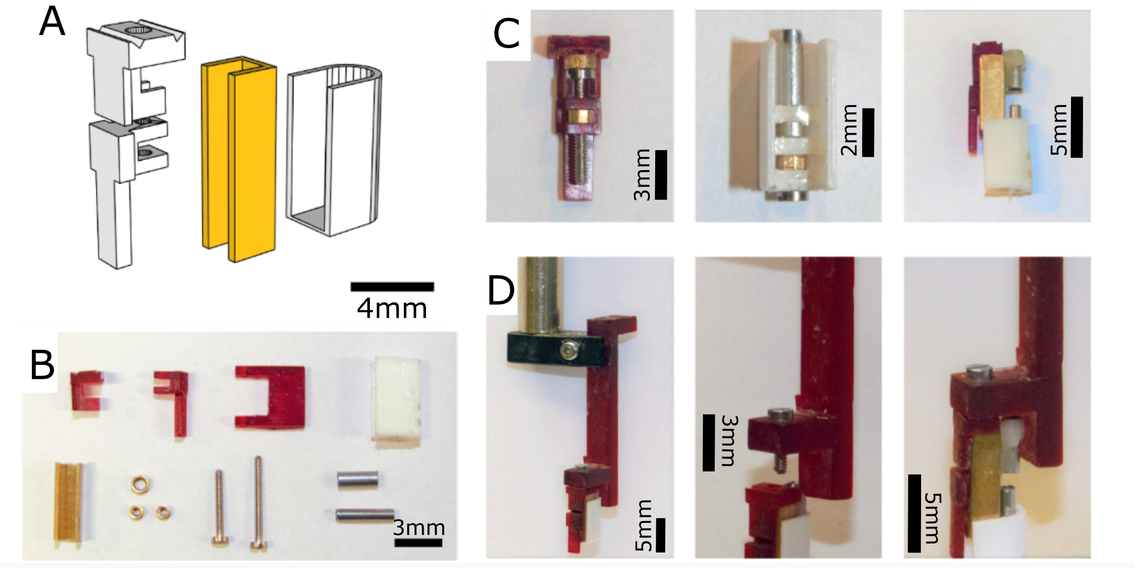

Figure 19: *Custom made microdrive A) 3D model of the microdrive. B) all pieces requiring assembly of the microdrive. C) stage of assembly of the microdrive D) picture during surgery for implantation and deimplantation. Figure adapted from Schwesig et al., in prep.*

3.2.4 *Microdrive design*

A custom-made probe drive and holder parts were 3D printed and manufactured in order to ensure a small scale and low cost movable implant which was also recoverable and re-implantable (Figure 19). During behavioral recordings a probe was also reinserted into a contralateral side of the hippocampus. This implant sits on top of a removable cranial floor which is attached to a base ring that is fixed to the skull of the animal. During removal for reuse a small nut sits in a white sleeve allowing the removal of the shuttle which is encapsulated in a brass railing (Figure 19 C). This allowed me to reimplant from one side to another using the same silicone probe in 2 animals after decrease in unit yield from one hippocampus side, while the behavior stayed consistent.

3.2.5 *Base ring manufacturing*

The assembly of the base ring required printing the base ring in-house and later cleaning and drilling holes for nuts to be cemented in order to attach other parts of the cranial implant, such as protective covers or a base plate, which covers the craniotomy and allows a precise positioning of the silicone probe. The assembly

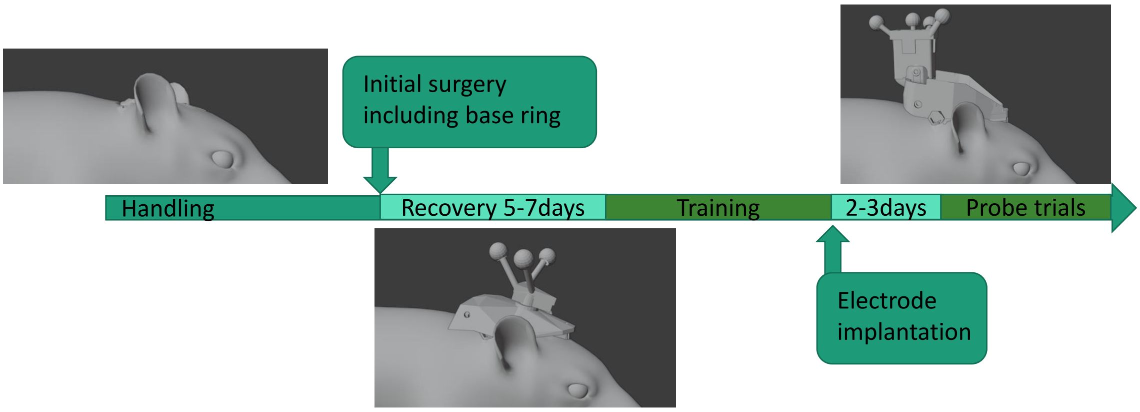

Figure 20: *Timeline of surgeries and training. Surgery was split to 2 parts with initial base ring implantation which required longer recovery time. This was then followed by training and the electrode implantation which did not require long recovery time due to shorter and less invasive surgery.*

is highly specific and requires testing and sub-mm precision adjustments in order ensure a proper fit on the skull and long lasting implant.

3.2.6 *Surgical procedure*

Surgery on the animals was done after habituation to the arena but before any behavioral training (Figure 20). The initial cranial implant was an invasive surgery and required a long recovery time of up to one week before the start of training. The initial surgery exposed but did not disturb the skull yet. Subsequent addition of a craniotomy and switch from a low to a high profile cover were fast and ensured a shorter recovery time after the surgery. This helped to keep a stable level of behavior training and allowed for recording good quality units from the hippocampus due to the recency of implantation.

Standard stereotaxic surgery was performed. Before the surgery all cranial implant pieces were tested and cleaned with ethanol (90 %). A three component solution consisting of Medetomidine Midazolam and Fentanyl (0.15 mg/kg, 2 mg/kg, 0.005 mg/kg) was injected IM (intramuscularly) to anesthetize animals and keep the anesthesia for the duration of the surgery. After 1-1.5 hours, the anesthesia source was switched to Isofluoran (.5-1.5 %) via inhalation followed by an injection of Metamizol Subcutaneously (SC). During the procedure animals were head-fixed in a stereotaxic frame (Stoeling Co.) and placed on a large heating pad with a thermometer to maintain their body temperature at 36 °C. During the initial surgery the base ring, which included the protective cover and base plate, was cemented to the skull together with drilled 8-10 M1 1 mm screws, anchored to the frontal, left parietal and occipital bones (Dental cement, Paladur). Two screws were inserted into the occipital bone and served as electrical ground. Skin was sutured around the implant and wound to prevent scratching and infection. At the end of the surgery a three component antagonist consisiting of Naloxon, Flumazenil and Atipamezol (0.12 mg/kg, .02 mg/kg, 0.75 mg/kg) was injected (SC) in order to stop the lingering effects of the initial three component agonist mixture and to stimulate normal behavior in the animal after the surgery, such as eating and drinking. The post-surgical treatment included injection of Metamizol (110 mg/kg) (SC) and at least five days of daily injections of antibiotics (Baytril, 10 mg/kg) (SC) and at least three days of analgesics (Meloxicam, 1 mg/kg) (SC) starting from the day after surgery. The recordings started only after complete animal recovery.

3.2.6.1 *Craniotomy*

During the second stage of surgery, the same anesthesia protocol was performed. The probe was dipped in DiI, a red fluorescent lipophilic dye (DiCarlo et al. 1996) the night before implantation. The implant cover was removed along with the base plate and cleaned from debris. After cleaning, all animals were initially implanted in the right hippocampus. One was a bilateral implantation into both hemispheres and another two were reimplantations during which a probe was moved from the right to the left hemisphere. The neural probe attached to the microdrive was oriented 15° to the vertical plane. It was inserted into a 2 mm wide squared craniotomy window, center of which was at (AP -4.6 mm ML 2.4 mm) the probe was then centered at the same coordinates (AP -4.6 mm ML 2.4 mm) and lowered about 2 mm into the surface of the brain avoiding veins in the brain. If a vessel was in the way of the probe the probe was angled slightly in the horizontal plane or moved to avoid hitting a vessel and inserted at slow speed. Bone wax was then melted over the craniotomy to prevent leakage of cerebral spinal fluid, while providing a flexible membrane and reduced pulsation of the brain. The microdrive was cemented onto a base plate which was screwed to the base ring. After the implantation a higher base ring cover was attached to the base ring and an electrophysiological signal was tested before the injection of 3 component antagonists. The post surgical procedures were the same as during the first surgery, only the recovery period could be shortened due to less bleeding as the surgery was overall less invasive. There was a very high integrity of the cranial implants. Our implants lasted on average 180 days ± 53 days (SD) with a minimum of 86 and maximum of 246 days. A typical length of my experiment was 6 months.

3.3 RECORDING PROCEDURES

After the animal's complete recovery from the second surgery, electrodes were adjusted to lower the probe tips with recording channels to the pyramidal layer of hippocampal CA1.The lowering of the electrodes was done in small steps (31.25 to 62.5 μm) not exceeding 125 μm per day to avoid damaging neural tissue and missing the correct hippocampal layer. After the adjustment the live activity of the probe was recorded for an hour in order to estimate the position and amount of cells present at that layer. The correct placement of the electrodes was defined looking at the electrophysiological signal. Specificaly the presence of sharp waves (György Buzsáki 1986) and ripples (John O'Keefe and Dostrovsky 1971) in the Local Field Potential (LFP) signal during immobility periods, as well as the presence of bursts of spikes corresponding to the putative pyramidal cell activity in this region. When the correct layer was reached, adjusting of the electrodes stopped until the signal worsened sometimes over a period of days.

3.3.1 *Froot loop switch*

During the switch of different loggers due to low battery level or removal of the logger at the end of the recording day a specific procedure was followed. A Froot Loop (Kellogg's ®) was offered to the animal to distract it from unscrewing the logger from the implant cover or during lowering of the drive with microelectrodes into the brain. This procedure took about one minute.

3.4 HISTOLOGY

In order to confirm the location of the electrodes, besides the analysis of the electrophysiological signal a histological analysis on the animal's brain tissue was performed. Animals were deeply anesthetized with pentobarbital and then perfused with 4 % paraformaldehyde. Following perfusion, brains were extracted and stored in paraformaldehyde for at least one day. Brains were sliced sagittally in 40-80 μm thick slices. Slices were then mounted and stained with 4,6-diamidino-2-phenylindole (DAPI) vectashield (Vectorlabs). Histological verification of recording sites was performed using standard light microscopy with 10x magnification. Imaging of red was done on a confocal microscope. Images were stitched together using Microsoft Image Composition Editor (ICE) (Microsoft) and overlaid using Fiji is just image J (FIJI) (Figure 21).

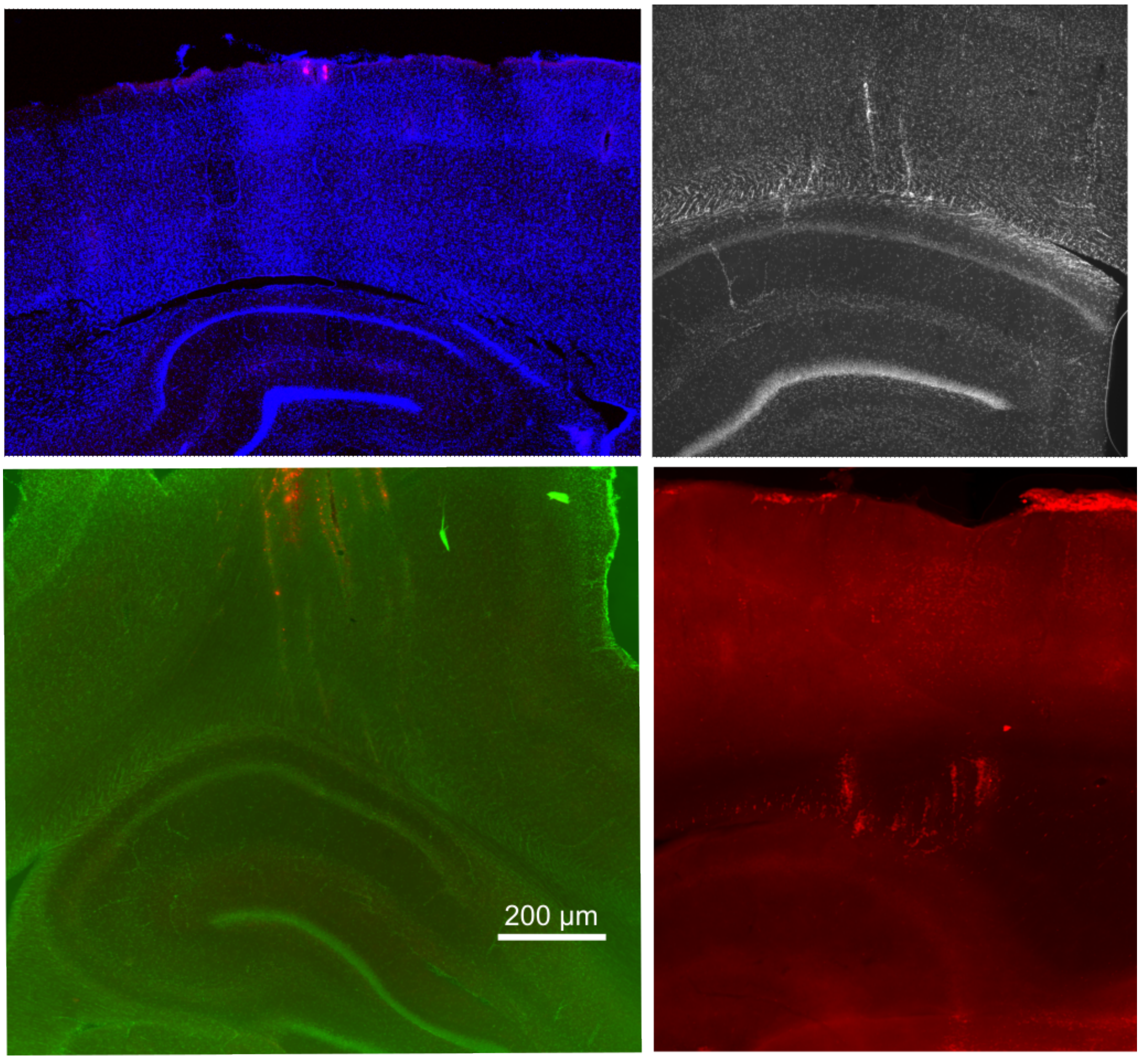

Figure 21: *Histology slices from different animals highlighted are traces of the dyed probe showing 6 shanks targeting the CA1 pyramidal layer.*

3.5 ACQUISITION SYSTEM

Acquisition of behavioral data was done via a custom written software based on the ratCAVE package (Del Grosso and Sirota 2019) and the Motive tracking software (OptiTrack, NaturalPoint Inc., United States). Acquisition of electrophysiological data was done via a White Matter wireless acquisition software (White Matter) at a 25 kHz sampling rate with a 10m radio link for remote control and online monitoring. The 64-chanel silicone probes (Cambridge Neurotech) were attached to a White Matter headstage connected to the HS-W wireless headstage logger with a battery. At the end of each recording or before the battery ran out of power, which occurred at about 4 hours with 64 ch recordings, the logger would be replaced for a charged one to continue recording.

3.5.1 *Standard recording timeline*

During a standard recording day about 5 hours of electrophysiological recording were amassed. In the morning, after checking the electrophysiological traces and possibly moving the probe lower into the brain, sleep would be recorded. When the animal was not resting during many of these 5 hours we used a freely moving virtual reality setup. I will now describe in detail our virtual reality setup.

3.6 USE OF VIRTUAL REALITY IN NEUROSCIENCE

The uses of virtual reality systems have been described in Thurley and Ayaz (Thurley and Ayaz 2017). In this case we are using a freely moving VR system, not a floating ball, which assures the vestibular system inputs are aligned with the given visual cues as they would be in a natural environment. Another advantage is that the freely moving VR does not require timely habituation of the animal to the system. The advantages are of course that the object are represented in 3D which plays an important role in objects representation, important for creating correct object vector cells (Høydal et al. 2019).

3.7 PATH INTEGRATION PARADIGMS

In comparison to other path integration paradigms as discussed in the introduction we wanted to have a flexible task. The two segments of the task can be

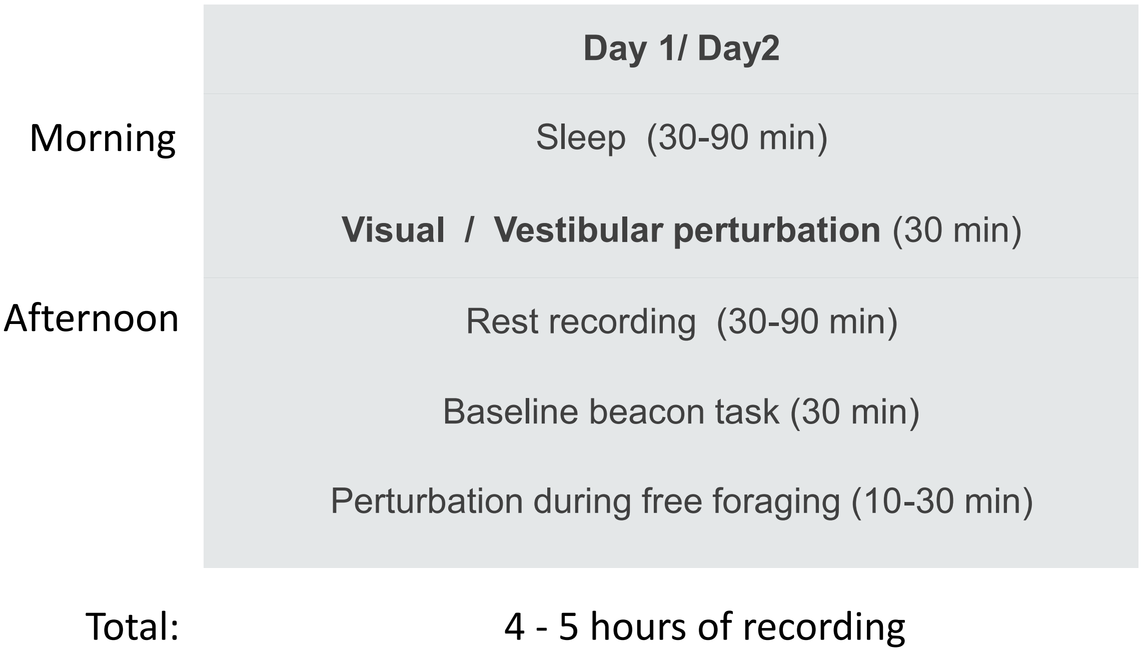

Figure 22: *Schematic schedule of a standard recording day. The circadian rhythm of the animals would switch from light to night cycle (more active) around 14:00. Baseline and vestibular tasks were pseudorandomly mixed in between the morning and afternoon.*

compared to a short term memory task. In many such paradigms an animal has to remember its past movement similar to alternating T-maze task, where it has to take a left and then a right turn at the decision point. In our case this is expanded to having remembered the path which the animal took and to being able to retrieve it to find a straight way or path back to where the last trial ended.

