A Dual Investigation of a Modified Sharp Wave Ripple Detector and the Effects of Hippocampal Growth Hormone on Sharp Wave Ripple Properties

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1 ABSTRACT

Hippocampus is established as an essential brain region for cognitive processes across species. Studies on hippocampus has generated vast amount of information, including discoveries of fundamental neurobiological mechanisms of neural plasticity and higher cognitive functions. One such key mechanism originating from hippocampal research is the discovery of the sharp wave ripple. The sharp wave ripple is proposed to be a cognitive bio-marker for consolidation of memory and planning, and represents a defining property of the hippocampus. While the sharp wave ripple has established its position as an integral part of the capacity for memory consolidation, there are still a lot to discover. Little known about how the locally expressed neuromodulatory growth hormone, affects functional neural processes in the hippocampus. The current thesis thus had a dual purpose; first, it attempted at solving relevant challenges concerning detection of sharp wave ripples, before the possible effects of growth hormone on properties of sharp wave ripples associated with memory were investigated. The detector was deemed acceptable, however, there are issues with the involved method of the implementation of the specific detector type used in the current project. After setting up the detector, it was applied to local field potential recordings from CA1 in rats, and the detected sharp wave ripples was used in a subsequent comparison between rats with different amount of hippocampal growth hormone, manipulated by viral infections. Unfortunately, there was not found any differences between the growth hormone manipulated groups.

Hippocampus, Sharp wave ripple, local field potential, growth hormone, episodic memory, memory consolidation

2 SAMMENDRAG

Hippocampus er etablert som et viktig hjerneområde for kognitive mekanismer, En spesifikk mekanisme som utspringer fra hippocampal aktivitet, sterkt relatert til hukommelse, er sharp wave ripple. Sharp wave ripple er en foreslått kognitiv biomarkør for minnekonsolidering og planlegging. Selv om sharp wave ripple har befestet seg som en viktig aktør i hukommelsesprosesser, er det fortsatt mye som gjenstår å oppdage når det gjelder de underliggende faktorene for generering påvirkning av den nevrale aktiviteten. En relevant, men lite studert, aktør i forbindelse med disse underliggende faktorene for sharp wave ripple, er vekst hormon. Veksthormon er indikert å påvirke plastiske egenskaper ved nevroner og kan derfor antas å påvirke den nevrale aktiviteten man ser under en sharp wave ripple. Men vi møter på utfordringer når det gjelder deteksjon av sharp wave ripples, i tillegg til at det ikke er gjort mye forskning på veksthormonets virkninger i nervesystemet. Denne oppgaven har derfor et todelt mål, først forsøker vi nødt til å løse utfordringer knyttet til deteksjon av sharp wave ripple, deretter, er hensikten å kunne utforske mulige effekter av veksthormon på observerte egenskaper ved de detekterte sharp wave ripples-hendelsene. Etter å ha evaluert og akseptert detektoren, riktignok ikke uten problemer, ble den benyttet på local field potential-opptak fra CA1 hippocampus der det ble detektert sharp wave ripple. De detekterte hendelsene ble brukt i en påfølgende sammenlikning mellom grupper av rotter med manipulert mengde veksthormon i hippocampus. Det ble dessverre ikke funnet signifikante forskjeller mellom veksthormongruppene.

Hippocampus, sharp wave ripple, local field potential, veksthormon, episodisk hukommelse, minnekonsolidering

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4 INNHOLD

In this introduction I will provide the theoretical foundation for the conduction of the current project based on scientific findings and theoretical models. I hope to provide the reader with an understanding of the phenomena and concepts of neural processes underlying memory and hormonal influence on these processes, which constitute the emphasis of this thesis, while at the same time create a rationale for conducting the current study and how it may be of importance to gain a better understanding of age-related hormonal influence on the cognitive process of memory. The purpose of this study is two-fold. The overarching goal is to investigate whether the observed effects of growth hormone on cognitive processes may be attributed to alterations of the properties of hippocampal sharp wave ripples related to memory processes. However, as detection of sharp wave ripple events involves several challenges necessary to overcome, involving varying event amplitude and present noise and artifacts, a specific focus of the current project was to optimize the detection of sharp wave ripple event by modifying a pre-existing and widely used sharp wave ripple detector.

4.1 PROJECT OVERVIEW

Hormonal changes related to both normal ageing and disorders are thought to influence cognitive processes (Nyberg & Hallberg, 2013). A hormone of particular interest is growth hormone (GH). Although not as widely studied in terms of its involvement in cognitive processes as it has in general tissue functions, this hormone is associated with cognitive performance, in particular memory functions (Nieuwpoort & Drent, 2008). Emerging indications of GH-involvement in key neurobiological processes underlying memory abilities, coupled with the observed behavioural effects of patients with GH deficiency and age-related GH-reduction, triggers the wish to closer examine the possible involvement it may have in neural activity patterns associated with specific memory processes (Carroll et al., 2000; Falleti et al., 2006; Nyberg & Hallberg, 2013).

One such memory related process that GH may modulate is the sharp wave ripple (SWR), a proposed mechanism for consolidation of hippocampal dependent memories. SWRs are distinct activities seen in the local field potential (LFP) in the hippocampus, reflecting large synchronous activity of hippocampal neurons. As will be further described in the subsequent sections, hippocampus is thought to encode a specific type of memory, and SWRs is the proposed measurable neural activity responsible for the long-term storage of these memories, also known as memory consolidation (Buzsáki, 1989, 2015; Joo & Frank, 2018).

The amount of GH is naturally decreasing with increased age, as does the memory abilities (Hersch & Merriam, 2008; Herzog & Rodgers, 1989). In addition, age is also associated with changes in SWR properties (Cowen et al., 2020; Wiegand et al., 2016). The involvement both GH and SWR exhibits in cognitive processes and memory performance encourage the current investigation of whether manipulation of GH may show potential effects on properties of SWR related to memory performance. I will start this thesis by describing how and why the hippocampus has emerged as a relevant brain structure in the study of memory in both humans and animals. To understand how the hippocampus accomplishes its mnemonic functions, it necessary to turn our attention to its architecture and the properties of the principal excitatory neurons constituting the hippocampus before I describe the underlying biological processes proposed to underly long-term storage of memories. After that, I will give a description about the dynamics of the LFP, with emphasis on the SWR, describing how this activity is thought to reflect consolidation of memories and related challenges in detection of these specific neural events. Finally, I will provide a brief description of GH, its proposed involvement in cognitive processes, and why manipulation of GH may affect properties of the SWRs.

4.2 HIPPOCAMPUS AS THE REGION OF STUDY

The ability to remember has fascinated both scientists and laypeople alike (Staniloiu & Markowitsch, 2017). It is such an obvious property of the human mind, enriching our lives and essential to adaptive behaviour in both humans and animals, however, when attempting to grasp what *memory* actually is, it appears elusive. Therefore, it was of great importance when the striking effects of lesions to areas in the medial temporal lobe were observed, creating a region of interest for memory research (Scoville & Milner, 1957). Lesions to the hippocampus in the medial temporal lobe in humans lead to impairments of memories of experiences, establishing both a brain region of interest in the study of memory and the separation of different types of memory (Ferguson et al., 2019; Squire, 2009). Subsequent examinations contributed to creating a taxonomy of different memory systems which differentiated between different types of memories (Tulving, 2002). The novel nomenclature of memory helped operationalise the phenomena, enabling scientific research of memory, with focus on the brain region of hippocampus

4.2.1 Development of a memory taxonomy

A substantial reason for studying the hippocampus within the branch of behavioural neuroscience is its association with mnemonic processes. This originates from the findings from studies assessing hippocampal lesions or pathological conditions, causally linking the region to memory abilities, and perhaps the most wide known are the case studies of the patient H.M. (later known to be named Henry Molaison) (Scoville & Milner, 1957). Molaison had areas of his bilateral medial temporal lobe removed as a surgical operation to treat his epilepsy. Later MRI studies inspecting the lesions found the lesioned areas to include most of amygdala and half of the hippocampal formation which includes the entorhinal cortex, dentate gyrus, hippocampus proper and subiculum complex (Corkin et al., 1997). This had a pronounced impact on his mnemonic abilities, particularly his abilities to form new memories for experienced events after the lesion, although preserving other aspects of his cognitive- and perceptual abilities (Squire, 2009). For example, he was able to hold a normal conversation and remember shorter strings of numbers for brief periods of time. However, after a couple of minutes, or when his attention was brought to other matters, this briefly held information of the conversational topic or string of numbers was lost. Another notable discovery from studying HM was the apparent types of memories not affected by the lesion, his ability to remember learned movements for instance. He was able to improve his performance on a movement task, however he could not remember he had completed the actual training sessions. Therefore, this led to the distinction between a memory system responsible for experiences, *episodic memory*, and another type of memory responsible for movement, called *procedural* memory.

In another study that builds further upon the notion of separate memory types, Vargha-Khadem et al. (1997) followed three individuals with bilateral hippocampal pathology. The individuals exhibited an impaired ability to remember experiences, however they all showed almost normal levels of intelligence and were able to progress through the education system. This has contributed to the organisation of the human memory system by suggesting a *semantic* form of memory. Semantic memory together with episodic memory makes up the declarative, or explicit, form of memory. Also, findings from the study of Varga-Khadem et al. (1997) suggests that this semantic memory are somewhat independent of hippocampus, while the earlier studies mentioned in above paragraphs suggests that episodic memory, in contrast, are dependent on hippocampus. The notion of a hippocampal dependent memory system responsible for episodic memories, which logically involves a spatial component, are interesting when later studies found the prominent neural response of the cells constituting the

hippocampus to represent spatial information, in addition to being sensitive to sensory and contextual information, tying together the information in the environment with a spatial component (Colgin et al., 2008; Kentros, 2006; O'Keefe & Dostrovsky, 1971). This will be described in more detail in later sections.

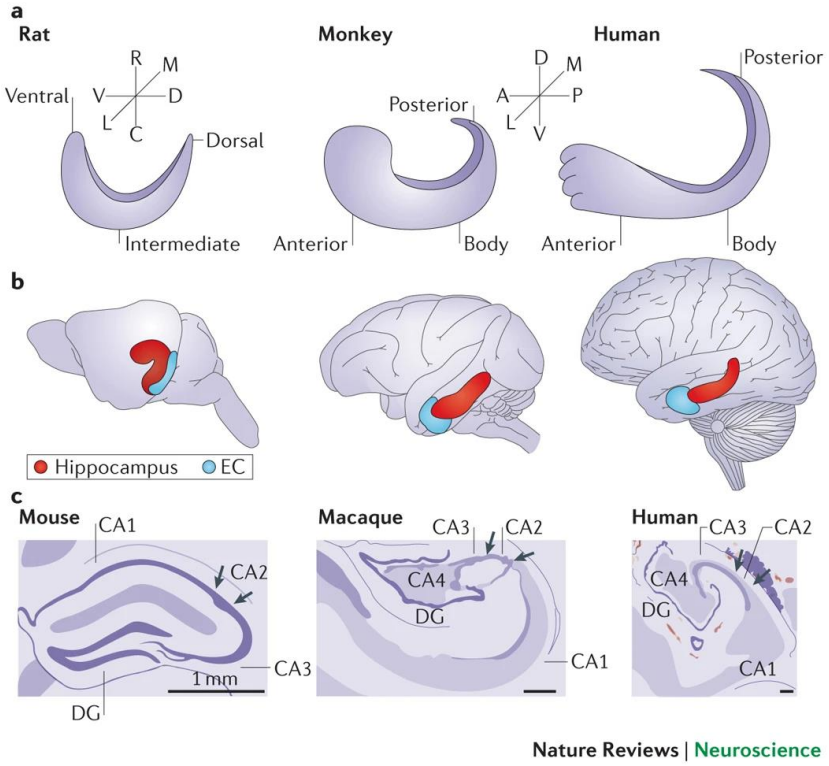
The studies and conclusions mentioned above mostly involve humans and focus of memory concepts in humans inferred from the observed effects of lesions. Interestingly, similar effects of impaired memory following hippocampal lesions are also found in animals (Squire, 1992; Steckler et al., 1998). In primates, bilateral hippocampal formation lesion is shown to impair performance on object recognition task (Zola et al., 2000). In rats, hippocampal lesions also lead to impairment in several memory tasks (Sutherland & McDonald, 1990). This seemingly implication for a memory function of the hippocampus preserved across species enables the use of animal models in memory research. The results from which can be carefully applied to indicate aspects of memory processes in humans. Importantly, the architecture of the hippocampal formation is relatively preserved across species as well, and further establishes the possibility of using animal models in memory research (Andersen et al., 2006; Squire, 1992).

4.2.2 Historical background of hippocampus as a contributor in neuroscience

The name hippocampus was introduced around 500 years ago, owing to the entire structure's physical resemblance of the sea horse, when assessed in dissections (Andersen et al., 2006). Subsequent studies of this brain structure have helped establish an understanding of the detailed organisation of different subregions and the intrinsic connectivity between these regions, in addition to widespread connections with other brain areas (Maller et al., 2019; Strange et al., 2014; Witter, 2010). These hippocampal subregions are named after the cell organisation's resemblance of a ram's horn, thus termed Cornu Ammonis (latin for ram's horn, abbreviated CA), and is divided into three subfields, *CA1*, *CA2* and *CA3*. Together, they make the *hippocampus proper* (figure 1). The hippocampus proper is part of the greater network in the medial temporal lobe, termed *the hippocampal formation*, which also includes the adjacent structures, *entorhinal cortex*, *pre-* and *parasubiculum*, *subiculum* and *dentate gyrus*.

Figure 1.