3.8 VIRTUAL REALITY SETUP

All experiments except for full body tracking ethological experiments were designed to be conducted in a 3D virtual reality setup, ratCAVE (Del Grosso, Graboski, et al. 2017). The arena setup is a combination of a large rectangular arena (floor area 162 cm × 72 cm, about one meter squared, and walls of 60 cm height, placed at a 70 degree angle to accommodate the visual projection). A set of 7 infra-red tracking cameras (Prime 13W 240 fps, OptiTrack, NaturalPoint Inc., United States) and 4 infra-red tracking cameras (Prime 17 240 fps, OptiTrack, NaturalPoint Inc., United States) are located above the arena. A high-frequency projector (PROPixx 1440hz projector VPixx Technologies Inc., Canada) was used to project a 3D virtual environment on the bottom as well as the slanted walls of the arena.

For each experimental session a 3D-printed set of 3-4 spherical reflective markers was magnetically attached to the cranial implant of the animal. In principle the tracking software scanned the position of infrared reflective markers in 3D space with multiple cameras. The position of the animal was used to render the projection around the animal such that the animal is in first user perspective inside of the virtual reality - the images are updated from the animal's perspective. Blender software was used to design the virtual environment and export it to .obj files, used by the custom written 3D graphics python software (Del Grosso and Sirota 2019) for rendering.

3.8.1 *Arena movement*

Two linear actuators and a bearing system on rails were installed below the arena to physically move the arena along one coordinate axis. The maximum move was 30cm, limited by the borders of the projection on the floor of the arena. The speed profile was sinusoidal to achieve a more stable vestibular perturbation (Figure 16 A).This type of stimulation resulted in a more even stimulation of the vestibular system then a sudden onset and offset. During the time of perturbation the animal was freely moving around the arena, resulting in an active and passive stimulation of the vestibular system stimulating the otoliths and semicircular canals (Carriot, Brooks, and Cullen 2013). The actuators were controlled by a microcontroler (Arduino UNO) with a motor shield, connected via USB serial port to the computer with the experiment control software and were triggered via a closed loop trigger upon the animal rearing in a visible beacon for the outbound part of the invisible segment.

3.8.2 *Trial schedule and reward system*

Our reward system consisted of a pellet dispenser (Campden Instruments Ltd.) and 2 speakers (Logitech), which were both positioned above the arena. The pellet dispenser was triggered by a closed loop with a microcontroller (Arduino UNO) based on a command from adapted ratCAVE software whenever the animal met the conditions to trigger the reward. Following the trigger, the speaker played a pre-recorded sound of pellets shaking which was used during training and habituation to stimulate faster training of the animals. The conditions to trigger were met if the animal stayed in a beacon zone for a certain amount of time or reared; the exact condition depended on the task type. During the control trials the reward was triggered every minute. The pellet would randomly scatter on

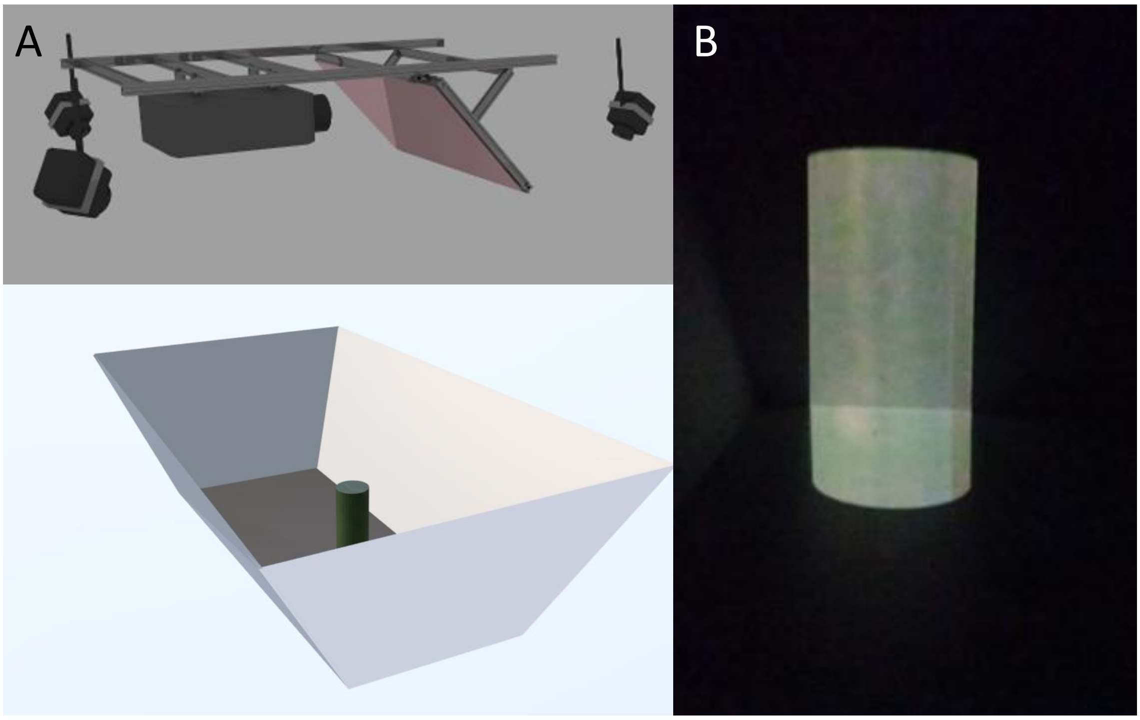

Figure 23: The ratCAVE freely moving virtual system for rodents used in the experiment. A) 3D model of the arena (adapted from Del Grosso, Graboski, et al. 2017, licensed under https://creativecommons.org/licenses/by/4.0/, permission not required. B) View from the animal's perspective as it is approaching the green beacon during the task.

the floor of the arena.The closed loop trial and reward system were implemented through custom written python code based on (Del Grosso and Sirota 2019).

3.8.3 *Synchronization*

Our synchronization was achieved by recording the time stamp in the Optitrack recording software (OptiTrack, NaturalPoint Inc., United States) and transforming it into our behavioral software (Del Grosso and Sirota 2019) to associate time stamps with the movement of the animal and include behaviorally relevant triggered data. A separate behavioral dataset was recorded by Optitrack itself including the timestamp of recording for each frame, but lacked the behaviorally relevant information. The optitrack recording timestamp could then be used to synchronize both behaviorally relevant task time stamps and normal position of the animal. In order to synchronize with a freely moving electrophysiology acquisition system the Optitrack software would send a Transistor-Transistor Logic (TTL) trigger pulse at the beginning of the recording to the wireless receiver which would then send the radio signal to the logger to start recording. This way the electrophysiology data was synchronized with movement data from Motive software which was subsequently synchronized with behaviorally relevant information provided by the ratCAVE software. A 120hz sync clock was also sent from the Motive recording software with TTL cable connection to wireless transceiver to assure no delay happened during the recording itself. During initial wireless acquisition a photodiode box was used instead of Bayonet Neill–Concelman (BNC) cable due a non functioning wireless receiver output. Photodiode was then connected to a BNC cable to Motive software to start behavioral recordings (Figure 24).

3.9 TRAINING

Animals went through 3 training stages (Figure 25) in order to master the behavioral paradigm. Following the recovery from the surgery of base ring implantation, they were put on mild food deprivation around 90-95% of body weight from before the surgery. They were trained about 5 times per week.

At first animals are habituated to a sound of rattling pellets which provides them with task food enrichments in their home cage. This sound was later played from a speaker above the arena. In the first stage, animals are introduced to the task by having to approach and stay in a green virtual beacon.

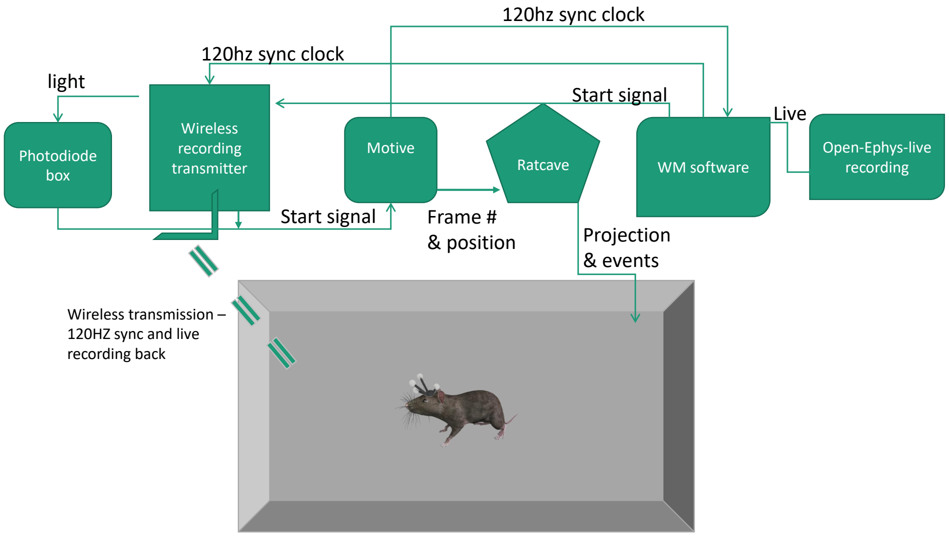

Figure 24: *Overview of synchronization steps necessarily for fully connected wireless electrophysiological recording with freely moving virtual reality. Optitrack software sends a TTL trigger pulse at the beginning of the recording to the wireless receiver which would then send the radio signal to the logger to start recording. This way the electrophysiology data was synchronized with movement data from Motive software which was subsequently synchronized with behaviorally relevant information provided by the ratCAVE software. A 120hz sync clock was also sent from the Motive recording software with TTL cable connection to wireless transceiver to assure no delay happened during the recording itself. During initial wireless acquisition a photodiode box was used instead of BNC cable due a non-functioning wireless receiver output. Photodiode was then connected to a BNC cable to Motive software to start behavioral recordings*

The sessions in the first stage required the animal to simply enter the zone where the beacon was presented to trigger pellet release and sound. For each trigger only one pellet was distributed. The later trials increased the time that was required for a successful trigger. Animals could trigger the beacon if they reared in the zone at any point (starting from the second animal batch). The design with the increase of the time animals needed to stay in the arena was individualized for each rat based on their performance on previous sessions. Once they learned to associate the beacon with a pellet and they successfully triggered it on 3 consecutive days at least 24 times while managing to walk more than 250 meters in 30 minutes, then they entered the second stage. It took anywhere between 4-7 training sessions for an animal to reach the second stage.

In the second stage the beacon moves to different locations, but still stays visible at all times. The location was at first moved every 20 beacon triggers but this would decrease to eventually settle at every 10, again based on individual rat performance. During the first set of trials, the beacon is always present in the same location as in the first part of the stage. Animals also increased the proportion of the triggered beacons by rearing more during that stage.

In the third stage, after the beacon changes location every 10 appearances it starts initially to be invisible every 10th presentation (before the switch to a new location) and then this decreases by even numbers all the way to being invisible every other presentation: achieving the full task at about 3 weeks of training. The animal has to perform and show the remembrance of the previous beacon location by rearing in the location on its hind legs and reaching a sufficient height to trigger the reward, or staying within the boundary of the beacon for over 2 seconds. After the initial training period, 90% of the triggers are triggered by the animal rearing in the arena. Animals have to exit the beacon location in order to "load" another pellet. The size of the sensitive beacon trigger area increases to twice the radius for the invisible trials and if the animal cannot achieve the beacon within 60 seconds, the beacon is shown switching to a visible condition. The training and perturbation sessions are all the same length as regular recording sessions which are 30 minutes long.

Animals were observed during the task and notes were kept about the potential increase or decrease of the difficulty of the task. In general when the new difficulty of the task was introduced, there was a slight drop off in the simple measure of performance by looking at pellets triggered in 30 minutes (Figure 25). The training had to be individualized to each rat in order to prevent frustration in the task and to promote locomotion around the arena.

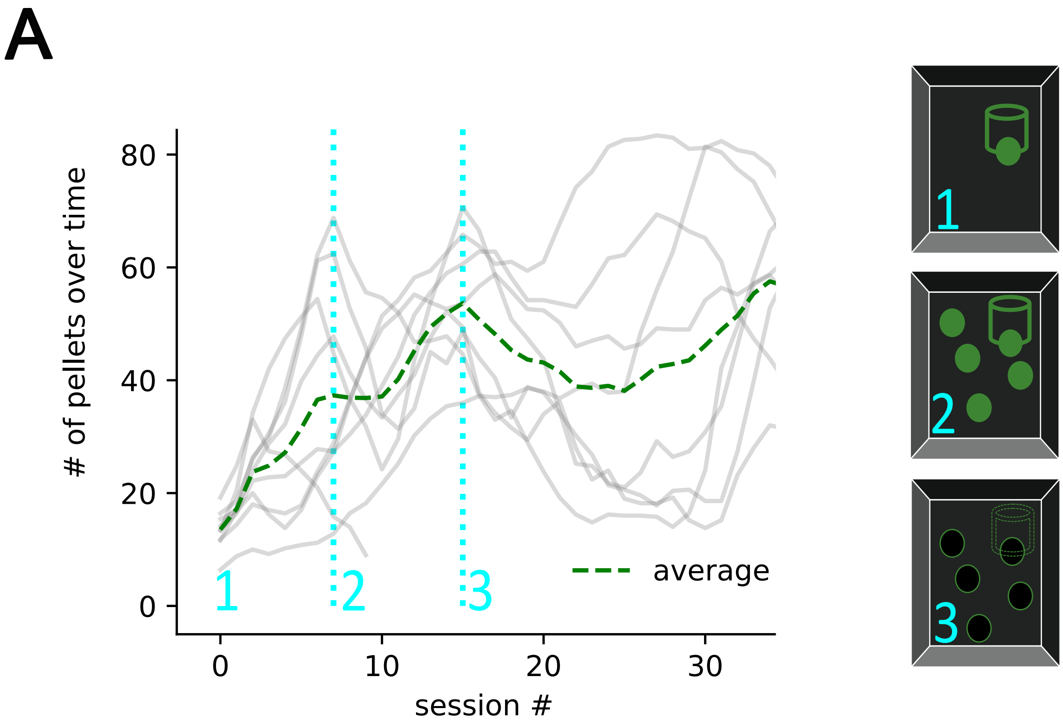

Figure 25: Training stages reflect in performance measures. A) same as 19A. Notice drop of performance after the next stage of the training was introduced.

3.10 DATA ACQUISITION AND PROCESSING

Most of the data analysis was done using Python 3 and its standard math packages including numpy, scipy, scikit-learn,matplotlib, plotly and other utility packages. Some of the analysis was done in Matlab (R2018b) using standard libraries (local minima detection for detecting the center of mass of place fields). The majority of behavioral analysis was conducted using Jupyter notebooks for better visualization. All final code for the project is available in the github project repository.

3.11 SINGLE UNIT IDENTIFICATION

KlustaKwik and NeuroSuite were used to perform automatic spike sorting and to manually curate out noise clusters and to be able to merge similar clusters based on their waveshape similarity and cross/auto-correlations. Every recording session in a day was concatenated to use resting periods in between the sessions to validate the stability of given cells and improve the quality of spike sorting. Interneuron clusters, identified by their spike width and average firing rate, were also separated from pyramidal cell clusters for further analysis. All units were then filtered for interneurons and pyramidal cells based on their firing rate.

3.12 SPATIAL FIRING MAPS

For each recording session a spatial firing map was calculated based on the density of neuronal firing plotted over animal trajectory to create a heat density maps (Figure 34). This was then overlaid with the location of the beacons in the arena. We also used patch analysis for multi-field place fields in order to get a peak firing rate for each unit (Figure 34).

4

RESULTS

In this section I want to focus on a three-part analysis. First, I want to show a proof of behavior using results from the task. In the second section I will present results from electrophysiology, including our place cell descriptions. I then combine it with behavior descriptions to describe at least a part of the neural mechanism responsible for path integration.

4.1 BEHAVIOR

4.1.1 *General behavioral description*

In order to prove that this novel task is a path integration task, we need to focus on the animal behavior in the arena. At first I examined general locomotion patterns the animals exhibit in the arena. I found very few inter-individual differences, likely associated with the training in the task. For animals who were taught a rearing behavior, there was a distinct difference in the amount of time spent rearing compared to animals from pilot trials not habituated to the rearing demands (Figure 14).