Overview of Hippocampus



Note. Visualisation of hippocampus across three different species (from left to right: Mouse, Macaque and Human) assessed at three levels (a-c). a) Depiction of form of the isolated hippocampus and its orientation according to the anatomical axes. b) Hippocampus proper and entorhinal cortex position in the brain. C) Coronal section of the different regions of hippocampus proper. (Strange et al., 2014)

These interconnected structures, and the hippocampus proper in particular, has been of great importance for neuroscientific research. A large amount of knowledge has emerged from studies of these specific regions in the temporal lobe (Lømo, 1971; Moser et al., 2015; O'Keefe & Dostrovsky, 1971; Vanderwolf, 1969). Important findings include the fundamental neurobiological principle of activity dependent synaptic plasticity, an important general principle applicable in the entire nervous system (Lømo, 1971). And, with electrophysiological recordings of living animals allows for a deeper understanding of the processes underlying higher cognitive functions seen in the studies of place cells and specific oscillatory activity pattern like the sharp-wave ripple and theta oscillation (O'Keefe & Dostrovsky, 1971; Vanderwolf, 1969). In the 1950's, one of the most profound findings in

research on memory appeared in the case of the epilepsy patient H.M., which following a bilateral hippocampi lesion showed impaired mnemonic abilities, especially the ability to form new memories of experiences was impaired (Scoville & Milner, 1957). The combination of the development of enhanced methods to study the activity of single neurons and neuron populations, both *in vivo* and *in vitro* slice preparations, and development of behavioural study paradigms, in addition to the clear association with cognitive functions, has led to hippocampus being one of the most studied brain structures, having a profound impact for neuroscientific research in general, and for the cognitive function of memory particularly (Andersen et al., 2006).

4.2.3 Architecture

Without knowing the constituents of a system, it is impossible to understand how it may carry out its functional properties. Therefore, to be able to draw conclusions of the possible functions of a brain region, it is necessary to establish an understanding of the parts constituting it and how the constituents are connected within the structure as well as to other parts of the brain. There are still things we do not know about the hippocampus in terms of its components and connectivity, but there is certainly a lot we do know. As hippocampus is one of the brain structures that has one of the more prominent functions and is relatively accessible for studying, either in slice preparations in vitro or in vivo electrical recordings of exploring animals, there is a lot of studies covering vast amounts of aspects of the hippocampus (see Andersen et al., 2006). One of the things that made hippocampus popular to use in neurobiological studies is its organised, single layering of neurons and the prominent unidirectional intrinsic connections between its subregions and the connections with adjacent regions.

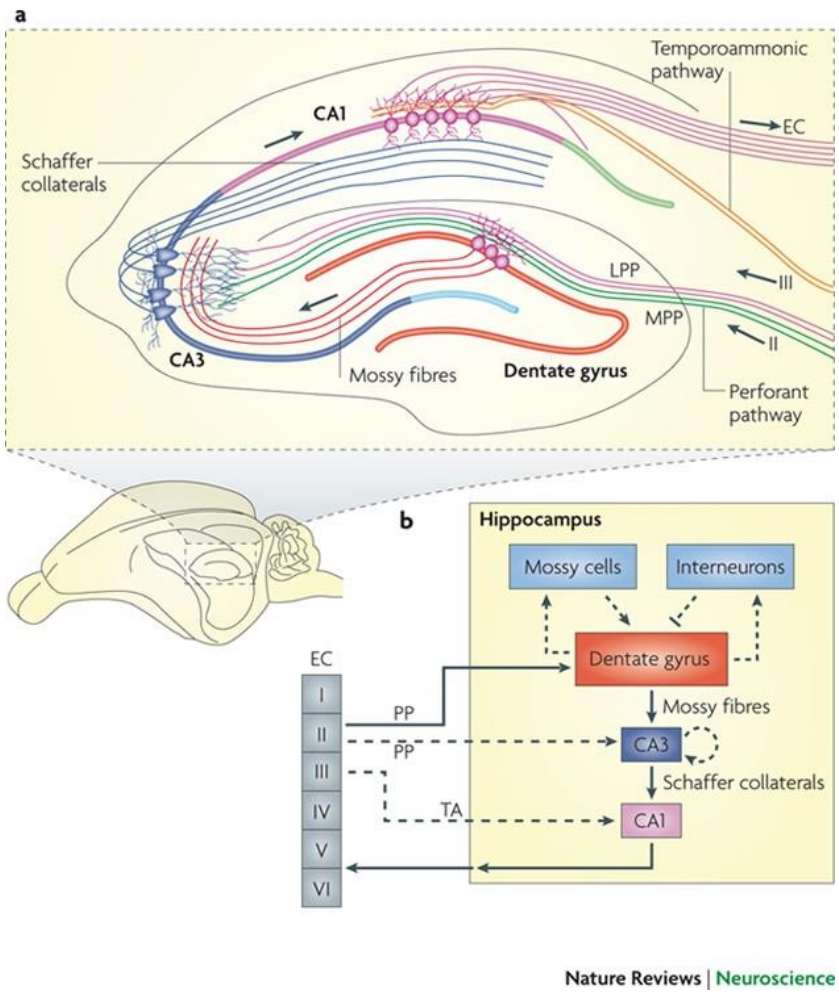
Physical proximity, in the form of synaptic connections, are preliminary for neurons to be able to transmit signal between them and communicate with each other. Early staining techniques found the hippocampus exhibiting a relatively simple organisation in terms of distinct cellular layering and intrinsic connectivity (Andersen et al., 1971, 2006). The hippocampus proper is divided in relatively distinct subregions, each with distinct properties and connectivity. The subregions constituting the hippocampus is cornu ammonis (CA) 1, 2 and 3. Together with the dentate gyrus (DG) and entorhinal cortex (EC), a simplified circuit is often used to describe the flow of information carried by the excitatory neurons. This was named the tri-synaptic circuit, after the number of synapses included, and is mainly

unidirectional (figure 2). The start of this circuit begins with the neurons in the EC which sends its axons to the DG, in the *perforant path*. Then, the information propagates further to the CA3, in the *mossy fibres*. And from the CA3, the information travels through the *Schaffer collaterals* to CA1. From CA1, the information are diverging to arrive at both, subiculum, which innervates large portions of subcortical structures, and back to the EC from which the information may be distributed to further higher neocortical areas (Andersen et al., 2006).

It is necessary to understand the proponents of the local circuits of CA3 and CA1 when assessing the LFP signal measured from this region, because this organisation reveals the foundation for the essential properties of sharp wave-ripples and contributes to evaluate its potential function. As the Figure 2 depicts, CA3 receives afferents from layer 2 EC neurons and from the Mossy fibres of DG. Importantly, CA3 is reciprocally organised, where a large portion of the neurons innervates other neurons in the same region, creating a recurrent system (Andersen et al., 2006; Hopfield, 1982). This recurrent system is proposed to carry out intriguing functions for memory, i.e., the ability of auto-association and pattern completion, reflecting an ability to initiate a reinstatement of an earlier activity pattern from only partial activation. This may be the neural representation of how some sensory stimulation may trigger a memory recall of a precious experience, for example, a certain smell may trigger memories of events related to that specific smell. Each subregion is also divided in different layers. The principal neurons of the hippocampal subregions have similar orientation (Fig x A). For CA1, the basal dendrites are in the superficial layer of stratum oriens, and apical dendrites in stratum radiatum, while the cell bodies are found in the stratum pyramidale (Andersen et al., 2006). Excitatory synaptic input to CA1 from the CA3 arrives at both stratum radiatum and stratum oriens. In addition, there are also observed a variety of interneurons with both local and widespread connections (Freund & Buzsáki, 1996; Sik et al., 1995). In contrast to the excitatory connections, interneurons have inhibiting effects, through release of GABA binding to GABA receptors in the excitatory principal neurons, the membrane potential of the principal cells are hyperpolarised. This continuous competition between excitatory drive and interneuron inhibition are seen as oscillations in the field potentials when measuring neural activity, and these oscillations are thought to contribute to coherent communication between regions and are therefore important for cognitive functions (Buzsáki, 2006). This mutual effect of inhibition and excitation are what contributes to the neural pattern of sharp wave ripples.

Figure 2.

Connectivity of Hippocampus



Note. a) Visual overview of the connectivity of the hippocampus proper. b) Tri-synaptic connections indicated with solid lines. Additional connections shown with dashed lines. (Deng et al., 2010)

It is also important to understand what different types of information are arriving at the hippocampus. By examining the regions which innervates the region, it is indicated that areas from several neocortical areas innervates the hippocampus via the EC (Canto et al., 2008). Interestingly, it seems that the information is topographically organised and the amount of already processed information are different across species, with larger part of hippocampus in humans are receiving more higher order processed information, compared to that of rodents where most of the hippocampus are receiving less processed sensory motor information (Buzsáki & Tingley, 2018).

However, what pioneered the understanding of hippocampal function came after assessing the single units constituting the circuit, and was the finding of the *place cells* (O'Keefe & Dostrovsky, 1971). This created a clear neural correlate of behaviour or experience and generated the opportunity to study the function of hippocampus at a neuronal level.

4.2.4 Place cells

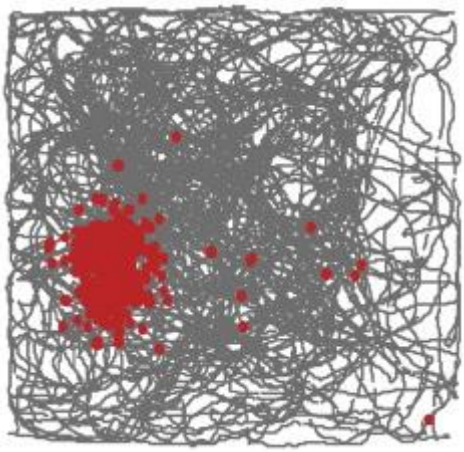
A profound discovery, which has largely shaped the neuroscientific study of memory, is the finding of the place cell (O'Keefe & Dostrovsky, 1971). In the 60's and 70's, methodological advances improved the neuroscientific research in terms of novel ways to study the brain using among others in vivo electrophysiological recordings and use of circuit stimulation in slice preparations (Andersen et al., 2006). As Buzsaki (2020) has written, a lot of neuroscientific research are perturbation-based. Not just by lesioning, but also by manipulating certain stimuli parameters while measuring the activity of single neurons, and from the corresponding change of activity of these neurons, inferring plausible functions of the neurons. For example, manipulation of light stimuli on the retina while recording the neural activity of cells in the primary visual cortex has contributed to the great understanding, we now have of how visual information are processed (Wurtz, 2015). The stimuli that affect neural activity is relatively easy to manipulate concerning neurons in the primary sensory cortices, however, the further away from these primary sensory neurons we get, the more difficult it is to manipulate and establish a functional correlation between the stimuli and the neural response. Hippocampus, which is not a primary sensory area, is therefore difficult to study in terms of determining what the function of its neural activity is (Andersen et al., 2006). Therefore, an important breakthrough was made with the discovery of hippocampal place cells (O'Keefe & Dostrovsky, 1971).

Early electrophysiological studies of the hippocampus found a clear correlate between the animal's location in the environment and increased firing of the principal cells of hippocampus, thereby getting the name, place cells (O'Keefe & Dostrovsky, 1971). Such place cells was discovered in rodents, but similar spatial sensitive neurons have later also been observed in other species, including humans (Ekstrom et al., 2003, 2005). O'Keefe and Dostrovsky (1971) noticed that the place cell in a rat exploring a room is mostly inactive unless the animal approaches the place field of that particular place cell. The place field being any location in the environment, and when the animal arrived within that place field, the cell increased the electrical activity manifolds. Later studies shows that the place fields may both

gradually emerge after exploration, or being manifested upon a single exposure (Frank et al., 2004).

Figure 3.

Visualisation of Place Representation of Hippocampal Place Cell



Note. This figure shows the trajectory of the animal in grey line and the location of the rat's head when place cell spikes were detected. A clearly visible place field is depicted the lower left part of the environment. Edited figure from (Derdikman & Moser, 2010).

The place cells were later found to represent not only spatial information, but also incorporate non-spatial information, including sensory information, contextual factors and task demands (Wood et al., 1999). Other representational content of the environment where also found to be represented by different cells in entorhinal cortex, for instance head-direction cells which was sensitive for the animal's orientation in the environment and border cells sensitive for borders in the environment (Moser & Moser, 2011).

Together, these cells encoding spatial information forms ensembles of many cells representing an environment, and the hippocampus was therefore considered responsible for creating and storing a cognitive map of the environment, a representation of space in the mind, according to the cognitive map theory (Eichenbaum, 2017). According to the theory first formulated by Tolman (1948), the environment is proposed to be fully represented by a large configuration of hippocampal place cells, and that this representation of the environment allows for intelligent and creative behaviour. By having a cognitive map of an environment, one may be able to construct a representational model and act upon creative solutions even though they have not been experienced before. For instance, the ability to see and take a short-cut even though one has never actually been through that route. Thereby deviating from

the strict behaviouristic thinking prevalent at the time, reducing most behaviour to originate from learned stimuli-response connections.

An important property of place cells for the proposed cognitive map they produce, is their ability to show a stable configuration for the same environment. Single-unit recordings from dorsal hippocampus in freely moving rats showed significant stability of place fields for periods of up to 153 days (Thompson & Best, 1990). In addition to exhibiting a stable representation of space, it is necessary for the place cells to be able to separate different environments and be part of several cognitive maps. This may be solved by creating orthogonal maps. The following section will explain how the property of remapping may reflect this ability to distinguish between different environments.

4.2.4.1 Remapping

An important property of the hippocampal place cells is their observed ability to change their activity following changes in the environment (Colgin et al., 2008; Kentros, 2006; Muller & Kubie, 1987). This reflects that the place cells are sensitive to sensory information and other external contextual cues and allows for the possibility of the same place cells to be involved in different maps of different environments.

Remapping of place cells encompasses the change of activity in response to changes in sensory information, task demands or motivational states (Colgin et al., 2008). This change of activity manifests in different ways. Place fields can appear in areas of the environment, change their position, or disappear. In addition, the firing rate of the place cell is also able to be altered. Kubie and Muller (1987) set out to test how isolated changes in the environment altered the place cell activity patterns. Manipulating several features of the environment, one at a time, they discovered that different types of remapping occurred in response to the different manipulations. More thoroughly differentiated and clearly categorised remapping is presented in newer papers. For instance, Leutgeb et al. (2005) recorded hippocampal neurons in rats while the box enclosure was changed, but remained in the same location, and another condition were the enclosure was the same but in different locations. When the enclosure was changed, and the location stayed the same, a type of remapping where the firing rates changes but the place fields remain in the same position, occurred. However, in the other condition, both place fields and firing rate was changed. These two types of remapping is called rate – and global remapping, respectively (Colgin et al., 2008).