Previous research from our laboratory highlights the difference between states of behavior when animals are locomoting (Graboski et al., *in prep.*). In general rats have two forms of distinct walk. The first mode is a high walk typically signified by a higher head height and higher head pitch from the neck angle (Figure 26). This is also connected to a distinct breathing pattern. The second mode is a low walk which is made distinctive by the animal having its head pitched more towards the arena floor with its overall head height lower as well. These two types of locomotion behavior or two walking modes can be found across different research arenas. A third state would be a rear state, which does not happen as

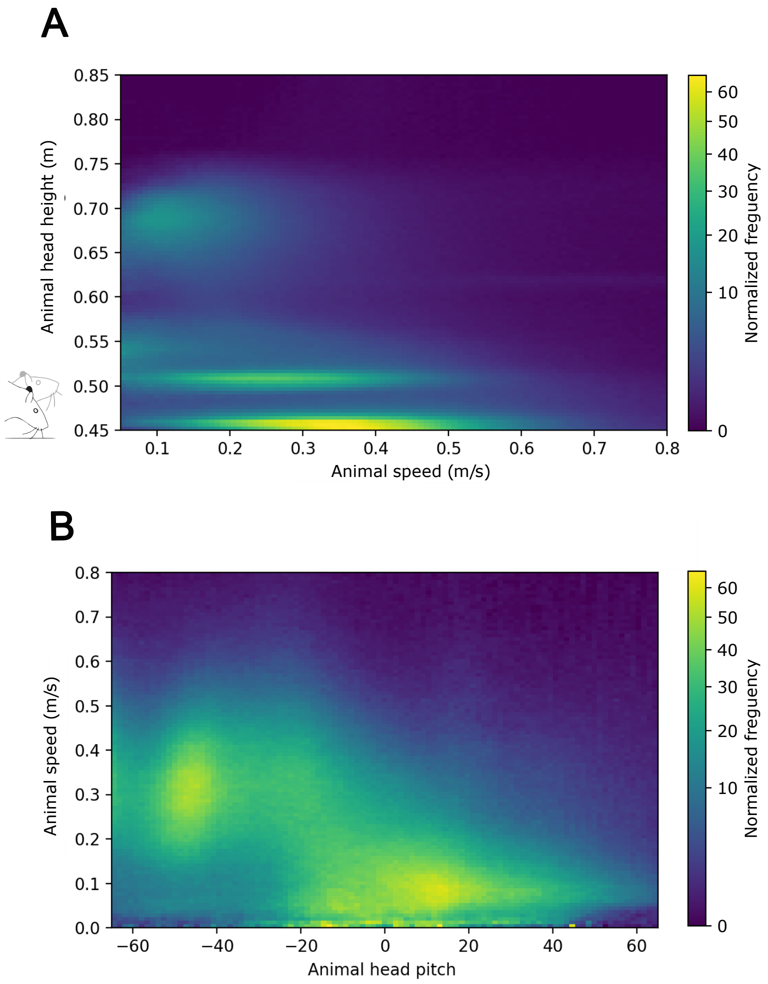

Figure 26: *Example of general behavior of an animal gathered across 200+ sessions. A) Joint probability distribution function for speed and head height across all sessions for one animal, unveiling distinct modes as depicted by cartoon inset. Distinct clouds can be observed for rearing and a clear separation between high and low walk. Note the .62 m rearing cutoff. B) Joint distribution for head pitch and animal speeds across all sessions for one animal.*

often as the first two, but in our case the rearing state serves an important role in completing our task as described above and hence it is overrepresented in our data when compared to the behavior of naive rats.

In order to prove our novel path integration task we wanted to show the following:

- Animals can navigate to a previously known location in darkness.
- Animals navigate to this location using path integration and not other navigational strategies.

- The accuracy of the task behaviour is lower when the animal's vestibular system or egocentric visual flow are perturbed during the path integration.
- Goal (beacon) directed and reward search navigation are associated with a different behavioural state.

4.1.2 *Darkness location retrieval*

At first we want to show that the animal can navigate to a previously known location in darkness. In the previous section we already introduced the training the animal has to undergo in order to successfully complete the full task.

The training took on average 3 weeks. If we simply measure the behavior output in the amount of pellets the animal triggered in 30 minutes, we see a clear upward trajectory for each of the animals. It is important to note that during each of the consecutive steps the task gets more challenging yet the animals prove they can get better at each point in the task (see Methods).

4.1.3 *Straightness measure*

Besides showing the animals' trial learning curves as a measure of success in the task, we also focused on the straightness of the trajectory given a running window before the beacon approach. This measure has been reported as a single value in the past, sometimes as the only metric to evaluate the precision of path integration (Maaswinkel and Whishaw 1999). The hypothesis is that if animals can store a vector to a given location in their brain then they must be able to retrieve it when they want to make an inbound trip and should do so in the straightest way possible. If they actually can integrate over a path, they show significantly straighter trajectories to the beacon, and it is a sign that they have learned the task. If they are not using any other cues to do so in darkness, then it is a successful path integration attempt.

In our case we use straightness as a sliding window measure in time away from the beacon trigger and compare it to bootstrapped data of an animal's random trajectory within a session (Figure 28). The more detailed analysis proved that compared to sham the rate of significance for two dimensional straightness increases from back in time from two seconds before the beacon trigger. These last 2 seconds are often part of the rearing dynamics of the animal as can be seen from the average rear and therefore cause the dip in straightness (29).

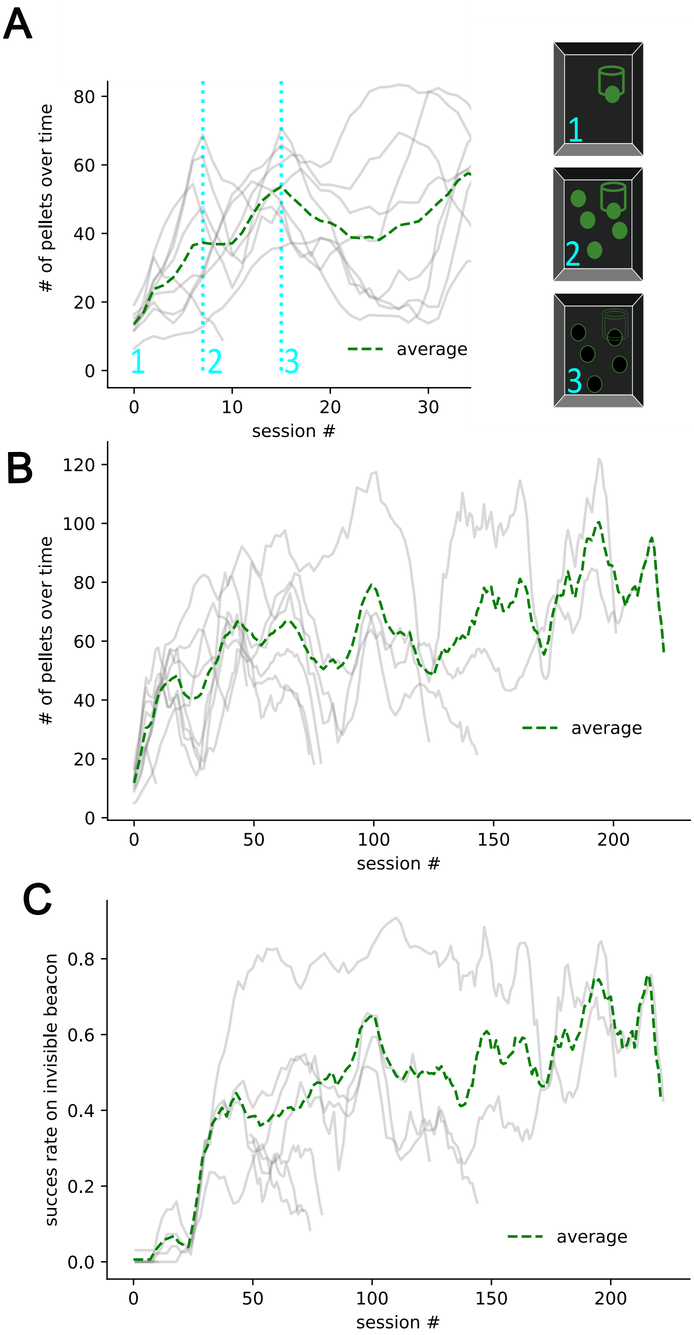

Figure 27: *Overall animal performance. A) Different stages of the training.* ***1.*** *Visible only in one location* ***2.*** *Visible in different locations* ***3.*** *Invisible every other presentation with different locations. Notice the slight dip after each training step due to increased difficulty of the task, which was increased within the stage as well adjusted to the performance of each individual rat. B) Performance on pellets achieved over time across all sessions and all animals. C) Success rate ratio of rearing inside of the invisible beacon area before timeout of 60 s or not.*

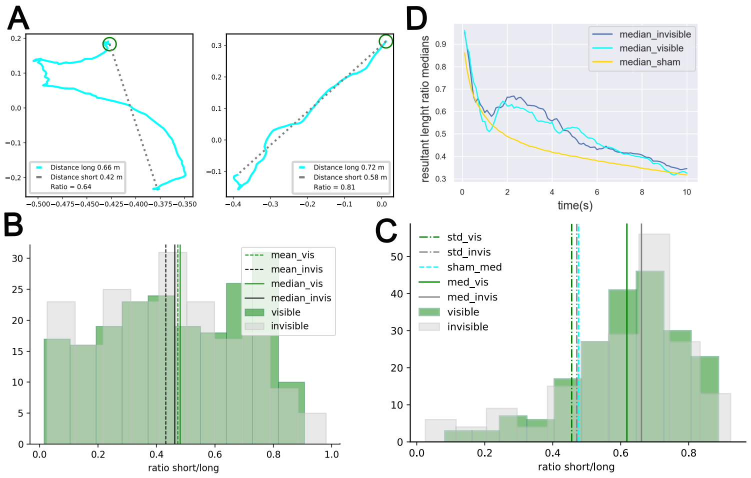

Figure 28: *Straightness of trajectory before approaching a beacon, a continuous measure. A) Two individual examples from a trajectory up to 3.5 seconds back from the beacon trigger.* left *in complete darkness and* right *with visible beacon. In the legend is the straightness ratio, calculated by dividing the length of the straightest possible trajectory from point to point by the length of the actual trajectory. B) Ratios for a set of bootstrapped sham trajectories at a 3 second point away from the beacon, shown as a histogram comparing visible beacon sham (green) and complete darkness or invisible condition sham (gray). C) Same as B but for actual trajectories in the training task and with addition of the location of sham on the graph representing 3 seconds back from the beacon. D) Running average on a set of animals over all sessions on straightness of path when approaching a visible (dark blue) or invisible (cyan) beacon versus a generated sham from 10 000 random 10 second trajectory samples.*

4.1.4 *Rearing dynamics*

In this section I want to explore the behavior which became the key to accomplish our path integration task and focus on how it gives us a readout of the animal behavior more precisely than the animal staying in a given spot.

After a behavioral pilot trial of two animals and detailed analysis of behavior we observed that rats tended to rear in the vicinity of the beacon. We then focused on adding this as one of the ways to trigger the reward. Rats have a hard time keeping completely still in such a small space, and the approach to the beacon is each time different, underlining the need for a more precise estimation. When rearing was reinforced the rats transitioned to use rearing as the only way to trigger the beacon. After the initial pilot experiment we identified a local minimum on a height histogram, a transition between high walk and rearing and used it as our threshold measure; .62meters above the floor of the room (Figure 15). This threshold was kept consistent across animals since all were male and weighed approximately similar throughout the experiment. Animals were also rearing on the walls of the arena. Due to our pseudo random distribution of the beacon and no presentation of beacons which were touching the wall of the

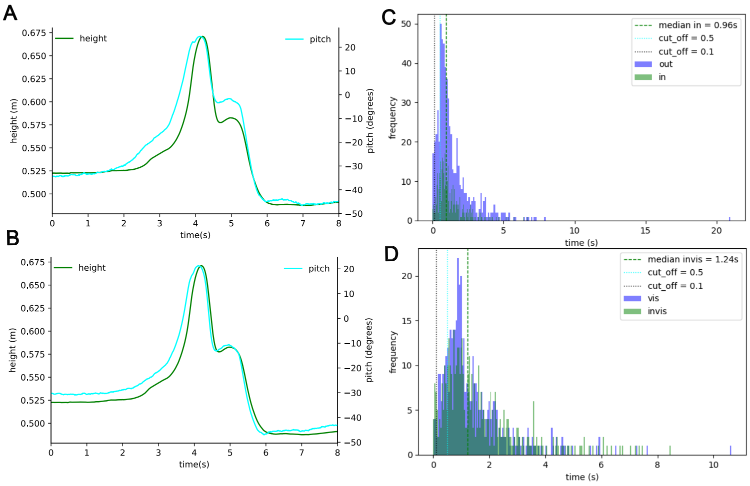

Figure 29: *Average rear and duration. A) An average rear dynamic across one animal across all sessions. Green represents the head height of the animal on the y axis and cyan represents the pitch of the head of the animal on the second y axis. Note how the pitch precedes the height of the animal on the way up to the rear. B) Same as A but across the whole population. C) A histogram of duration of rears for the first seven training sessions in one animal. Blue is duration of the rear which is outside of the beacon zone. In green are duration of rears which successfully triggered beacon. Median for both populations is plotted in dashed green. Time horizontal lines are at .5 s and .1 s for comparison. D) Same as C but for sessions 16-24; here the duration of the median rear increased slightly after training.*

arena, the animals reared very often in the center of the arena, which we focused our analysis on, such that most triggers are self supported rears and show very similar rearing dynamics.

We specifically focus on the rearing dynamic and at first plot an average rear (Figure 29). During the rear is when the animal appears to have dipped in XY speed.

4.1.5 *Rearing density*

The other feature we focused on in our behavioral analysis were the rearing episodes the animals learned to make throughout the task. If the animal rears, we can estimate its intended location with higher precision to assess its path integration intention. For the overall analysis we have focused on the density of rears around the location of the beacon. On average animals tend to make 2-3 unsupported rears for every beacon presented in the arena, even in complete darkness. The average rear is preceded by the animal's head pitch changing. The duration of the rear increases slightly with training (Figure 29).

We show that the density of the rearing is heavily concentrated on the outer perimeter of the beacon. If we account for the space available to rear by measuring the area in concentric circles away from the beacon we can normalize for space available for rearing and plot rearing density corrected for location (Figure 30 B). For this rearing analysis we focused only on rears which are not supported by the wall. We looked at how this rearing density compares to rearing in an invisible or visible beacon and compared it to shuffled condition. This analysis showed that even in the invisible condition the rearing is surrounded near the center of the beacon with a much higher likelihood then sham proving that animals can remember this location of the beacon in darkness. (Figure 30).

4.1.6 *Use of allocentric cues*

The next step was to prove that the developed task is indeed purely path integration dependent and rats cannot rely on the allocentric cues. We wanted to investigate differences in success in early or late trials after location change within a block of trials with the same beacon location. We reasoned that later in the block animals could associate beacon (homing) location to subtle but likely available allocentric cues, if any exist, which would result in performance improvement from earlier to late trials within a block. In contrast, if the animals can still complete the task, even after the beacon location changes, then we have proven that the buildup of allocentric cues, especially olfactory cues, are not helping the animals to achieve the task and it is purely dependent on the path integration.

If the animal were to use olfactory cues to successfully retrieve the beacon location, its success would likely be higher on the later trials within a location block. This perfomance difference would be because it has developed an allocentric based strategy. In our case we don't see this difference. In fact, if the animals were to learn only to use allocentric information it would be very misleading especially for the early trials of that given block. This would also prove the use of other allocentric cues or a habitual motor behavior. We show that the early trials are as likely to be successful as the late trials within the same location of the beacon (Figure 31).

4.1.7 *Inbound and outbound differences*

Our task can also be thought of as a short-term memory task with an outbound path being the encoding of the navigational memory and the inbound trajectory being the readout of that memory. Rats are then tasked to summarize the

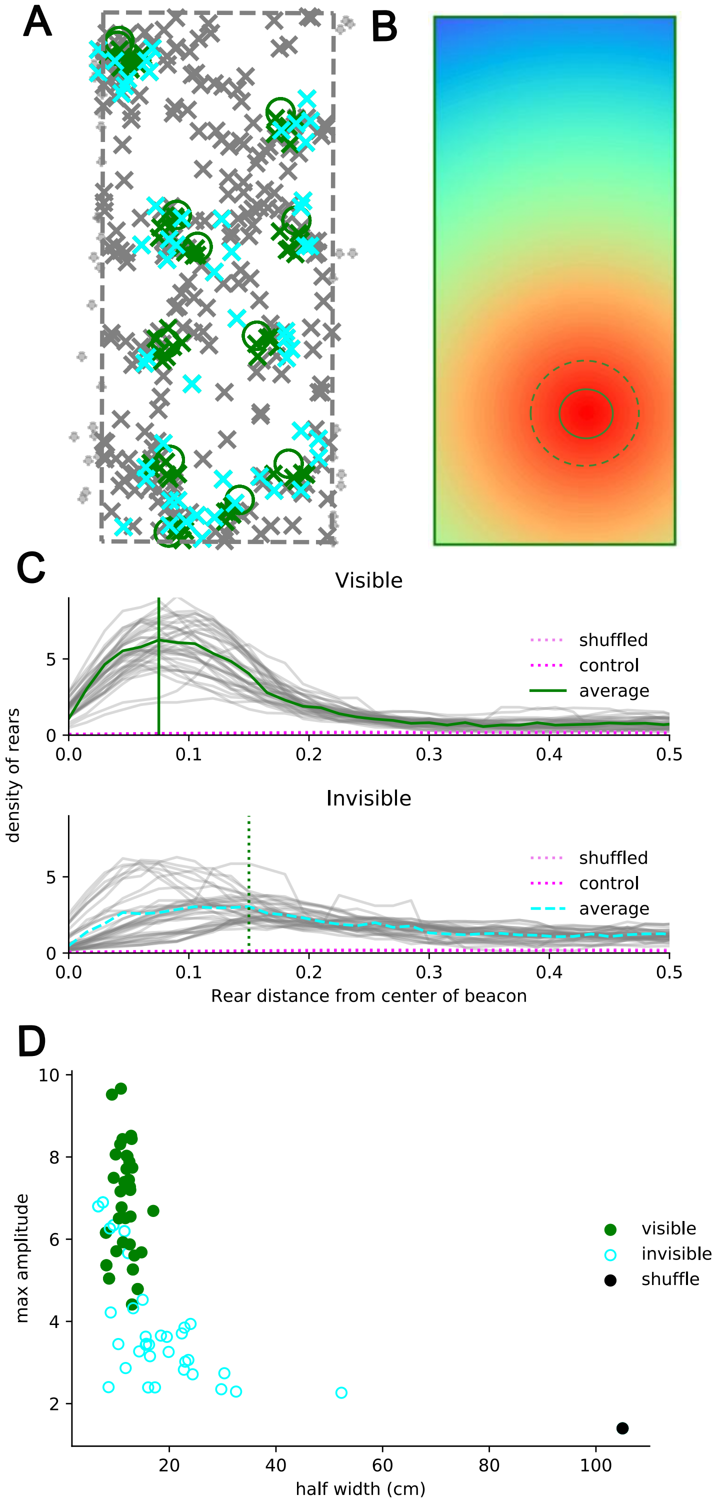

Figure 30: A) A single session example as shown in Figure 13. B) Simulated calculation of rearing density, normalized for area available to rear at a given distance away from the beacon for each location of the beacon for each session. C) Density curve per 1000 rears of an animal during visible and invisible conditions. Vertical line signifies the beacon trigger area for visible and invisible conditions. D) Summary figure of maximal amplitude and half widths from C). Invisible beacon rearing density has a much broader distribution then the visible one.