This ability to represent different types of information by altering its activity, as well as being able to show stable representations over time, reflects important properties of the place cells in terms of its association with memory processes. Kentros (Kentros, 2006) proposes that an analogy can be made between the stabilisation of place fields and the consolidation of memories. The hippocampal network forms unique place maps for different environment. Early studies on place cells indicated this, but only assessed a low number of cells and environment. Elaborating these initial indications, Alme et al. (2014) recorded from an average of 30 place cells in CA3 in rats in 11 different environments. They found that in each environment a unique place cell representation was formed during the first trial. Also, the place field map was reinstated upon subsequent exposure to the environments, speaking in favour of a memory for that environment.

Results from a recent study indicated that rats with increased hippocampal GH showed more global remapping in a novel environment compared with a control group (Haugland, 2021). The findings from that study serves as the inspiration for the current study to assess the possible involvement of SWR. A prerequisite for remapping is the ability of the place cells to change their activity. This ability is an intrinsic property of most neurons and will be further explained in the next section.

4.3 PLASTICITY

The way our brain is thought to permanently store experiences involves changes in synaptic weights and synaptic connections, reflected in the property named *synaptic plasticity* (Abraham et al., 2019; Neves et al., 2008). Therefore, to better understand memory it is important to understand the mechanisms underlying this general ability of the nervous system to alter its form and function. By changes in the synaptic properties, the network is thought to be able to encode, store and later retrieve patterns of neural activity that represents a given experience. And in relation with the focus in the current project, studies have found that during normal aging, plasticity mechanisms in rat are found to decline, together with the decline of cognitive abilities associated with aging (see review (Rosenzweig & Barnes, 2003).

There are several types of synaptic plasticity. One involves morphological changes of the neurons which alters the number of connections with other neurons. Another includes change in the strength of the signal transmission in the synapse (Lømo, 1971).

Synaptic plasticity were first demonstrated in the classical study of Lømo (1971). There, it was demonstrated how high frequency stimulation of the axons of neurons in the entorhinal cortex resulted in increased excitatory post synaptic potentials (EPSP) in the innervated granule cells of the dentate gyrus. In subsequent studies, changes in synaptic efficacy is found to be able to include both increase and decrease the EPSP (Dudek & Bear, 1992; Whitlock et al., 2006). The EPSP reflects depolarisation of the individual neuron after stimulation from the presynaptic neuron. The findings from Lømo implies that after high frequency activity of EC-neurons, the innervated cells in DG requires less stimulation to trigger subsequent action potentials.

There are several mechanisms underlying the changes in the synapse. One mechanism relevant for memory are involving the relative timing of neural activity, the spike time-dependent plasticity (STDP) (Tazerart et al., 2020). Here, the timing of activity in the post synaptic and presynaptic neuron determines the synaptic change. Depending on the precise timing and frequency of the activity of the neurons, different changes can occur, either increasing synaptic efficacy, potentiation, or weaking the signal transmission, depression. In the potentiation of the synapse, the postsynaptic neuron needs to be depolarised as the presynaptic neuron is active, while depression of the synapse is caused by postsynaptic neural activity preceding presynaptic activity (Tazerart et al., 2020). These changes in synaptic weights may last for different temporal periods. Some for only brief periods of time, or as have been assessed in in vivo animal studies, long term potentiation (LTP) was observed for at least a year (Abraham & Williams, 2003).

The biological mechanisms underlying synaptic changes are complex and involve many biochemical processes. For potentiation, this will involve change of properties increasing the probability of presynaptic activity to generate a sufficient EPSP to cause post synaptic action potentials (AP). That is, presynaptic activity before synaptic changes causes a lower EPSP than presynaptic activity after the synaptic changes. This is what Lømo and Bliss (1971) observed in their seminal paper. Of the observed changes in the postsynaptic neurons causing the increase of the probability to fire APs are upregulation of the glutamate receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA), in the post synaptic membrane (Lee & Kirkwood, 2011; Lüscher & Malenka, 2012). AMPARs are involved in the initial depolarisation of the neuron because the NMDAR-channel is blocked by a magnesium ion. However, after stimulation of AMPARs has caused sufficient depolarisation, the magnesium ion is expelled, and the NMDAR-channel opens. An

important proponent in hippocampal neuron long-term potentiation involves the opening of NMDA channels. The NMDA-channel is permeable to calcium which triggers subsequent molecular cascades contributing to synaptic changes. For example, calcium initiates calcium/calmodulin-dependent kinase II (CaMKII)-modulated phosphorylation of AMPAR and AMPAR subunits, leading to upregulation of AMPARs in the postsynaptic membrane and increased conductance of AMPARs (Lisman et al., 2002). Such upregulation of AMPARs are associated with increased spine size, which also is observed in GH-treated animals (Haugland et al., 2020; Lüscher & Malenka, 2012). NMDAR activation are also suggested to be partially involved in changes of gene expression, prolonging the potentiation effect for even longer periods of time (Abraham et al., 2002).

Factors contributing to synaptic changes, such as the mentioned up-/down-regulation of ion-channels and dendritic spine growth, are studied and found to be associated with cognitive abilities (Haugland et al., 2020; Whitlock et al., 2006). And, an experimental study showed how manipulation of the NMDA receptor impaired spatial memory in rats, using a water maze paradigm in which the rats were searching for a hidden platform in water located in the centre of either quadrant (Morris et al., 1986). Here, they blocked NMDA receptors with D,L-AP5 which resulted in inhibited performance on the spatial learning task. This blocking of NMDA receptor and thus inhibiting LTP demonstrates the link between NMDA mediated synaptic changes and memory function.

The synaptic changes may be subdivided into early and late phases reflecting how long the changes lasts. In early phases, there is no new synthesis of proteins, and the synaptic change is relatively short lasting. The later phase on the other hand involves gene expression and is longer lasting. In vivo recordings spanning over several years is difficult to conduct, making our understanding of factors influencing the synaptic strength over a longer period of time limited. Initial experiences may be represented by early phase LTP. In accordance with the synaptic tag and capture hypothesis, in which weakly potentiated synapses gets a “tag”, a sequence of place cell activity as a result of exploring the environment may get these “tags” (Redondo & Morris, 2011). Then, later activation of the place cell sequences may initiate a more persistent synaptic change in a distributed neocortical network, as late phase LTP. This synaptic tagging hypothesis is interesting in terms of the SPW-R replay of preceding activity further described in the following section. Altogether, synaptic plasticity is strongly indicated to be involved in memory formation, and among other agents involved in these plasticity changes is the growth hormone.

4.4 GROWTH HORMONE

Hormones works as signalling agents in the organism (Nussey & Whitehead, 2001). From the site of synthetisation, they are released, often into the blood system which distributes the hormones around in the body, before binding to receptors in the cellular membrane of the target cells, causing a range of effects in the target cell. As opposed to the other main signalling agent in the nervous system, the neurotransmitters, hormones generally have a longer lasting effect on its target cell, than do neurotransmitters which are involved in causing rapid changes in the electric potential of the neuron. However, hormones are involved in many cellular processes and carries numerous different functions all over the body as well as in the brain. By binding to specific receptors in the target cells, hormones initiate intracellular activities in that specific target cell involving cascades of biochemical processes. These initiated intracellular activities may lead to expression of proteins which in turn alters the many functions of the cell.

Growth hormone (GH) are found being involved in several different processes, in the brain and in the rest of the body (Nyberg & Hallberg, 2013; Ranke & Wit, 2018). Prominently, as the name implies, it is involved in the growth of different types of tissue and are also involved in metabolism. It acts by binding to the GH receptor, initiating various intracellular cascades, which in turn affects expression of several genes in the target cell. It is secreted by the pituitary into the bloodstream and are shown to be able to crossing the blood-brain barrier, but are also shown to be locally expressed in hippocampus in mice (Nyberg & Hallberg, 2013; Sun et al., 2005). GH also has properties that are interesting for memory research, and studies has indicated its involvement in several cognitive processes (Nyberg & Hallberg, 2013).

Associational studies established the link between GH and cognitive processes (Nyberg & Hallberg, 2013). A meta-analysis from 2006 shows that GH deficiency were associated with moderate-large cognitive impairments, and that GH treatment improved these impairments, and memory performance in particular were improved after GH treatment (Falleti et al., 2006). Lower GH levels are also observed in normal ageing and following sleep deprivation (Hersch & Merriam, 2008; Kim et al., 2010). The paradoxical case of Ames dwarf mice shows the possible importance of hippocampal GH (Sun et al., 2005). These animals have generally lower GH levels due to an impaired development of the GH secreting anterior pituitary. However, they exhibit normal cognition and lifespan. It was found that they had

local increases of hippocampal GH-level, and in addition did not exhibit the usual cognitive decline with ageing.

The associations observed between GH and cognitive processes are of great inspiration for the reasons to conduct the current study. Specifically, the association with memory triggers the question about the plausible involvement in hippocampus. Interestingly, there have been found mRNA coding for GH receptor in the brain of the rat (Burton et al., 1992; Mathews et al., 1989). Also, many of the observed functions of GH are mediated through insulin-like-growth-factor (IGF) (Ranke & Wit, 2018).

The known biological mechanisms of GH in hippocampus may contribute to understand why this effect was observed in the Ames dwarf mice. The link between synaptic plasticity and cognitive performance, and the crucial role of the NMDA receptor, has already been established in above sections. GH, and the related IGF-1 and IGF-2, have been found to enhance LTP, potentially through increasing the gene expression for NMDA receptor subunit GluN2B and growth of dendritic spines (Nyberg & Hallberg, 2013). Also, a recent study have shown increased spine density on the dendrites of hippocampal neurons (Haugland et al., 2020). In the same study, the rats showed enhanced performance in a spatial task, further establishing the involvement of GH in the cognitive process of memory.

Based on the information presented in this and above sections, it is plausible that GH may affect cognitive performance by mediating properties of SWR. By upregulating NMDA and AMPA receptor channels in hippocampal neurons, GH contributes to making the neurons more susceptible for exhibiting excitatory activity and other aspects of plasticity. This action of GH may interfere with observable properties of the SWR, length and rate of occurrence, both associated with memory. Although it should be noted that applying NMDA antagonists in ventral hippocampus CA3 increased the size of SWR using in vitro model (Colgin et al., 2005). However, due to the concurrent indications of upregulation of other glutamatergic receptors, i.e., AMPA, and the joint effect of GH and SWR on memory, it could still be expected that GH will affect SWR properties associated with better memory performance.

4.5 SHARP WAVE RIPPLE

The measurement of electrical potentials in the extracellular space can detect the spiking activity of single neurons, and it is a common practice to use this single unit activity to infer something about behaviour outcomes and cognitive functions (Buzsáki, 2006;

Csicsvari et al., 2003). Often be ascribing function to a neuron based on whether their activity increases or remains unchanged. Both the discovery of hippocampal place cells and the concept of synaptic plasticity are based largely on this form of extracellular recording that measures the activity of single neurons. However, recordings of the extracellular potential can also measure the activity of larger neuronal populations, when extracting the lower frequency parts of the signal, up to ~500Hz. This electrophysiological signal is termed local field potential (LFP) and measures the electrical transmembrane currents of larger neuronal populations in units of volt (V).

The origin of these LFP signals found in the extracellular space has multiple neural sources (Einevoll et al., 2013). Although the exact contribution of different sources of the signal are considered ambiguous, a main proponent of the LFP signatures is contributed by transmembrane fluctuations in the dendrites of active synapses. However, essentially all transmembrane fluctuations contribute to the signal, whether it is in the synapse or action potentials along the axon etc. (Einevoll et al., 2013). But, for the recorded LFP, synaptic activity is considered the main contributor. Synaptic activity drives the excitatory- and inhibitory post synaptic potentials (EPSP and IPSP, respectively), by the opening of selective permeable ion channels. The stimulation of these dendritic ion channels may last for longer than the activity of ion channels in the axon, responsible for the action potential and subsequently extracellular recorded spikes, and thus, these EPSPs and IPSPs are longer lasting than the spiking activity and accessible when extracting the lower frequency part of the LFP signal. The resulting measured extracellular signal appears as fluctuating potentials in the LFP.

These fluctuations are commonly called oscillations and have been organised and categorised in distinct frequency bands expressed in hertz (Hz) (Buzsáki, 2006). The different frequency bands are correlated with distinct behavioural states and functions related to sensory processing and cognitive processes (Colgin, 2016; M. E. Hasselmo, 2005). The frequency bands and its correlated states and functions have been observed across species and also in humans (Buzsáki, 2006). For example, hippocampal theta frequency in rodent (~6-10Hz) is correlated with exploratory activity and associated with memory encoding (Buzsáki, 1989, 2015; M. E. Hasselmo, 2005). And in sensory cortical areas, LFP activity in the gamma band are indicated to encode sensory information (Mazzoni et al., 2008).

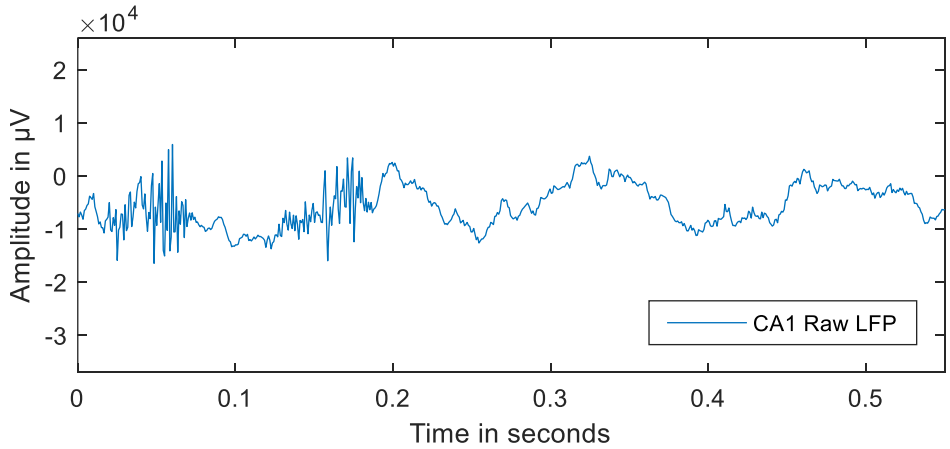
Oscillations are thought to provide effective communication between different parts of the brain, making functional assemblies of several neural populations in different part of the

brain by creating temporally coordinated windows of increased possibility of information from one area to reach downstream areas (Buzsáki, 2006). This is achieved by the organised activity of excitatory drive of the principal neurons and inhibitory effect of interneurons. Also, during exploration, place cells spikes are found to show preferential spike timing relative to the phase of the theta (Leibold & Monsalve-Mercado, 2017; O'Keefe & Recce, 1993). The place fields have not strict boundaries and show some overlap. This leads to co-activity of several place cells with different, overlapping, place fields ordered on the theta. The place cell of a place field just entered, are active early on the theta phase, while place cells with place fields the animal just went through are active on late theta phase. This ordering of place cell spikes on the theta oscillation are called phase precession and may be a mechanism ensuring synaptic coupling between the place cells involved due to the temporal proximity of the activity, according to the spike time dependent plasticity rule.