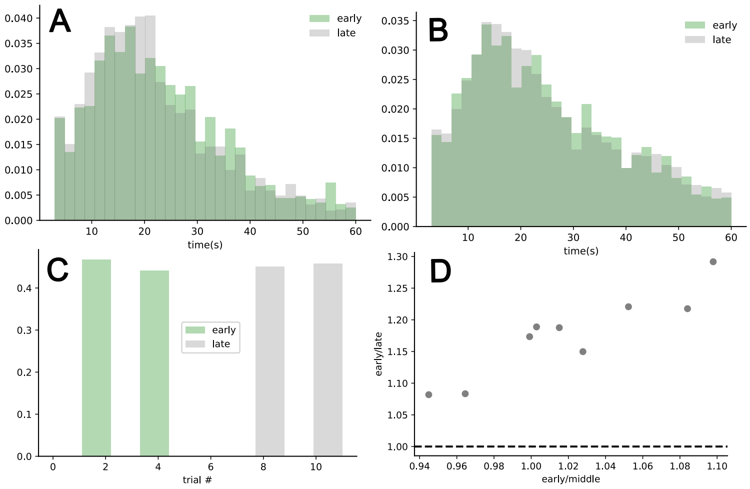

Figure 31: Early vs. late performance in a block of trials to disprove allocentric strategy. A) One rat example of a histogram of times it took to trigger an invisible beacon. Highlighted in green are early trials 2 and 4; in gray trials 8 and 10 and their respective time to achieve invisible beacon. B) Same as A but for all rats. C) Showing early and late trials and their likelihood of being successfully triggered in an invisible condition. D) Ratio of successful and unsuccessful invisible beacon presentations where early trials are divided by later trials on the x axis or early trials are divided by middle trials. Early trials are slightly more successful than middle trials than late trials.

outbound memory by creating the shortest path back to the beacon similar to a short-term memory path integration task in humans (Shrager, Kirwan, and Squire 2008). There is also some evidence that the behavioral state of the animal is different when it is approaching a goal like a beacon or when it is searching for something. In our case this would be a pellet. After comparing the straightness analysis we focused on seven seconds before and after beacon trigger and compared together the head pitch, head height and the speed. More discrete analysis might uncover differences but, our overall analysis did not reveal any differences in these measures between a goal-oriented approach or a presumable search strategy.

4.1.8 *Behavioral analysis of perturbation*

We also focused on analyzing the beacon approach and the rearing density around the beacon to compare how the animals behaved during perturbations. We found that animals decreased the precision of their rearing to the center of the beacon during visual perturbation. We also found similar results for the vestibular perturbation. In both cases the rear density remained above sham level (Figure 33). This decrease in precision is clearly a result of the perturbations.

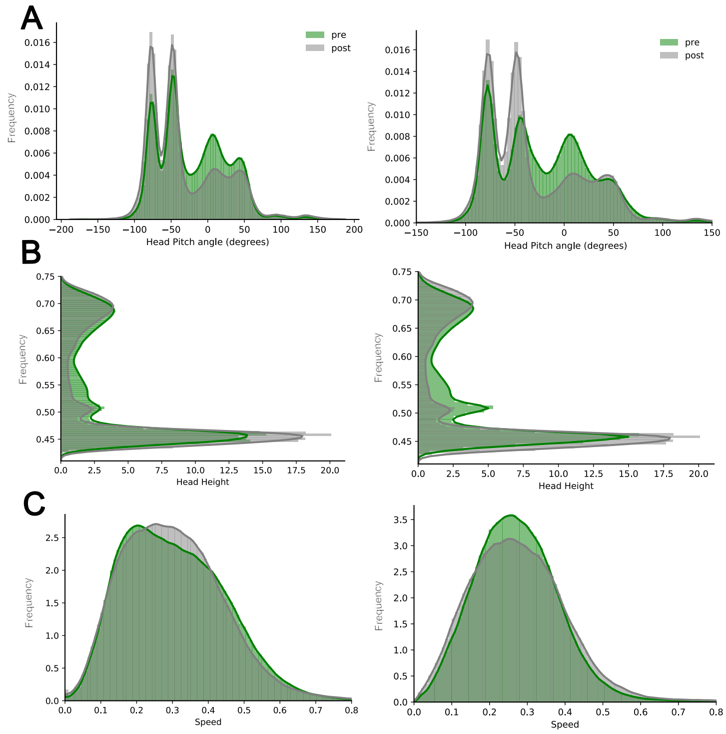

Figure 32: *Behavioral differences before and after beacon trigger. A) Head pitch differences for first seven seconds before (green) a beacon trigger and 7 seconds after (grey).* Left *are individual differences and on the* right *population differences. B) Same as A) but for head height, here again one can notice the .62 m rearing threshold. C) Same as A) and B), but for speed. No significant difference between the two populations were found using Kolmogorov-Smirnov Kolmogorov-Smirnov (K-S) test.*

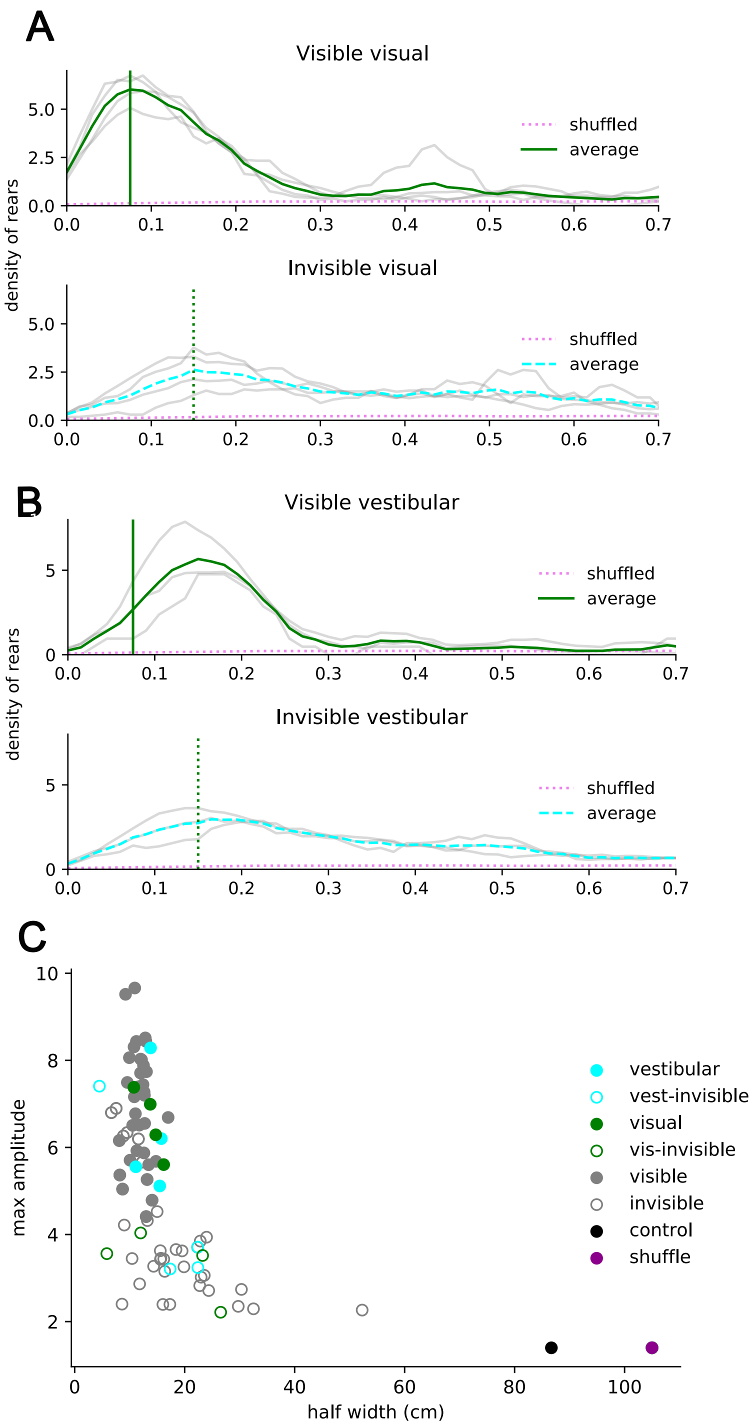

Figure 33: *A) Detail of rearing density around beacon for visual perturbation for visible and invisible beacons respectively. Green vertical lines signify the beacon trigger area for the respective condition. B) Same as A) but for vestibular perturbation. C) Summary of the max amplitude and half widths for different sets of thousands of rears and their proximity to the center of the beacon. Filled circles signify any invisible rear (complete darkness). Green color signifies the visual perturbation, Cyan for vestibular perturbation, blue shuffle, black control. Half width is much smaller for invisible conditions, yet significantly above zero.*

4.1.9 *Behavioral Summary*

In the section above we showed that rats are capable of finding a recently visited location in darkness. We show that the task is majorly reliant on the use of egocentric cues, because if the animals used allocentric cues, this would improve the performances during later trials for a given beacon location. We have also shown that we can perturb this behavior: vestibular and visual perturbations cause a drop in accuracy in the task performance pointing to clear contributions of the two egocentric sources, visual flow and vestibular sense of heading in our path integration task. These perturbations do not prevent the behavior from happening.

4.2 ELECTROPHYSIOLOGY RESULTS

In the next sections of the results I will now focus on the neuronal readout from the CA1 layer of the hippocampus in order to get more insight about its contribution to the path integration task. We will compare different properties of spatially selective cells across task and behavior of the animal. I recorded cells from the CA1 hippocampal layer of 6 animals; the data set includes over 600 hours of electrophysiological recordings spanning over 250 task sessions including both perturbation paradigms and control tasks. Here I will only present a preliminary analysis and describe a few of the many manually curated spatially selective cells in a subset of sessions. There is more to be explored in the data set that unfortunately did not fit in the span and timeline of this thesis.

4.2.1 *Examples of Place cells*

Our data set includes a large variety of place cells, some encoding for the location of the beacon or the border of the arena (Figure 34 A,B). Keeping track of the arena as it displaced allowed comparing place fields in the room and arena reference frames and clearly indicated that the cells were anchored to the arena reference frame (Figure 35 E). We identified a number of cells with wmultiple place fields (up to 3), some co-localized to the beacon location and also many cells which firing most strongly only when the animal was rearing (Figure 35 C). We split the place field 2D rate map calculation to the epochs of theta state and locomotion excluding rear, which matches a conventional place cells analysis. In order to see the effect of the task segment - approach to the beacon, or goal-directed vs departure, or path integration, we restricted rate map computation to 10 seconds of approach or departure (Figure 35). Most cells preserved a place field in the same location, while very few remapped between approach and departure.

4.2.2 *Place cells remapping between task phases under perturbation*

We sought to quantify the effect the perturbation during path integration segment (departure) has on the rate of place cells compared to that during goal-directed approach. Figure 35 shows that while in-field firing rates were largely correlated between approach and departure, a number of cells exhibited 2-fold and stronger change of the firing rate (Figure 35 H), consistent with the rate remapping phenomenon. Future quantification of the difference controlling for the effect of low occupancy and variability across trials depending on the location of the beacon is needed to provide solid interpretation of this result.

4.2.2.1 *High place cells*

In our behavioral and electrophysiological analysis we focused on different behavioral states of the animal. In this case we take advantage of our 3D recording and show state dependent firing of place cells. We found a variety of state dependent cells in the dataset including low cells (Figure 35 B) and rear cells (Figure 35 C). These latter ones are of particular importance due to the nature of our task, which requires animals to rear in order to receive a reward at a specified location in the arena.

4.3 ELECTROPHYSIOLOGY SUMMARY

With our few place cell examples we wanted to sketch a possible road map ahead to discover how the brain encodes for our novel path integration task. We initially set out to prove that a vector to a memorized location is updated during path integration and retrieved during goal-directed navigation via theta-coordinated predictive dynamics. We can only show a glimpse of the possible mechanism by showing the different remapping of the place cells depending on whether the animal is on an inbound trip to the beacon or if it has already triggered the beacon and is now searching for a reward.

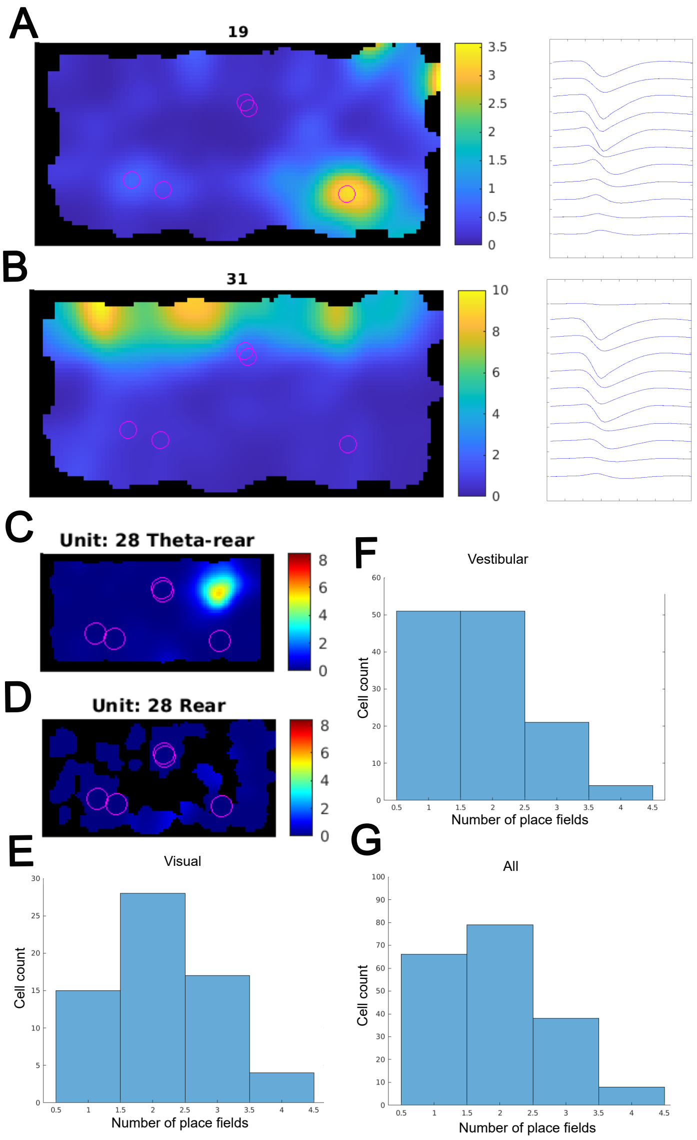

Figure 34: *A) Place cell rate map examples. Color represents the firing rate (Hz) of the cell for the given location for the whole period of the session including rears. In this example firing is the highest in a location of a beacon.* Right *Average spike waveshape for the example cells. B) Same as A) above but encoding for one wall of the arena a possible border vector cell. C) Example place cell rate maps computed over running period where theta oscillations are the strongest, above 2 cm/s excluding rear states. D) Same as C) but only using rearing trajectories E) Overview summary for curated set of 6 sessions from 3 animals from the data set and their respective amount of single or multi place fields cells in the data set, here only showing cells from visual perturbation sessions. F) Same as E) but for vestibular sessions. G) For all sessions combined*

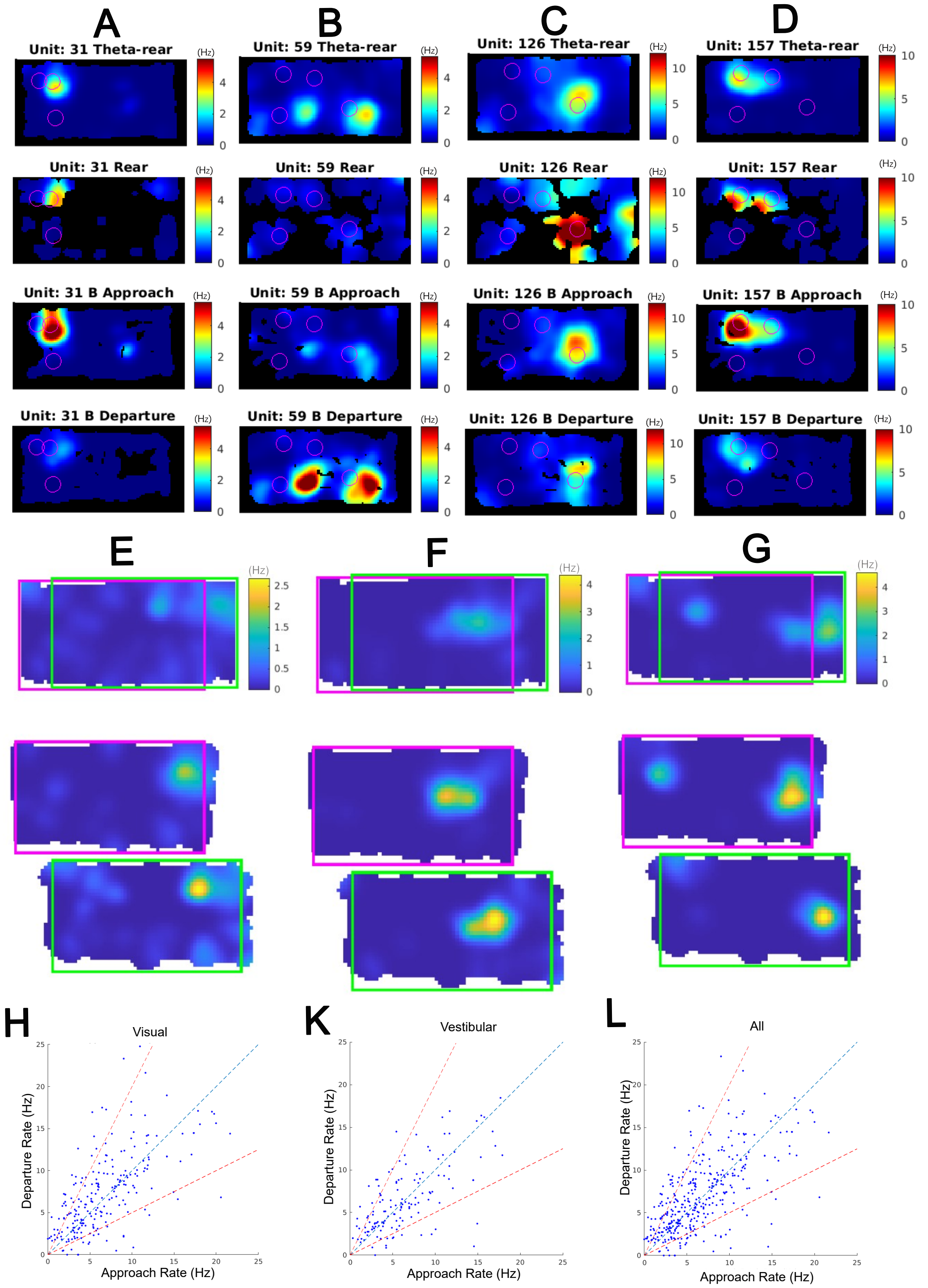

Figure 35: A) Similar to Figure 34 *C).* Below *Same as* top *but only for 10 seconds before the trigger of the beacon (approach) and* below *for 10 seconds after the beacon (departure). Note cell remaps after the trigger of the beacon and is mainly active during the approach and rearing . B) Here the cells appears to fire most during an outbound segment of the task. C) Cell which fires highest during rearing at a beacon location. D) Cell firing highest during approach and rearing. For this cell, there appear to be two distinct population encoding for a different beacon but from the same angle and mainly in the rearing state. E) Showing place cell examples during vestibular perturbation. Cell stays locked in an allocentric frame not dependent on the borders of the arena. F) Cell remaps slightly towards the shift of the arena G) Cell appears to be responding mainly to the physical border for the arena. H) Overview of the same group cells as in Figure* 34 *E), but here showing the in-field firing rate of each individual place field during approach to and that during departure away from the beacon, here in the visual perturbation. Red dashed line denotes 2 fold change in in-field firing rate K) Same as H), but for vestibular pertubation L) Across all curated cells.*

5

DISCUSSION

In our work we teased apart contributions of allocentric and egocentric frames using a novel path integration task. With 3D tracking, closed loop conditioning and freely moving virtual reality we were able to revolutionize the approach to making a rodent behavioral task with the use of a 3rd dimension for conditioning. We explored how the animal integrates and acts upon a summed path with homing behavior. We showed novel ways of interpreting path integration behavior with a continuous straightness measure and rearing density around the beacon. We also proved that the animals are not using allocentric information by focusing on success in early and late trials for the same location. With these methods, we show that the performance on the task, utilizing the integrated vector composed of egocentric information, is deteriorated using visual and vestibular perturbations. The use of idiothetic sensors such as the vestibular system in a path integration task is supported from the past literature (Wallace et al. 2002; Zheng et al. 2009; Meer et al. 2010). By probing the idiothetic system with perturbations we additionally show that the task relies on the correct use of path integration. We show examples of place cells which are correlated with the allocentric and the egocentric reference frame. We also show cells which adapt their firing based on the task demands - change of beacon location or inbound and outbound beacon travel. We also highlight cells which are modulated by the state of the animal.

5.1 NOVEL PATH INTEGRATION TASK

The results from other path integration tasks were often confounded by uncontrolled variables due to their inflexibility for lack of trial repetitions (H. Mittelstaedt and M.-L. Mittelstaedt 1982,Heinemans and Moita 2022; Shamash et al. 2021), head restraint (Madhav, Jayakumar, Lashkari, et al. 2022) and inability to record the full 3D motion of an animal during the task (Rossier et al.

2000; Maaswinkel and Whishaw 1999). This hindered the recording of the full behavioral dynamics which aids the neural understanding of path integration.

Our task requires 3 weeks of learning to master, consists of high throughput of trials in a closed loop manner based on 3D motion of the animal. It allows long term recordings from key structures in the brain during the task in a freely moving animal. With such a freely moving animal, our unique idiothetic perturbation paradigms allowed us to influence the systems under standard behaving conditions, unlike restrained VR experiments (Laurens and Angelaki 2018). This way we were able to expand on previous work with a novel paradigm. Our results are based on large amounts of precise behavioral data to understand the changing neural dynamics of the navigation in different spatial reference frames. We find for example the influence of the height of the animal on the encoding of the place, which would not be possible without 3D tracking as it is in many previous path integration tasks.

5.2 OTHER BEHAVIORAL STRATEGIES

The ability of the animals to find the beacon in darkness already hints at a use of path integration to complete the task, but it does not fully support a pure use of path integration. In order to solve the task animals could still be using other behavioral strategies like random search, wall-hugging, circling or chaining (Ruediger et al. 2012; Wolfer and Lipp 2000) and other allocentrically driven strategies such as using the built up of odorants by the animal throughout the task. The possibility to move the location of the beacon allows us to ensure that the animals are not using allocentric cues such as olfaction to help navigate to the beacon. By measuring the success of the animals on early and late trials where the location of the beacon has changed we prove that the animals are not using these other behavioral strategies such as arena borders or other allocentric features in order to find the location of the goal.

This is important in showing the dependence on pure idiothetic cues like proprioception, visual flow or the vestibular signal (7) which help to define the animal's position in the egocentric coordinate system. Many allocentric cues can be overlooked as in previous tasks (Arnold et al. 2014; Etienne et al. 1998; Rossier et al. 2000; Maaswinkel and Whishaw 1999). Based on past research we know that the vestibular system plays a critical role in a dead reckoning (path integration) task without light, due to vestibular lesions (Zheng et al. 2009; Wallace et al. 2002; Stackman and Herbert 2002), and perturbations to the head direction system (Golob and Taube 1999; Meer et al. 2010). Recent studies also showed the importance of unanchored visual flow to the perception of self motion (Madhav, Jayakumar, Li, et al. 2022). We designed two types of perturbations, tackling besides other senses, vestibular sense and visual sense, specifically unanchored visual flow that contribute to the head direction system used for path integration.

5.3 PERTURBATIONS

It has been argued that passive vestibular perturbations have different effects on the animal with regard to their representation of space if they are done in a restrained or freely moving rat (Laurens and Angelaki 2018). In our case we manage the perturbations in a freely moving virtual reality system, providing us with sensory input as naturalistic as possible especially for the vestibular system when compared to head fixed virtual reality. Achieving a naturalistic stimulation to the inner ear is essential in studying path integration due to its contribution to the head direction signal.

Our visual flow perturbation was done in an unanchored manner to disrupt part of optic flow which constitutes a signal to the head direction system, but it did not allow for the use of the visual system to align an egocentric frame with an allocentric one by showing a visual cue that is stable in the environment.

5.4 DISCRETE MEASURES OF PATH INTEGRATION

We have unveiled new measures adapted to the task, namely the continuous straightness measure and rearing density. First, these metrics help to prove that animals are not using a circling strategy, chaining or random search to be able to complete the task. Second, they also portray how precise the animals are in the task and to study the perturbation effects we enact on them.

A straightness and an angle to home location has been measured in previous studies, but usually only from one point (Maaswinkel and Whishaw 1999; Shettleworth and Sutton 2005). This simple kind of analysis might be missing the precise moment when the animal will recall the actual vector back and enact it. During our task, this vector becomes a continuous measure. On top, no previously published experiments to my knowledge have rewarded rats for a rearing behavior in a closed loop manner. This procedure has been suggested before (Lever, Burton, and O'Keefe 2006) and similarly enacted in rearing mouse lemurs and mice using optogenetic manipulation as a reward as well as operant conditioning (Nourizonoz et al. 2020). By supporting rearing behavior with a reward we have also changed the amount of state transitions the animals normally do from walking to rearing (Figure 14 A). With the use of the rearing behavior as a trigger we can use dispersed beacon placement and its specific height position for detection instead of having the animal stay in one spot. There are some walls which are supported by the slated wall of the arena, but we have not use those in our analysis (Figure 13 C).

5.4.1 *Behavioral state classification during path integration*

Another important aspect of the animal navigation is its behavioral state. Above, we have already mentioned that animals which are given access to water and are food deprived will represent space differently when they are thirsty (Low and Giocomo 2022). Simple hunger state can have a large influence on whether a bee will stick with a certain reward or will search for an uncertain one (Katz and Naug 2015). Another behavioral state can simply be defined via the animals' locomotion. When rats are locomoting they have 3 different states: a low walk, a high walk and rear. During low and high walk there are differences between how the animal is breathing and what kind of environmental sampling it is doing (Graboski et al. *in prep.*). The behavioral states the animal is doing can be correlated to different parts of a path integration task.

Through our research on discrete measures of path integration we have noticed different states of animals when it comes to an inbound trip or an outbound trip. On the first look it is obvious that the animals tend to rear much more on the inbound trip (towards the beacon, where they need to rear) and much less on an outbound trip where they need to collect the pellet from the task. Some of this can be easily explained by the demands of the task, like rearing vs. picking a pellet from the ground, but there are other behavioral constants beside just height of the head which are consistent among inbound and outbound parts of the task.

This observation also holds for the many other experimental works when it comes to other species in the animal kingdom. We can observe many behavioral differences between exploitation and exploration (Hills et al. 2015; Mobbs et al. 2018). In our analysis based on our lab's research from the past (Graboski et al. *in prep.*), we have also focussed on differences between animals' behavioral dynamics when it is integrating the path on an outbound trip and also when it is focused on retrieving the correct trajectory back to the original spot on an inbound trip.

5.4.2 *Inbound and outbound part of the path integration*

How the animal combines different sources of information, allocentric and egocentric has been described previously (Angelaki, Shaikh, et al. 2004; Gu et al. 2006; Harootonian, Ekstrom, and R. C. Wilson 2022; Stavropoulos et al. 2022; Maaswinkel and Whishaw 1999) , but the connection to how a low walk or a high walk might be connected to different environmental sensory collection as well as different parts of a path integration task like inbound and outbound behavior has not yet been described in detail. The two segments present themselves in our task: The outbound part of the task, when the animal is actually integrating the sensory information and the inbound part of the task which starts with a retrieval of the vector or the integrated information followed by the actual movement towards the goal is also described as inbound locomotion. We were not able to prove any significant difference between the inbound and outbound segments of the task (Figure 32).

5.5 ELECTROPHYSIOLOGICAL ANALYSIS

In the last decades, research on path integration has revealed connections to head direction cells, grid cells and boundary vector cells among others. From modeling work it has become more clear that a purely idiothetic system requires calibration from the outside world (Savelli and Knierim 2019). The allocentric frame of reference calibrates for the drift of the egocentric one. This way, rats are capable of navigating in complete darkness during which they integrate vestibular information which in turn updates where on the cognitive map they are located and which place cells would be firing. In our data set we find examples of boundary vector cells and cells which rate remap due visual and vestibular perturbation, as well as cells which remap on a state dependent manner, showing different remaping on an inbound and an outbound trip from the beacon. Rate remapping (both increase or decrease) in response to perturbation suggests that their effect is comparable at both the level of behavior performance, but also on the hippocampal place cells.

5.6 TYING SPATIALLY SELECTIVE CELLS TO OUR TASK.

How do spatially specific firing cells of the animal behave during our task?

There can be many effects on a given behavioral task on the spatial specific encoding like in case of a mismatch (Gothard, W. E. Skaggs, and Bruce L McNaughton 1996) or goal, valence dependence (Markus, Qin, et al. 1995; Pettit, Yuan, and Harvey 2022; Boccara et al. 2019; Gauthier and Tank 2018) and attention (Markus, Barnes, et al. 1994; Kentros et al. 2004) among other behavioral variables. Previous research (Grieves, Jedidi-Ayoub, et al. 2020) has highlighted 3D grid cells and place cells in an arena, but it has stopped short of describing a place code during different states of the animal such as during rearing. Research in our lab suggests the presence of place cells firing only when the animal is rearing (Graboski et al.*in prep.*). Due to our rearing paradigm we find some of these in our data set (Figure 27).

We expected the beacon in our VR arena to be represented with OVC whose firing is very similar to a kind of reward expectation cells due to the Pavlovian instrumental transfer. The animal learns to associate the noise with the beacon with the pellet reward, leading to subsequent reward seeking behavior (Cartoni, Balleine, and Baldassarre 2016, Cartoni, Puglisi-Allegra, and Baldassarre 2013). With trained animals there might be OVCs which encode only the beacon location and these would dictate the firing of the spatially selective cells which have a tendency to have higher firing rates around rewarded or areas with more valence (Low and Giocomo 2022). We find many examples of cells which code for the position of the beacon and whose rate of firing seems to increase the closer to the beacon the animal is.

Many of the cells which we have recorded are multimodal cells such that they could be encoding for a vector of the beacon and act as place cells in front of the beacon as well. These cell properties get easily mixed within the CA1 of the hippocampus. Research suggests a differential effect of deep and superficial layers on encoding of objects. Geiller et al. 2017; Sharif et al. 2021). Cells in the deeper layers of the CA1 are more controlled by landmarks Geiller et al. 2017, whereas superficial layers are more likely to have a single firing field and are very slow to respond to changes in the environment. These cells in the hippocampus are also driven by theta oscillations which arise from the medial septum.

Research from Fattahi et al. 2018 suggests that inactivation of MS, a theta oscillation generator, leads to a deterioration of spatial information provided by place cells. This also furthers previous findings that superficial CA1 cells help global positioning more than deep CA1 cells which are more bound towards encoding objects within an environment. In our arena the peculiarity in the tasks is that the object with the same identity keep moving to different places in the environment dissociating the allocentric reference frame with the need to realign along the egocentric reference frame. We find many exmaples of cells which remap during an inbound or an outbound trip to the beacon trigger location when we only look into the firing of cells when the animal is locomoting at a rate faster then 2 cm/s, during which more stable theta dynamics emerge.

5.6.1 *Beacon encoding*

One hypothesis coming from the paragraph above is that the neurons in deeper layers of CA1 would represent the beacon location and identity and that the more superficial cells of the CA1 would enable the flexible processing of spatial information mainly based on egocentric cues. This would then create a conflict between the changed location of the beacon and the egocentric understanding of space. The superficial CA1 layers would be less likely to adapt to this new beacon location, but the deep cell layers could take into account the change of location of the beacon. This hypothesis remains to be tested using our dataset.

The deep layer would only get updated when the landmark would be visible or change, but the superficial CA1 layers would be continuously updated due to the input of the egocentric cues which support path integration. The update would be expected to create a shift of place cells which keep track of a given reward. This is also shown in many papers where the place cells tend to fire closer to such a salient location (Jercog et al. 2019; Gothard, W. E. Skaggs, and Bruce L McNaughton 1996). The differences between egocentric and allocentric cues as well as deep and superficial layers of the hippocampus are likely not as stark as might be thought of. In the CA1 there is not a single threshold where deep cells all the sudden become superficial. Along the same blurred line, I want to highlight the possibility that the sensory systems also don't align exactly along idiothetic and allocentric lines either.

5.7 FRAME SPECTRUM HYPOTHESIS

Literature on modeling path integration often portrays two boxes which are introduced into the model: allocentric and egocentric frames of reference (Savelli and Knierim 2019). Other research supports the idea that these two reference frames work in synergy with each other (Volcic and Kappers 2008) .

Having delved in deep research in this topic, I want to argue for a continuity of the frames which bleed into each other while the animal is using different senses. Vision is often described as contributing mainly to allocentric input while an animal is navigating. On the other hand, proprioception or efference copies serve large inputs for the idiothetic reference frame. Both allocentric and egocentric

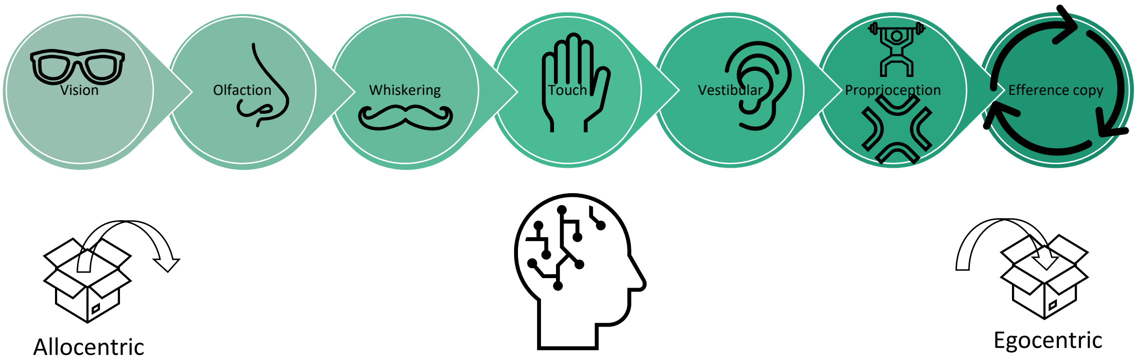

Figure 36: *The Continuity framework hypothesis where the line between which sense creates more of an allocentric or an egocentric reference frame is blurred.*

inputs contribute to an internal model of position in space, a continuous attractor. But what if we are discussing olfactory systems or whiskers' sense of touching objects? I would argue that smell over time gives us allocentric information , especially when there are different gradients of a particular substance which we sense in time as we explore through the environment. Odor has been shown to be enough to keep stable head direction even in congenitally blind mice (Asumbisa, Peyrache, and Trenholm 2022). The gradient would give us the understanding of how smell relates to the arena as a gradient of space. Places where an animal spends more time might accumulate more odors than places nearby. Other research on odor representation in a spatial context shows that a mix of stimuli including odor is responsible for firing of a given place cell (Lebedev, Pimashkin, and Ossadtchi 2018; Liu et al. 2020; Kulvicius et al. 2008). With whisking the rat can also relate two objects to each other by sensing a similar texture of an object on each side and relating them to each other. Vision is often seen as an allocentric sense, but what if we can only see one object and everything else is in complete darkness? That would be in a pure sense an egocentric perspective. What if we can observe two different parts of just one object? Would this object's representation be created from an allocentric perspective since we can relate those parts to each other, or egocentric as it is still one object and we can refer it only to ourselves? A theory has been proposed which assigns all the object locations next to each other in a grid-cell like column within the neocortex hence it would put each part of the object on an internal representation map and those could be connected due to our internal model of the world completing the objects together (Hawkins et al. 2019).