A prominent type of activity found in the LFP, called large amplitude irregular activity (LIA) showing a pronounced deviation from the baseline theta activity was discovered by Case Vandervolf (1969). These neural activities caught the attention of other researchers, and in time lead to the discovery of sharp wave coupled fast oscillatory patterns of large amplitude in the hippocampus, termed sharp wave ripples (SWR) (Buzsáki, 2015). However, determining the satisfactory power to identify the activity to be a true SWR is problematic due to variable number of neurons active in each SWR, as will be explained later.

Figure 4.

Raw Local Field Potential

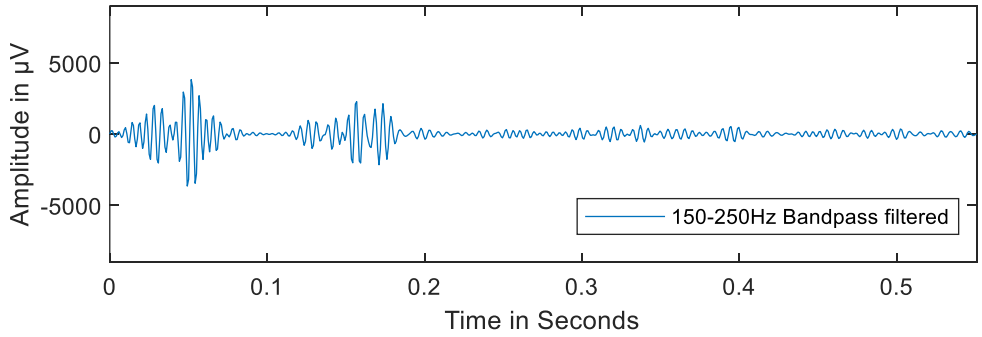


Note. Example of SWRs and theta activity from the raw LFP signal from the current study. Note the two events of large amplitude high frequency oscillation activity in the first 200ms period in addition to the subsequent prominent theta activity.

Sharp-wave ripple event are a highly synchronous activity of neural populations in hippocampus. Characterised as a large deflection of the local field potential in CA3/str radiatum CA1 and a subsequent high frequency oscillation (approx. 150-250Hz in rats) in the stratum pyramidale layer in CA1. This neural event is prominent during sleep, but are also present during awake periods, then, most occurring during pauses of locomotor behaviour and temporary disengagement of the exploration the environment, such as awake resting states, termed consummatory states (Buzsáki, 2015). It exhibits several properties intriguing for a mnemonic function, and studies indicates its causal involvement in memory (Carr et al., 2011; Girardeau et al., 2009; Jadhav et al., 2012).

Figure 5.

Bandpass-filtered Signal



Note. Signal filtered with a 12th order FIR1 bandpass filter (150-250Hz). The same signal as Figure 4.

The activity seems to reflect an intrinsic property of the hippocampal network as the EC cells, the main carrier of cortical information to hippocampus, are found to be less active during sharp waves, thus not responsible for initiation of the SWR (Chrobak & Buzsáki, 1994). The event is initiated by a transient cessation of inhibitory influence from subcortical structures to CA3, thus creating a gradual build-up of activity in the CA3 recurrent network, before releasing a big burst of excitatory activity seen as the large sharp wave deflection in the LFP (Csicsvari et al., 2000; M. Hasselmo et al., 1995; Schlingloff et al., 2014). This, brings about a huge excitatory effect on the CA1 region and the subsequent high frequency ripple oscillations caused by the coordinated activity of excitatory principal cells and inhibitory interneurons (Buzsáki, 2015; Nguyen et al., 2009; Schlingloff et al., 2014). However, the neurons engaged in each distinct SWR event varies greatly, and thus affects the resulting LFP amplitude, which leads to issues for detection of the events (Buzsáki, 2015; Hagen et al., 2020).

Precise detection of true SWRs is essential, but in detecting the events one is faced with several issues. First, in vivo electrophysiological recordings entail various sources of noise thus exhibiting a reduced signal-to-noise ratio. Originating from, for example, physical touches to and movement of the microdrives, muscle activity in the vicinity of the electrode interfering with the recording of hippocampal neuron activity i.e., chewing, external noise from the electric line, or otherwise other signals recorded in the LFP not caused by brain activity. Another factor affecting the accuracy of SWR detection is the variable LFP power (Hagen et al., 2020). The prevalent existing methods of SWR detection originate from the implementation of the methods used in the study by Csicsvari et al. (1999), where the detection are based on pre-defined SD from the normalised squared signal, or, a similar method using pre-defined SDs from the mean of the smoothed envelope of the bandpassed signal (i.e. (Karlsson & Frank, 2009)). Due to the prevalence of varying SWR amplitude, a single fixed threshold for detection of SWR, pre-defined based on for example other studies, may reduce the accuracy of the detector, and presents a relevant issue in the SWR research (Hagen et al., 2020).

As any other oscillatory activity, the properties of the SWR includes the oscillation frequency, in Hz, and amplitude, in volts. In addition, relevant parameters of the event are its duration and rate of occurrence, both causally implicated to the involved in memory functions (Fernández-Ruiz et al., 2019; Girardeau et al., 2009). By detecting the onset of an event, Girardeau and colleagues (2009) disrupted the SWR events in sleep periods after a spatial training session. The results from this study showed an impaired performance on subsequent tests in rats receiving electrical disruption of SWR compared to other groups receiving electrical stimulation outside SWR periods and a control group, and therefore indicates a role in consolidation of spatial memory. The proposed consolidation function of SWRs are further strengthened by other studies, correlating SWR length and rate of occurrence with memory performance (Eschenko et al., 2008; Fernández-Ruiz et al., 2019).