I believe we should consider thinking about the between senses and their respective contributions to the allocentric or idiothetic frame as a spectrum rather than having a firm line of one sense to another.

5.8 NEURAL MECHANISM

By drawing conclusions from the literature and our own research we want to highlight a possible neural mechanism to enable path integration. We initially set out to prove that the update of the path integrator happens online via theta sweeps within continuous attractor dynamics. As our research highlights, disruption of the vestibular code affects animals performance on our path integration task. Similar disruption to the vestibular code also heavily affects theta and subsequent decodability of position from CA1 place cells (Terrazas et al. 2005). Similarly, inactivation of the medial septum, a theta oscillation generator, leads to a deterioration of spatial information provided by place cells (Fattahi et al. 2018). Hence vestibular signal is needed to drive movement related theta oscillations. Theta sequences in turn have been used to describe how an animal can readout its past and future trajectories (Pfeiffer and Foster 2013; Papale et al. 2016; Xu et al. 2019).

As introduced earlier (Figure 8 -10), theta phase precession is when the rate of a place cell firing changes independently of the phase of theta. It is possible to fit 6-9 place cell assemblies within a single theta cycle given a normal speed of a rat being 5-6 cm/s. This one theta cycle represents the past, present and the future within the theta traveling wave (Figure 8). This gives researchers the possibility to try to decode where the animal goes next before it travels there (Pfeiffer and Foster 2013; Green et al. 2022; Grieves, Wood, and Dudchenko 2016). Theta sequences could be serving as a possible vector generator for paths taken by the animal.

The readout from the vector can be executed similarly to the way this occurs in the fly brain, where the central complex and the mushroom body show a sinusoidal signal giving a 360 degree reference which can be updated at any given point depending on the allocentric or egocentric cue changes (Lyu, Abbott, and Maimon 2022). In the case of a fly this would be the strength of the wind against its head. In a rat's perspective this would be the reliability of allocentric cues, such as a visible beacon. Both the rat and the fly can then read out from the sinusoidal vector to improve their travel.

In the case of the fly, it uses a 2D vector with the amplitude of the sinusoid representing the length of the path and the phase reflecting the direction of travel (Lyu, Abbott, and Maimon 2022). This is a very good example of how an allocentric signal and egocentric signal (heading direction) can be combined together to represent a specific vector of travel using relatively few neurons. In the case of the rat this does not have to be any different. With our data we were not able to prove this hypothesis.

One question which also remains is how this signal is read out downstream and where it is stored. We are also missing the link of how this signal is retrieved in case of an imminent threat or when the animal starts a homing trajectory.

Using research from Sobolev et al. *in prep.*. we can estimate that some place cells are more egocentrically driven, hence their firing rate would likely be more induced by fast head movement of the rat due to the vestibular signal integrating more of the acceleration. In this regard we could look into the effect of theta precession onto these more path integration-driven or egocentrically-driven cells and juxtapose it with the effect on more allocentrically-driven cells near the boundary of the arena. We can stipulate that the precession of these cells is more influenced by the theta when the animal moves its head rather than the cells which are mainly driven by the visual cues where the optic flow makes up more of the self motion information.

Population of not only place cells but also grid cells plays a role in path integration. Many papers have shown dependence of grid cells on correct working of place cells especially in the long term (Bonnevie et al. 2013). On the other hand, it is still debated whether grid cells are the full solution or likely just part of the picture due to their stability and error correction (Fernandez-Leon, Uysal, and Ji 2022). In our case error correction could help to aid the ability of place cells to adjust to a goal being in a different location and to code for this location in a flexible way.

5.9 FUTURE TRAJECTORY

In order to investigate some of these questions further, this novel path integration task could be easily adopted even without a freely moving virtual reality in other labs. Novel tools like DeepLabCut (Mathis et al. 2018; Kosourikhina et al. 2022) provide the ability for markerless tracking of the 3D position of a freely moving animal in an arena, providing a possibility for closed loop feedback based on different dynamics of the animals, such as reward at an experimenter defined spot when an animal is rearing. This has been done previously in marmosets (Nourizonoz et al. 2020). It is also possible to replace the beacon presentation with an Light Emitting Diode (LED) array from the floor or the ceiling or any focused light on part of the arena. This task could also be used with pure auditory stimulation similar to a recently developed sensory island task but with the involvement of rearing instead of waiting and randomly scattered reward. (Ferreiro et al. 2020). The trial segmentation profile of this task makes it easier to analyze and offers the ability to be done without the use of experimenter supervision. Overall our task is adaptable to use in labs without 3D tracking and virtual reality and shows a future promise for in depth exploration of path integration behavior with varied, even circuit level perturbations.

Our task can be adapted into the future to take advantage of the freely moving virtual reality by controlling the location in an allocentric rich virtual reality reference frame. By shifting, rotating or changing the gain as a way of perturbation, we could force the rat to rely only on allocentric cues, helping to gain an understanding of the role uncertain allocentric cues play in navigating and if navigation can be rescued only by using egocentric cues.

6

CONCLUSION

In conclusion, we have shown that animals make use of visual egocentric information as well as vestibular information to integrate pieces of their path over time in a novel path integration task. We have also conceived of new measures to probe a path integration task which is segmentable, can be recorded over a long term and results in a high throughput of trials. During our paradigms we detected over 100 000 rears during more than 50 000 trials across 11 subjects. Using these statistics we show that by perturbing the egocentric frame of reference with visual and vestibular perturbations we deteriorate but do not abolish the performance of the animals on the task. We argue that both of the above mentioned sensory inputs are important to estimate distance traveled.

We delve into the neural mechanism by recording place cells from the CA1 hippocampus and we show place cells which are correlated with each, the allocentric and the egocentric reference frames. We also show cells which adapt their firing based on the task demands, e.g.change of beacon location, and cells which are modulated by the state of the animal. This position could be read out from theta bound mechanism in order to update the integrator.

Our contributions illuminate the use of egocentric reference frame during path integration. Our task provides a basis for future work to investigate the mechanism even further.

BIBLIOGRAPHY

Abeles, M (1982). *Local circuits: an electrophysiological study.*

Aitken, Phillip, Yiwen Zheng, and Paul F Smith (2018). “The modulation of hippocampal theta rhythm by the vestibular system”. In: *Journal of Neurophysiology* 119.2, pp. 548–562.

Alefantis, Panos et al. (2022). “Sensory evidence accumulation using optic flow in a naturalistic navigation task”. In: *Journal of Neuroscience* 42.27, pp. 5451–5462.

Allen, Timothy A and Norbert J Fortin (2013). “The evolution of episodic memory”. In: *Proceedings of the National Academy of Sciences* 110.supplement_2, pp. 10379–10386.

Alyan, S and BL McNaughton (1999). “Hippocampectomized rats are capable of homing by path integration.” In: *Behavioral neuroscience* 113.1, p. 19.

Andersson, Sebastian O, Edvard I Moser, and May-Britt Moser (2021). “Visual stimulus features that elicit activity in object-vector cells”. In: *Communications Biology* 4.1, pp. 1–13.

Angelaki, Dora E and Kathleen E Cullen (2008). “Vestibular system: the many facets of a multimodal sense”. In: *Annual Review of Neuroscience* 31.1, pp. 125–150.

Angelaki, Dora E and Jean Laurens (2020). “The head direction cell network: attractor dynamics, integration within the navigation system, and three-dimensional properties”. In: *Current Opinion in Neurobiology* 60, pp. 136–144.

Angelaki, Dora E, Aasef G Shaikh, et al. (2004). “Neurons compute internal models of the physical laws of motion”. In: *Nature* 430.6999, pp. 560–564.

Arleo, Angelo and Laure Rondi-Reig (2007). “Multimodal sensory integration and concurrent navigation strategies for spatial cognition in real and artificial organisms”. In: *Journal of Integrative Neuroscience* 6.03, pp. 327–366.

Arnold, Aiden EG F et al. (2014). “Differential neural network configuration during human path integration”. In: *Frontiers in Human Neuroscience* 8, p. 263.

Asumbisa, Kadjita, Adrien Peyrache, and Stuart Trenholm (2022). “Flexible cue anchoring strategies enable stable head direction coding in both sighted and blind animals”. In: *Nature Communications* 13.1, p. 5483.

Barry, Caswell et al. (2006). “The boundary vector cell model of place cell firing and spatial memory”. In: *Reviews in the Neurosciences* 17.1-2, pp. 71–98.

Benchenane, Karim et al. (2010). "Coherent theta oscillations and reorganization of spike timing in the hippocampal-prefrontal network upon learning". In: *Neuron* 66.6, pp. 921–936.

Blair, Hugh T and Patricia E Sharp (1995). "Anticipatory head direction signals in anterior thalamus: evidence for a thalamocortical circuit that integrates angular head motion to compute head direction". In: *Journal of Neuroscience* 15.9, pp. 6260–6270.

Boccara, Charlotte N et al. (2019). "The entorhinal cognitive map is attracted to goals". In: *Science* 363.6434, pp. 1443–1447.

Bonnevie, Tora et al. (2013). "Grid cells require excitatory drive from the hippocampus". In: *Nature neuroscience* 16.3, pp. 309–317.

Boroditsky, Lera (2000). "Metaphoric structuring: Understanding time through spatial metaphors". In: *Cognition* 75.1, pp. 1–28.

Boroditsky, Lera and Alice Gaby (2010). "Remembrances of times East: absolute spatial representations of time in an Australian aboriginal community". In: *Psychological Science* 21.11, pp. 1635–1639.

Bowler, John C and Attila Losonczy (2022). "Direct Cortical Inputs to Hippocampal Area CA1 Transmit Complementary Signals for Goal-directed Navigation". In: *bioRxiv*.

Brandt, Thomas et al. (2005). "Vestibular loss causes hippocampal atrophy and impaired spatial memory in humans". In: *Brain* 128.11, pp. 2732–2741.

Brun, Vegard Heimly et al. (2008). "Progressive increase in grid scale from dorsal to ventral medial entorhinal cortex". In: *Hippocampus* 18.12, pp. 1200–1212.

Burak, Yoram and Ila R Fiete (2009). "Accurate path integration in continuous attractor network models of grid cells". In: *PLoS Computational Biology* 5.2, e1000291.

Burda, H et al. (1991). "Magnetic orientation in subterranean mole rats of the superspecies Spalax ehrenbergi: Experiments, patterns, and memory". In: *Isr. J. Zool* 37, pp. 182–183.

Burgess, Neil (2006). "Spatial memory: how egocentric and allocentric combine". In: *Trends in Cognitive Sciences* 10.12, pp. 551–557.

— (2008). "Grid cells and theta as oscillatory interference: theory and predictions". In: *Hippocampus* 18.12, pp. 1157–1174.

Burgess, Neil and John O'Keefe (1996). "Neuronal computations underlying the firing of place cells and their role in navigation". In: *Hippocampus* 6.6, pp. 749–762.

Butler, William N, Kiah Hardcastle, and Lisa M Giocomo (2019). "Remembered reward locations restructure entorhinal spatial maps". In: *Science* 363.6434, pp. 1447–1452.

Buzsáki, György (1986). "Hippocampal sharp waves: their origin and significance". In: *Brain Research* 398.2, pp. 242–252.

Buzsáki, György (2005). “Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory”. In: *Hippocampus* 15.7, pp. 827–840.

— (2019). *The brain from inside out*. Oxford University Press.

Buzsáki, György and J. Chrobak (2005). “Synaptic plasticity and self-organization in the hippocampus”. In: *Nature Neuroscience* 8.11, pp. 1418–1420.

Buzsáki, György and Edvard Moser (2013). “Memory, navigation and theta rhythm in the hippocampal-entorhinal system”. In: *Nature Neuroscience* 16.2, pp. 130–138.

Buzsáki, György, Cornelius H Vanderwolf, et al. (1983). “Cellular bases of hippocampal EEG in the behaving rat”. In: *Brain Research Reviews* 6.2, pp. 139–171.

Carr, Margaret F, Shantanu P Jadhav, and Loren M Frank (2011). “Hippocampal replay in the awake state: a potential substrate for memory consolidation and retrieval”. In: *Nature Neuroscience* 14.2, pp. 147–153.

Carriot, Jerome, Jessica X Brooks, and Kathleen E Cullen (2013). “Multimodal integration of self-motion cues in the vestibular system: active versus passive translations”. In: *Journal of Neuroscience* 33.50, pp. 19555–19566.

Cartoni, Emilio, Bernard Balleine, and Gianluca Baldassarre (2016). “Appetitive Pavlovian-instrumental transfer: a review”. In: *Neuroscience & Biobehavioral Reviews* 71, pp. 829–848.

Cartoni, Emilio, Stefano Puglisi-Allegra, and Gianluca Baldassarre (2013). “The three principles of action: a Pavlovian-instrumental transfer hypothesis”. In: *Frontiers in Behavioral Neuroscience* 7, p. 153.

Casasanto, Daniel and Lera Boroditsky (2008). “Time in the mind: Using space to think about time”. In: *Cognition* 106.2, pp. 579–593.

Caspar, Kai R et al. (2020). “Eyes are essential for magnetoreception in a mammal”. In: *Journal of the Royal Society Interface* 17.170, p. 20200513.

Cheng, Zhixian and Yong Gu (2018). “Vestibular system and self-motion”. In: *Frontiers in Cellular Neuroscience* 12, p. 456.

Cholvin, Thibault, Thomas Hainmueller, and Marlene Bartos (2021). “The hippocampus converts dynamic entorhinal inputs into stable spatial maps”. In: *Neuron* 109.19, pp. 3135–3148.

Chrobak, JJ and G Buzsáki (1994). “Selective activation of deep layer (V-VI) retrohippocampal cortical neurons during hippocampal sharp waves in the behaving rat”. In: *Journal of Neuroscience* 14.10, pp. 6160–6170.

Clark, Benjamin J et al. (2010). “Impaired head direction cell representation in the anterodorsal thalamus after lesions of the retrosplenial cortex”. In: *Journal of Neuroscience* 30.15, pp. 5289–5302.

Crijns, Els and Hans Op de Beeck (2019). “The visual acuity of rats in touchscreen setups”. In: *Vision* 4.1, p. 4.

Cullen, Kathleen E (2012). “The vestibular system: multimodal integration and encoding of self-motion for motor control”. In: *Trends in Neurosciences* 35.3, pp. 185–196.

Cuthbert, Peter C et al. (2000). “Electrophysiological evidence for vestibular activation of the guinea pig hippocampus”. In: *Neuroreport* 11.7, pp. 1443–1447.

Darwin, Charles (1873). “Origin of certain instincts”. In: *Nature* 7, pp. 417–418.

Davidson, Thomas J, Fabian Kloosterman, and Matthew A Wilson (2009). “Hippocampal replay of extended experience”. In: *Neuron* 63.4, pp. 497–507.

Day, Brian L and Richard C Fitzpatrick (2005). “The vestibular system”. In: *Current biology* 15.15, R583–R586.

De No, Lorente (1933). “Vestibulo-ocular reflex arc”. In: *Arch Neurol Psychiat* 30, pp. 245–291.

Deadwyler, Sam A et al. (1975). “Physiological studies of the reciprocal connections between the hippocampus and entorhinal cortex”. In: *Experimental Neurology* 49.1, pp. 35–57.

Del Grosso, Nicholas A, Justin J Graboski, et al. (2017). “Virtual Reality system for freely-moving rodents”. In: *BioRxiv*, p. 161232.

Del Grosso, Nicholas A and Anton Sirota (2019). “Ratcave: A 3D graphics python package for cognitive psychology experiments”. In: *Behavior Research Methods* 51.5, pp. 2085–2093.

Deshmukh, Sachin S (2021). “Distal CA1 maintains a more coherent spatial representation than proximal CA1 when local and global cues conflict”. In: *Journal of Neuroscience* 41.47, pp. 9767–9781.

Deshmukh, Sachin S and James J Knierim (2013). “Influence of local objects on hippocampal representations: Landmark vectors and memory”. In: *Hippocampus* 23.4, pp. 253–267.

Diamanti, E Mika et al. (2021). “Spatial modulation of visual responses arises in cortex with active navigation”. In: *Elife* 10.

Diba, Kamran and György Buzsáki (2007). “Forward and reverse hippocampal place-cell sequences during ripples”. In: *Nature Neuroscience* 10.10, pp. 1241–1242.

DiCarlo, James J et al. (1996). “Marking microelectrode penetrations with fluorescent dyes”. In: *Journal of Neuroscience Methods* 64.1, pp. 75–81.

Dipoppa, Mario et al. (2018). “Vision and locomotion shape the interactions between neuron types in mouse visual cortex”. In: *Neuron* 98.3, pp. 602–615.

Dossani, Rimal Hanif, Symeon Missios, and Anil Nanda (2015). "The legacy of Henry Molaison (1926–2008) and the impact of his bilateral mesial temporal lobe surgery on the study of human memory". In: *World Neurosurgery* 84.4, pp. 1127–1135.

Dragoi, George and György Buzsáki (2006). "Temporal encoding of place sequences by hippocampal cell assemblies". In: *Neuron* 50.1, pp. 145–157.

Eichenbaum, Howard (2014). "Time cells in the hippocampus: a new dimension for mapping memories". In: *Nature Reviews Neuroscience* 15.11, pp. 732–744.

Eichenbaum, Howard et al. (1999). "The hippocampus, memory, and place cells: is it spatial memory or a memory space?" In: *Neuron* 23.2, pp. 209–226.

Ekstrom, Arne D et al. (2003). "Cellular networks underlying human spatial navigation". In: *Nature* 425.6954, pp. 184–188.

Esmaeili, Mohammad-Hossein et al. (2012). "Role of D1/D2 dopamine receptors in the CA1 region of the rat hippocampus in the rewarding effects of morphine administered into the ventral tegmental area". In: *Behavioural Brain Research* 231.1, pp. 111–115.

Etienne, Ariane S et al. (1998). "Role of dead reckoning in navigation." In.

Fattahi, Mohammad et al. (2018). "Differential representation of landmark and self-motion information along the CA1 radial axis: self-motion generated place fields shift toward landmarks during septal inactivation". In: *Journal of Neuroscience* 38.30, pp. 6766–6778.

Fernandez-Leon, Jose A, Ahmet Kerim Uysal, and Daoyun Ji (2022). "Place cells dynamically refine grid cell activities to reduce error accumulation during path integration in a continuous attractor model". In: *Scientific Reports* 12.1, pp. 1–21.

Ferreiro, Dardo N et al. (2020). "Sensory island task (SIT): a new behavioral paradigm to study sensory perception and neural processing in freely moving animals". In: *Frontiers in behavioral neuroscience* 14, p. 576154.

Fetsch, Christopher R et al. (2012). "Neural correlates of reliability-based cue weighting during multisensory integration". In: *Nature Neuroscience* 15.1, pp. 146–154.

Fetterhoff, Dustin, Andrey Sobolev, and Christian Leibold (2021). "Graded remapping of hippocampal ensembles under sensory conflicts". In: *Cell Reports* 36.11, p. 109661.

Fogwe, Leslie A., Vamsi Reddy, and Fassil B. Mesfin (2021). *Neuroanatomy, Hippocampus*. StatPearls Publishing, Treasure Island (FL).

Fukawa, Ayako et al. (2020). "Identifying core regions for path integration on medial entorhinal cortex of hippocampal formation". In: *Brain Sciences* 10.1, p. 28.

Fyhn, Marianne, Torkel Hafting, et al. (2008). "Grid cells in mice". In: *Hippocampus* 18.12, pp. 1230–1238.

Fyhn, Marianne, Sturla Molden, et al. (2004). "Spatial representation in the entorhinal cortex". In: *Science* 305.5688, pp. 1258–1264.

Gardner, Richard J et al. (2022). "Toroidal topology of population activity in grid cells". In: *Nature* 602.7895, pp. 123–128.

Gauthier, Jeffrey L and David W Tank (2018). "A dedicated population for reward coding in the hippocampus". In: *Neuron* 99.1, pp. 179–193.

Geiller, Tristan et al. (2017). "Place cells are more strongly tied to landmarks in deep than in superficial CA1". In: *Nature Communications* 8.1, pp. 1–11.

Gil, Mariana et al. (2018). "Impaired path integration in mice with disrupted grid cell firing". In: *Nature Neuroscience* 21.1, pp. 81–91.

Golob, Edward J and Jeffrey S Taube (1999). "Head direction cells in rats with hippocampal or overlying neocortical lesions: evidence for impaired angular path integration". In: *Journal of Neuroscience* 19.16, pp. 7198–7211.

Gothard, Katalin M, William E Skaggs, and Bruce L McNaughton (1996). "Dynamics of mismatch correction in the hippocampal ensemble code for space: interaction between path integration and environmental cues". In: *Journal of Neuroscience* 16.24, pp. 8027–8040.

Gothard, Katalin M, William E Skaggs, Kevin M Moore, et al. (1996). "Binding of hippocampal CA1 neural activity to multiple reference frames in a landmark-based navigation task". In: *Journal of Neuroscience* 16.2, pp. 823–835.

Green, Laura et al. (2022). "Action-driven remapping of hippocampal neuronal populations in jumping rats". In: *Proceedings of the National Academy of Sciences* 119.26, e2122141119.

Gridchyn, Igor et al. (2020). "Assembly-specific disruption of hippocampal replay leads to selective memory deficit". In: *Neuron* 106.2, pp. 291–300.

Grieves, Roddy M, Selim Jedidi-Ayoub, et al. (2020). "The place-cell representation of volumetric space in rats". In: *Nature Communications* 11.1, pp. 1–13.

Grieves, Roddy M, Emma R Wood, and Paul A Dudchenko (2016). "Place cells on a maze encode routes rather than destinations". In: *Elife* 5, e15986.

Gu, Yong et al. (2006). "Visual and nonvisual contributions to three-dimensional heading selectivity in the medial superior temporal area". In: *Journal of Neuroscience* 26.1, pp. 73–85.

Hafting, Torkel et al. (2005). "Microstructure of a spatial map in the entorhinal cortex". In: *Nature* 436.7052, pp. 801–806.

Haggerty, Daniel Christopher and Daoyun Ji (2015). "Activities of visual cortical and hippocampal neurons co-fluctuate in freely moving rats during spatial behavior". In: *Elife* 4, e08902.

Han, Yuan et al. (2020). "Excitatory VTA to DH projections provide a valence signal to memory circuits". In: *Nature Communications* 11.1, pp. 1–14.

Harootonian, Sevan K, Arne D Ekstrom, and Robert C Wilson (2022). “Combination and competition between path integration and landmark navigation in the estimation of heading direction”. In: *PLoS Computational Biology* 18.2, e1009222.

Haun, Daniel BM et al. (2011). “Plasticity of human spatial cognition: Spatial language and cognition covary across cultures”. In: *Cognition* 119.1, pp. 70–80.

Hawkins, Jeff et al. (2019). “A framework for intelligence and cortical function based on grid cells in the neocortex”. In: *Frontiers in Neural Circuits*, p. 121.

Hebb, DO (1949). “The organization of behavior. A neuropsychological theory”. In.

Heinemans, Mirjam and Marta A Moita (2022). “Looming stimuli reliably drive innate, but not learned, defensive responses in rats.” In: *bioRxiv*.

Hetherington, Phil A and Matthew L Shapiro (1997). “Hippocampal place fields are altered by the removal of single visual cues in a distance-dependent manner.” In: *Behavioral Neuroscience* 111.1, p. 20.

Hills, Thomas T et al. (2015). “Exploration versus exploitation in space, mind, and society”. In: *Trends in Cognitive Sciences* 19.1, pp. 46–54.

Hitier, Martin et al. (2021). “Stratification of hippocampal electrophysiological activation evoked by selective electrical stimulation of different angular and linear acceleration sensors in the rat peripheral vestibular system”. In: *Hearing Research* 403, p. 108173.

Honkanen, Anna et al. (2019). “The insect central complex and the neural basis of navigational strategies”. In: *Journal of Experimental Biology* 222.Suppl_1, jeb188854.

Hopfield, John J (1984). “Neurons with graded response have collective computational properties like those of two-state neurons.” In: *Proceedings of the National Academy of Sciences* 81.10, pp. 3088–3092.

Horii, Arata et al. (2004). “Vestibular influences on CA1 neurons in the rat hippocampus: an electrophysiological study in vivo”. In: *Experimental Brain Research* 155.2, pp. 245–250.

Høydal, Øyvind Arne et al. (2019). “Object-vector coding in the medial entorhinal cortex”. In: *Nature* 568.7752, pp. 400–404.

Inayat, Samsoon et al. (2022). “Conjunctive and complementary CA1 hippocampal cell populations relate sensory events to immobility and locomotion”. In: *bioRxiv*.

Jacob, Pierre-Yves et al. (2017). “An independent, landmark-dominated head-direction signal in dysgranular retrosplenial cortex”. In: *Nature Neuroscience* 20.2, pp. 173–175.

Jander, Rudolf (1957). “Die optische Richtungsorientierung der roten Waldameise (Formica rufa L.)” In: *Zeitschrift für vergleichende Physiologie* 40.2, pp. 162–238.

— (1963). “Insect orientation”. In: *Annual Review of Entomology* 8.1, pp. 95–114.

Jercog, Pablo E et al. (2019). "Heading direction with respect to a reference point modulates place-cell activity". In: *Nature Communications* 10.1, pp. 1–8.

Johnson, Adam and A David Redish (2007). "Neural ensembles in CA3 transiently encode paths forward of the animal at a decision point". In: *Journal of Neuroscience* 27.45, pp. 12176–12189.

Joo, Hannah R and Loren M Frank (2018). "The hippocampal sharp wave–ripple in memory retrieval for immediate use and consolidation". In: *Nature Reviews Neuroscience* 19.12, pp. 744–757.

Jun, James J et al. (2017). "Fully integrated silicon probes for high-density recording of neural activity". In: *Nature* 551.7679, pp. 232–236.

Katz, Keziah and Dhruba Naug (2015). "Energetic state regulates the exploration–exploitation trade-off in honeybees". In: *Behavioral Ecology* 26.4, pp. 1045–1050.

Kennedy, Jack P et al. (2022). "A Direct Comparison of Theta Power and Frequency to Speed and Acceleration". In: *Journal of Neuroscience* 42.21, pp. 4326–4341.

Kentros, Clifford G et al. (2004). "Increased attention to spatial context increases both place field stability and spatial memory". In: *Neuron* 42.2, pp. 283–295.

Keshavarzi, Sepiedeh et al. (2022). "Multisensory coding of angular head velocity in the retrosplenial cortex". In: *Neuron* 110.3, pp. 532–543.

Knierim, James J, Hemant S Kudrimoti, and Bruce L McNaughton (1995). "Place cells, head direction cells, and the learning of landmark stability". In: *Journal of Neuroscience* 15.3, pp. 1648–1659.

— (1998). "Interactions between idiothetic cues and external landmarks in the control of place cells and head direction cells". In: *Journal of Neurophysiology* 80.1, pp. 425–446.

Knight, Rebecca et al. (2014). "Weighted cue integration in the rodent head direction system". In: *Philosophical Transactions of the Royal Society B: Biological Sciences* 369.1635, p. 20120512.

Komorowski, Robert W, Joseph R Manns, and Howard Eichenbaum (2009). "Robust conjunctive item–place coding by hippocampal neurons parallels learning what happens where". In: *Journal of Neuroscience* 29.31, pp. 9918–9929.

Korotkova, Tatiana et al. (2018). "Reconciling the different faces of hippocampal theta: The role of theta oscillations in cognitive, emotional and innate behaviors". In: *Neuroscience & Biobehavioral Reviews* 85, pp. 65–80.

Kosourikhina, Veronika et al. (2022). "Validation of DeepLabCut as a tool for markerless 3D pose estimation". In: *bioRxiv.*

Kraus, Benjamin J et al. (2013). "Hippocampal "time cells": time versus path integration". In: *Neuron* 78.6, pp. 1090–1101.

Kremmyda, Olympia et al. (2016). "Beyond dizziness: virtual navigation, spatial anxiety and hippocampal volume in bilateral vestibulopathy". In: *Frontiers in Human Neuroscience* 10, p. 139.

Kropff, Emilio, James E Carmichael, Edvard I Moser, et al. (2021). "Frequency of theta rhythm is controlled by acceleration, but not speed, in running rats". In: *Neuron* 109.6, pp. 1029–1039.

Kropff, Emilio, James E Carmichael, May-Britt Moser, et al. (2015). "Speed cells in the medial entorhinal cortex". In: *Nature* 523.7561, pp. 419–424.

Kulvicius, Tomas et al. (2008). "Odor supported place cell model and goal navigation in rodents". In: *Journal of Computational Neuroscience* 25, pp. 481–500.

Kumar, Ashok (2011). "Long-term potentiation at CA3–CA1 hippocampal synapses with special emphasis on aging, disease, and stress". In: *Frontiers in Aging Neuroscience* 3, p. 7.

Larriva-Sahd, Jorge A (2014). "Some predictions of Rafael Lorente de Nó 80 years later". In: *Frontiers in Neuroanatomy* 8, p. 147.

Laurens, Jean and Dora E Angelaki (2018). "The brain compass: a perspective on how self-motion updates the head direction cell attractor". In: *Neuron* 97.2, pp. 275–289.

Laurens, Jean and Jacques Droulez (2007). "Bayesian processing of vestibular information". In: *Biological Cybernetics* 96.4, pp. 389–404.

Lavenex, Pamela Banta and Pierre Lavenex (2021). "A Critical Review of Spatial Abilities in Down and Williams Syndromes: Not All Space Is Created Equal". In: *Frontiers in Psychiatry* 12.

Lebedev, Mikhail A, Alexey Pimashkin, and Alexei Ossadtchi (2018). "Navigation patterns and scent marking: underappreciated contributors to hippocampal and entorhinal spatial representations?" In: *Frontiers in Behavioral Neuroscience* 12, p. 98.

Lever, Colin, Stephen Burton, Ali Jeewajee, et al. (2009). "Boundary vector cells in the subiculum of the hippocampal formation". In: *Journal of Neuroscience* 29.31, pp. 9771–9777.

Lever, Colin, Stephen Burton, and J O'Keefe (2006). "Rearing on hind legs, environmental novelty, and the hippocampal formation". In: *Reviews in the Neurosciences* 17.1-2, pp. 111–134.

Levy, Eliott RJ et al. (2021). "A neuronal code for space in hippocampal coactivity dynamics independent of place fields". In: *bioRxiv*, pp. 2021–07.

Lindeman, HH (1969). "Studies on the morphology of the sensory regions of the vestibular apparatus with 45 figures". In: *Ergebnisse der Anatomie und Entwicklungsgeschichte* 42.1, pp. 1–113.

Liu, Annie et al. (2020). “Mouse navigation strategies for odor source localization”. In: *Frontiers in Neuroscience* 14, p. 218.

Long, Xiaoyang and Sheng-Jia Zhang (2021). “A novel somatosensory spatial navigation system outside the hippocampal formation”. In: *Cell Research* 31.6, pp. 649–663.

Low, Isabel IC and Lisa M Giocomo (2022). “Task engagement turns on spatial maps”. In: *Nature Neuroscience* 25.5, pp. 534–535.

Ludwig, Casimir JH et al. (2018). “The influence of visual flow and perceptual load on locomotion speed”. In: *Attention, Perception, & Psychophysics* 80.1, pp. 69–81.

Lyu, Cheng, LF Abbott, and Gaby Maimon (2022). “Building an allocentric traveling direction signal via vector computation”. In: *Nature* 601.7891, pp. 92–97.

Maaswinkel, Hans, Leonard E Jarrard, and Ian Q Whishaw (1999). “Hippocampectomized rats are impaired in homing by path integration”. In: *Hippocampus* 9.5, pp. 553–561.

Maaswinkel, Hans and Ian Q Whishaw (1999). “Homing with locale, taxon, and dead reckoning strategies by foraging rats: sensory hierarchy in spatial navigation”. In: *Behavioural Brain Research* 99.2, pp. 143–152.

MacDonald, Christopher J et al. (2011). “Hippocampal “time cells” bridge the gap in memory for discontiguous events”. In: *Neuron* 71.4, pp. 737–749.

Madhav, Manu S, Ravikrishnan P Jayakumar, Shahin G Lashkari, et al. (2022). “The Dome: A virtual reality apparatus for freely locomoting rodents”. In: *Journal of Neuroscience Methods* 368, p. 109336.

Madhav, Manu S, Ravikrishnan P Jayakumar, Brian Y Li, et al. (2022). “Closed-loop control and recalibration of place cells by optic flow”. In: *bioRxiv*, pp. 2022–06.

Majid, Asifa et al. (2004). “Can language restructure cognition? The case for space”. In: *Trends in Cognitive Sciences* 8.3, pp. 108–114.

Malkemper, E Pascal et al. (2015). “Magnetoreception in the wood mouse (Apodemus sylvaticus): influence of weak frequency-modulated radio frequency fields”. In: *Scientific Reports* 5.1, pp. 1–6.

Mankin, Emily A et al. (2012). “Neuronal code for extended time in the hippocampus”. In: *Proceedings of the National Academy of Sciences* 109.47, pp. 19462–19467.

Manns, Joseph R, Marc W Howard, and Howard Eichenbaum (2007). “Gradual changes in hippocampal activity support remembering the order of events”. In: *Neuron* 56.3, pp. 530–540.

Markus, Etan J, Carol A Barnes, et al. (1994). “Spatial information content and reliability of hippocampal CA1 neurons: effects of visual input”. In: *Hippocampus* 4.4, pp. 410–421.

Markus, Etan J, Yu-Lin Qin, et al. (1995). "Interactions between location and task affect the spatial and directional firing of hippocampal neurons". In: *Journal of Neuroscience* 15.11, pp. 7079–7094.

Mathis, Alexander et al. (2018). "DeepLabCut: markerless pose estimation of user-defined body parts with deep learning". In: *Nature Neuroscience* 21.9, pp. 1281–1289.

McNaughton, Bruce L, Carol A Barnes, and Jeff O'Keefe (1983). "The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats". In: *Experimental Brain Research* 52.1, pp. 41–49.

McNaughton, Bruce L, Francesco P Battaglia, et al. (2006). "Path integration and the neural basis of the'cognitive map'". In: *Nature Reviews Neuroscience* 7.8, pp. 663–678.

McNaughton, Bruce L, James J Knierim, and Matthew A Wilson (1995). "Vector encoding and the vestibular foundations of spatial cognition: neurophysiological and computational mechanisms." In.

Meer, Matthijs AA van der et al. (2010). "Evidence for the use of an internal sense of direction in homing." In: *Behavioral Neuroscience* 124.1, p. 164.

Mehta, MR, AK Lee, and MA Wilson (2002). "Role of experience and oscillations in transforming a rate code into a temporal code". In: *Nature* 417.6890, pp. 741–746.

Mittelstaedt, Horst (2000). "Triple-loop model of path control by head direction and place cells". In: *Biological Cybernetics* 83.3, pp. 261–270.

Mittelstaedt, Horst and M-L Mittelstaedt (1982). "Homing by path integration". In: *Avian Navigation*. Springer, pp. 290–297.

Mobbs, Dean et al. (2018). "Foraging for foundations in decision neuroscience: insights from ethology". In: *Nature Reviews Neuroscience* 19.7, pp. 419–427.

Moore, Jason J et al. (2021). "Linking hippocampal multiplexed tuning, Hebbian plasticity and navigation". In: *Nature* 599.7885, pp. 442–448.

Moser, Edvard I, May-Britt Moser, and Bruce L McNaughton (2017). "Spatial representation in the hippocampal formation: a history". In: *Nature Neuroscience* 20.11, pp. 1448–1464.

Moser, May-Britt, David C Rowland, and Edvard I Moser (2015). "Place cells, grid cells, and memory". In: *Cold Spring Harbor perspectives in biology* 7.2, a021808.

Muir, Gary M and Jeffrey S Taube (2002). "The neural correlates of navigation: do head direction and place cells guide spatial behavior?" In: *Behavioral and Cognitive Neuroscience Reviews* 1.4, pp. 297–317.

Muller, Robert U and John L Kubie (1989). "The firing of hippocampal place cells predicts the future position of freely moving rats". In: *Journal of Neuroscience* 9.12, pp. 4101–4110.

Müller, Christina and Stefan Remy (2018). "Septo–hippocampal interaction". In: *Cell and tissue research* 373.3, pp. 565–575.

Murphy, Joseph John (1873). "Instinct: A mechanical analogy". In: *Nature* 7.182, pp. 483–483.

Murray, Elisabeth A et al. (2019). *The Evolutionary road to human memory*. Oxford University Press, USA.

Nagelhus, Anne et al. (2022). "Object-centered population coding in CA1 of the hippocampus". In: *bioRxiv*.

Naghavi, Farzaneh Sadat et al. (2019). "The involvement of intra-hippocampal dopamine receptors in the conditioned place preference induced by orexin administration into the rat ventral tegmental area". In: *Iranian Journal of Pharmaceutical Research: IJPR* 18.1, p. 328.

Nölle, Jonas et al. (2020). "Language as shaped by the environment: linguistic construal in a collaborative spatial task". In: *Palgrave Communications* 6.1, pp. 1–10.

Nourizonoz, Ali et al. (2020). "EthoLoop: automated closed-loop neuroethology in naturalistic environments". In: *Nature Methods* 17.10, pp. 1052–1059.

O'Keefe, John and Jonathan Dostrovsky (1971). "The hippocampus as a spatial map: Preliminary evidence from unit activity in the freely-moving rat." In: *Brain research*.

O'Keefe, John and Michael L Recce (1993). "Phase relationship between hippocampal place units and the EEG theta rhythm". In: *Hippocampus* 3.3, pp. 317–330.

Olry, Régis and Duane E Haines (1998). "Cerebral mythology: a skull stuffed with gods". In: *Journal of the History of the Neurosciences* 7.1, pp. 82–83.

Ormond, Jake and John O'Keefe (2022). "Hippocampal place cells have goal-oriented vector fields during navigation". In: *Nature* 607.7920, pp. 741–746.

Page, Hector JI et al. (2014). "A theoretical account of cue averaging in the rodent head direction system". In: *Philosophical Transactions of the Royal Society B: Biological Sciences* 369.1635, p. 20130283.

Papale, Andrew E et al. (2016). "Interplay between hippocampal sharp-wave-ripple events and vicarious trial and error behaviors in decision making". In: *Neuron* 92.5, pp. 975–982.

Pastalkova, Eva et al. (2008). "Internally generated cell assembly sequences in the rat hippocampus". In: *Science* 321.5894, pp. 1322–1327.

Pettit, Noah L, Xintong C Yuan, and Christopher D Harvey (2022). "Hippocampal place codes are gated by behavioral engagement". In: *Nature Neuroscience* 25.5, pp. 561–566.

Peyrache, Adrien and Adrian J Duszkiewicz (2021). "A spatial map out of place". In: *Cell Research* 31.6, pp. 605–606.

Peyrache, Adrien, Natalie Schieferstein, and Gyorgy Buzsáki (2017). "Transformation of the head-direction signal into a spatial code". In: *Nature Communications* 8.1, pp. 1–9.

Pfeiffer, Brad E and David J Foster (2013). "Hippocampal place-cell sequences depict future paths to remembered goals". In: *Nature* 497.7447, pp. 74–79.

Poo, Cindy et al. (2022). "Spatial maps in piriform cortex during olfactory navigation". In: *Nature* 601.7894, pp. 595–599.

Poulter, Steven, Tom Hartley, and Colin Lever (2018). "The neurobiology of mammalian navigation". In: *Current Biology* 28.17, R1023–R1042.

Prusky, Glen T, Paul WR West, and Robert M Douglas (2000). "Behavioral assessment of visual acuity in mice and rats". In: *Vision Research* 40.16, pp. 2201–2209.

Purandare, Chinmay S et al. (2022). "Moving bar of light evokes vectorial spatial selectivity in the immobile rat hippocampus". In: *Nature* 602.7897, pp. 461–467.

Quirk, Gregory J, Robert U Muller, and John L Kubie (1990). "The firing of hippocampal place cells in the dark depends on the rat's recent experience". In: *Journal of Neuroscience* 10.6, pp. 2008–2017.

Ramanoël, Stephen et al. (2022). "Future trends in brain aging research: Visuo-cognitive functions at stake during mobility and spatial navigation". In: *Aging Brain* 2, p. 100034.

Ranck Jr, JB (1984). "Head direction cells in the deep layer of dorsal presubiculum in freely moving rats". In: *Society of Neuroscience Abstract*. Vol. 10, p. 599.

Recce, Michael and Kenneth D Harris (1996). "Memory for places: A navigational model in support of Marr's theory of hippocampal function". In: *Hippocampus* 6.6, pp. 735–748.

Redish, A David, Adam N Elga, and David S Touretzky (1996). "A coupled attractor model of the rodent head direction system". In: *Network: Computation in Neural Systems* 7.4, p. 671.

Reynolds, John H, Leonardo Chelazzi, et al. (2004). "Attentional modulation of visual processing". In: *Annual Review of Neuroscience* 27.1, pp. 611–647.

Rogers, Lynn L (1988). "Homing tendencies of large mammals: a review". In: *Translocation of wild animals. Milwaukee: Wisconsin Humane Society*, pp. 76–92.

Rossier, Jérôme et al. (2000). "The place preference task: a new tool for studying the relation between behavior and place cell activity in rats." In: *Behavioral Neuroscience* 114.2, p. 273.

Ruediger, Sarah et al. (2012). "Goal-oriented searching mediated by ventral hippocampus early in trial-and-error learning". In: *Nature Neuroscience* 15.11, pp. 1563–1571.

Russell, Noah A et al. (2003). "Long-term effects of permanent vestibular lesions on hippocampal spatial firing". In: *Journal of Neuroscience* 23.16, pp. 6490–6498.

— (2006). “Lesions of the vestibular system disrupt hippocampal theta rhythm in the rat”. In: *Journal of Neurophysiology* 96.1, pp. 4–14.

Saleem, Aman B et al. (2018). “Coherent encoding of subjective spatial position in visual cortex and hippocampus”. In: *Nature* 562.7725, pp. 124–127.

Salz, Daniel M et al. (2016). “Time cells in hippocampal area CA3”. In: *Journal of Neuroscience* 36.28, pp. 7476–7484.

Sanders, Honi et al. (2019). “Temporal coding and rate remapping: Representation of nonspatial information in the hippocampus”. In: *Hippocampus* 29.2, pp. 111–127.

Sarel, Ayelet, Arseny Finkelstein, et al. (2017). “Vectorial representation of spatial goals in the hippocampus of bats”. In: *Science* 355.6321, pp. 176–180.

Sarel, Ayelet, Shaked Palgi, et al. (2022). “Natural switches in behaviour rapidly modulate hippocampal coding”. In: *Nature*, pp. 1–9.

Sargolini, Francesca et al. (2006). “Conjunctive representation of position, direction, and velocity in entorhinal cortex”. In: *Science* 312.5774, pp. 758–762.

Savelli, Francesco and James J Knierim (2019). “Origin and role of path integration in the cognitive representations of the hippocampus: computational insights into open questions”. In: *Journal of Experimental Biology* 222.Suppl_1, jeb188912.

Schneider, David M, Anders Nelson, and Richard Mooney (2014). “A synaptic and circuit basis for corollary discharge in the auditory cortex”. In: *Nature* 513.7517, pp. 189–194.

Shamash, Philip et al. (2021). “Mice learn multi-step routes by memorizing subgoal locations”. In: *Nature Neuroscience* 24.9, pp. 1270–1279.

Sharif, Farnaz et al. (2021). “Subcircuits of deep and superficial CA1 place cells support efficient spatial coding across heterogeneous environments”. In: *Neuron* 109.2, pp. 363–376.

Sharp, Patricia E, Hugh T Blair, and Jeiwon Cho (2001). “The anatomical and computational basis of the rat head-direction cell signal”. In: *Trends in Neurosciences* 24.5, pp. 289–294.

Sheintuch, Liron et al. (2020). “Multiple maps of the same spatial context can stably coexist in the mouse hippocampus”. In: *Current Biology* 30.8, pp. 1467–1476.

Sherrington, Charles Scott (1906). *The integrative action of the nervous system*. Vol. 35. Yale University Press.

Shettleworth, Sara J and Jennifer E Sutton (2005). “Multiple systems for spatial learning: dead reckoning and beacon homing in rats.” In: *Journal of Experimental Psychology: Animal Behavior Processes* 31.2, p. 125.

Shrager, Yael, C Brock Kirwan, and Larry R Squire (2008). “Neural basis of the cognitive map: path integration does not require hippocampus or entorhinal cortex”. In: *Proceedings of the National Academy of Sciences* 105.33, pp. 12034–12038.

Singer, Annabelle C et al. (2013). "Hippocampal SWR activity predicts correct decisions during the initial learning of an alternation task". In: *Neuron* 77.6, pp. 1163–1173.

Sit, Kevin K and Michael J Goard (2022). "Coregistration of heading to visual cues in retrosplenial cortex". In: *bioRxiv*.

Skaggs, William et al. (1994). "A model of the neural basis of the rat's sense of direction". In: *Advances in Neural Information Processing Systems* 7.

Skaggs, William E et al. (1996). "Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences". In: *Hippocampus* 6.2, pp. 149–172.

Smith, Paul F (1997). "Vestibular–hippocampal interactions". In: *Hippocampus* 7.5, pp. 465–471.

— (2017). "The vestibular system and cognition". In: *Current Opinion in Neurology* 30.1, pp. 84–89.

Solstad, Trygve et al. (2008). "Representation of geometric borders in the entorhinal cortex". In: *Science* 322.5909, pp. 1865–1868.

Stackman, Robert W and Aaron M Herbert (2002). "Rats with lesions of the vestibular system require a visual landmark for spatial navigation". In: *Behavioural Brain Research* 128.1, pp. 27–40.

Stangl, Matthias et al. (2020). "Sources of path integration error in young and aging humans". In: *Nature Communications* 11.1, pp. 1–15.

Stavropoulos, Akis et al. (2022). "Influence of sensory modality and control dynamics on human path integration". In: *Elife* 11, e63405.

Straka, Hans and Robert Baker (2013). "Vestibular blueprint in early vertebrates". In: *Frontiers in Neural Circuits* 7, p. 182.

Strange, Bryan A et al. (2014). "Functional organization of the hippocampal longitudinal axis". In: *Nature Reviews Neuroscience* 15.10, pp. 655–669.

Taube, Jeffrey S, Robert U Muller, and James B Ranck (1990). "Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis". In: *Journal of Neuroscience* 10.2, pp. 420–435.

Terrazas, Alejandro et al. (2005). "Self-motion and the hippocampal spatial metric". In: *Journal of Neuroscience* 25.35, pp. 8085–8096.

Thurley, Kay and Aslı Ayaz (2017). "Virtual reality systems for rodents". In: *Current zoology* 63.1, pp. 109–119.

Tolman, Edward C (1948). "Cognitive maps in rats and men." In: *Psychological Review* 55.4, p. 189.

Tulving, Endel (1972). "Episodic and semantic memory." In.

— (1985). "How many memory systems are there?" In: *American Psychologist* 40.4, p. 385.

— (2002). "Episodic memory: From mind to brain". In: *Annual Review of Psychology* 53.1, pp. 1–25.

Umbach, Gray et al. (2020). "Time cells in the human hippocampus and entorhinal cortex support episodic memory". In: *Proceedings of the National Academy of Sciences* 117.45, pp. 28463–28474.

Vale, Ruben et al. (2020). "A cortico-collicular circuit for accurate orientation to shelter during escape". In: *bioRxiv*.

Viejo, Guillaume and Adrien Peyrache (2020). "Precise coupling of the thalamic head-direction system to hippocampal ripples". In: *Nature Communications* 11.1, pp. 1–14.

Vijayabaskaran, Sandhiya and Sen Cheng (2022). "Navigation task and action space drive the emergence of egocentric and allocentric spatial representations". In: *PLOS Computational Biology* 18.10, e1010320.

Villette, Vincent et al. (2015). "Internally recurring hippocampal sequences as a population template of spatiotemporal information". In: *Neuron* 88.2, pp. 357–366.

Volcic, Robert and Astrid ML Kappers (2008). "Allocentric and egocentric reference frames in the processing of three-dimensional haptic space". In: *Experimental Brain Research* 188.2, pp. 199–213.

Wallace, Douglas G et al. (2002). "Vestibular information is required for dead reckoning in the rat". In: *Journal of Neuroscience* 22.22, pp. 10009–10017.

Wehner, Rüdiger, Mandyam V Srinivasan, et al. (2003). "Path integration in insects". In: *The Neurobiology of Spatial Behaviour*, pp. 9–30.

Wilber, Aaron A et al. (2014). "Interaction of egocentric and world-centered reference frames in the rat posterior parietal cortex". In: *Journal of Neuroscience* 34.16, pp. 5431–5446.

Winter, Shawn S, Benjamin J Clark, and Jeffrey S Taube (2015). "Disruption of the head direction cell network impairs the parahippocampal grid cell signal". In: *Science* 347.6224, pp. 870–874.

Witter, Menno P (2010). "Connectivity of the hippocampus". In: *Hippocampal Microcircuits*. Springer, pp. 5–26.

Witter, Menno P, Heidi Kleven, and Asgeir Kobro Flatmoen (2017). "Comparative contemplations on the hippocampus". In: *Brain, Behavior and Evolution* 90.1, pp. 15–24.

Wolfer, David P and Hans-Peter Lipp (2000). "Dissecting the behaviour of transgenic mice: is it the mutation, the genetic background, or the environment?" In: *Experimental Physiology* 85.6, pp. 627–634.

Wood, Emma R, Paul A Dudchenko, and Howard Eichenbaum (1999). “The global record of memory in hippocampal neuronal activity”. In: *Nature* 397.6720, pp. 613–616.

Wood, Emma R, Paul A Dudchenko, R Jonathan Robitsek, et al. (2000). “Hippocampal neurons encode information about different types of memory episodes occurring in the same location”. In: *Neuron* 27.3, pp. 623–633.

Xu, Haibing et al. (2019). “Assembly responses of hippocampal CA1 place cells predict learned behavior in goal-directed spatial tasks on the radial eight-arm maze”. In: *Neuron* 101.1, pp. 119–132.

Yoder, Ryan M, Benjamin J Clark, et al. (2011). “Both visual and idiothetic cues contribute to head direction cell stability during navigation along complex routes”. In: *Journal of Neurophysiology* 105.6, pp. 2989–3001.

Yoder, Ryan M, James R Peck, and Jeffrey S Taube (2015). “Visual landmark information gains control of the head direction signal at the lateral mammillary nuclei”. In: *Journal of Neuroscience* 35.4, pp. 1354–1367.

Yoder, Ryan M and Jeffrey S Taube (2009). “Head direction cell activity in mice: robust directional signal depends on intact otolith organs”. In: *Journal of Neuroscience* 29.4, pp. 1061–1076.

Zanchi, Silvia et al. (2022). “Interindividual differences influence multisensory processing during spatial navigation.” In: *Journal of Experimental Psychology: Human Perception and Performance* 48.2, p. 174.

Zhang, Kechen (1996). “Representation of spatial orientation by the intrinsic dynamics of the head-direction cell ensemble: a theory”. In: *Journal of Neuroscience* 16.6, pp. 2112–2126.

Zhang, Ningyu, Roddy M Grieves, and Kate J Jeffery (2022). “Environment symmetry drives a multidirectional code in rat retrosplenial cortex”. In: *Journal of Neuroscience* 42.49, pp. 9227–9241.

Zheng, Yiwen et al. (2009). “Long-term deficits on a foraging task after bilateral vestibular deafferentation in rats”. In: *Hippocampus* 19.5, pp. 480–486.

Zhu, Seren et al. (2022). “Eye movements reveal spatiotemporal dynamics of visually-informed planning in navigation”. In: *Elife* 11, e73097.

Ziv, Yaniv et al. (2013). “Long-term dynamics of CA1 hippocampal place codes”. In: *Nature Neuroscience* 16.3, pp. 264–266.

Zutshi, Ipshita et al. (2022). “Extrinsic control and intrinsic computation in the hippocampal CA1 circuit”. In: *Neuron* 110.4, pp. 658–673.

LIST OF PUBLICATIONS

Crego, A. C., **Štoček, F.**, Marchuk, A. G., Carmichael, J. E., van der Meer, M. A., Smith, K. S. (2020). Complementary control over habits and behavioral vigor by phasic activity in the dorsolateral striatum. Journal of Neuroscience, 40(10), 2139-2153.

AFFIDAVIT

Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation **"Disentangling multi sensory contribution to navigation using a novel freely moving virtual reality-enabled path integration task."** selbstständig angefertigt habe, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

I hereby confirm that this dissertation **"Disentangling multi sensory contribution to navigation using a novel freely moving virtual reality-enabled path integration task."** is the result of my own work and that I have only used sources or materials listed and specified in the dissertation.

Munich, March 1, 2023

Fabián Štoček

AUTHOR CONTRIBUTIONS

Fabián Štoček, Dr. Gerrit Schwesig, Jin Hwa Lee, Danai Eleni Vantaraki, Zagorka Bekjarova, Arvind Shankar, Prof. Dr. Hans Straka and Prof. Dr. Anton Sirota contributed to this research study.

The project was established by Anton Sirota and Hans Straka. The design of the experimental protocols and the virtual environment was done by Fabián Štoček with the support of Anton Sirota. Animal handling was performed by Fabián Štoček with help from Zagorka Bekjarova. 3D design, printing and assembling implants as well as the surgical procedures were done by Fabián Štoček. Spike sorting was done by Fabián Štoček, Justin Graboski, Danai Eleni Vantaraki and Arvind Shankar. Data analysis was done by Fabián Štoček with contributions from Jin Hwa Lee under review of Anton Sirota.

We assert that aforementioned author contributions are correct and accurate:

Munich, March 1, 2023

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