One of the aspects of SWR that makes it an interesting candidate memory process is its neuronal content. Mainly, the spiking content consists of replay of previous neural activity sequences during awake exploration (Atherton et al., 2015; Carr et al., 2011; Karlsson & Frank, 2009). As described in the place cell section, recording of place cell assemblies shows that they create a map of the environment, with the ability to store many maps and also show sensitivity to contextual aspects related to the experience in the environment. Interestingly, the cells that are active in waking periods in an environment show periods of reactivation

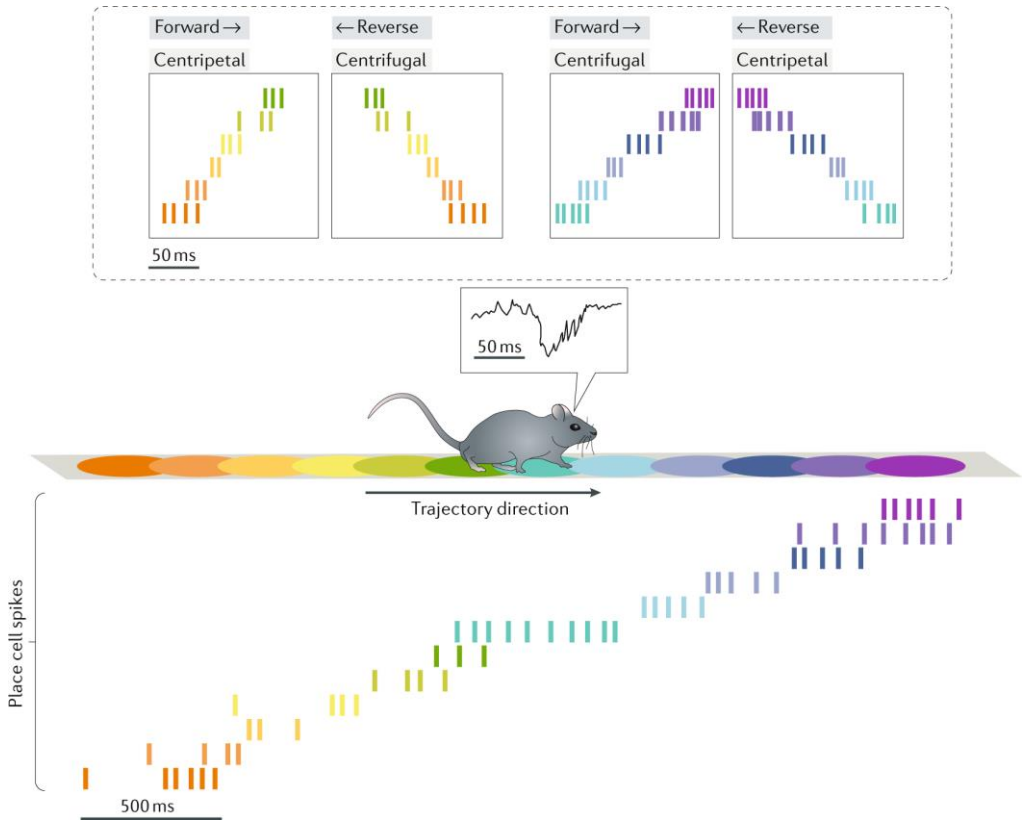
compressed in time during subsequent sleep, in similar sequential order as they occurred in the waking period. Davidson and colleagues (2009) showed that sequential active place cells in awake rodents during track running showed a replay of the sequential place cell activity in sleep after waking period, suggesting place cell assembly replay as plausible a neural correlate of a mental time travel. The proposed function of this replay of neural activity patterns is to store this neural correlate of experience on a more permanent basis, a neural mechanism for long term memory of experiences.

An observed effect of SWRs is stabilisation of place fields. Roux et al. (2017), used optogenetic inhibition of CA1 place cells during awake SWRs leading to reduced place field stability, and in subsequent exploration of the same environment these place cells showed a tendency of increased remapping. Similar results have also been found when disrupting SWR during sleep shortly after learning of an environment (van de Ven et al., 2016).

In additional studies, the neural content of SWRs was found to be played both forwards and backwards, in addition to exhibiting patterns corresponding to possible future activity in awake exploration (see Fig x) (Joo & Frank, 2018). This leads to the proposal of SWR not only being involved in consolidation of episodic memories, but also having a role in planning of possible futures guiding behavioural choices. This is interesting considering the proposal laid out by the cognitive map theory of hippocampal function, guiding creative solutions and intelligent behaviour. Studies have also established causal links between SWRs and planning in the awake state, in which disruption of SWR by electrical stimulation severely impaired performance on a spatial alterations task in rats (Jadhav et al., 2012).

Figure 6.

Informational Content of Hippocampal Sharp Wave Ripple



Note. Shows a colour coded sequential place cell activation in rat. The lower part of the figure shows place cell spikes corresponding to the specific parts of the linear track also visualised. The upper part shows a SWR event, and the different neural content that may be embedded in the SWR, either representing replay (the two leftmost) or pre-play of upcoming place cell activity (the two to the right). (Joo & Frank, 2018).

The hippocampus does not work in isolation, and the cooperation with the greater neocortex is a necessary aspect of neural representation of experience and involved in the storage of these experiences. As described in the section of hippocampal architecture, the hippocampus is connected to widespread neocortical areas. Accordingly, it has been shown that SWRs may affect neural activity in these downstream neocortical regions due to the large synchronous activity able to affect wide spread cortical areas (Buzsáki, 2015). For example, SWR are indicated to be correlated in time with cortical spindles during sleep (Siapas & Wilson, 1998). In addition, replay of the previous neural activity in both hippocampus and cortex are observed and were indicated to be coordinated in time (Ji & Wilson, 2007).

An important aspect considering hippocampal communication with the downstream neocortical areas are the coinciding activity of both the hippocampus and cortical areas. Prominent neocortical activity states are found and categorised in “down-“and “up”-states,

where in the “down”-state the neocortical neuron populations exhibit reduced activity, and vice-versa. Studies have indicated a correlation between the transition of these neocortical activity states and the occurrence of SWRs (Battaglia et al., 2004; Roumis & Frank, 2015). Here, it was found that SWR occurs more often in the transition from “down” to “up”-state, proposedly reflecting information temporally coordinated transfer from hippocampus to neocortex.

The above description of SWR establishes the foundation for the proposed consolidation function of SWR, which is portrayed by the two-stage model of memory consolidation (Buzsáki, 1989). The model posits that formation of memories occurs in two main stages. First, during exploration the hippocampus receives converging information from the neocortex and encodes this ongoing neural activity representing the experience by shifting the synaptic weights in hippocampal circuits. In this encoding stage, the neural activity is coordinated by the theta activity. The synaptic changes occurring during encoding creates a temporary storage of the neural representation of the experience in the hippocampus. Then, during subsequent “offline” periods of awake rest or sleep, when the animal is not engaged in awake exploratory activity, the hippocampus exhibits replay of the preceding neural activity patterns in a time compressed manner, embedded in the SWR. The proposed function of this replay is the consolidation of memories, a process contributing to making the memory more permanently stored by transferring it from the hippocampus to be represented in a distributed neocortical network. The replay of neural activity patterns during SWRs occur within a time frame allowing synaptic changes to happen according to spike-time -dependent plasticity. As will be further described below, GH are indicated to be involved in synaptic plasticity mechanisms, and therefore, may also have an additional role in the representation of the initial encoding of experience for later replay during SWR.

4.6 Aim of the Current Study

The current project is part of a greater study investigating the possible effects of growth hormone modulation of hippocampal activity. Earlier findings from the study of Haugland (2021) indicated hippocampal GH-overexpression to promote global remapping in a novel environment, a mechanism of the hippocampal network thought to reflect the ability to store different memories with a spatial dimension, in humans termed an episodic memory, by creating orthogonal maps for different environments, or different experiences in same

environment (Colgin et al., 2008). The current project will therefore examine whether the same rodents exhibit observable effects of GH manipulation for a related hippocampal memory process, SWR.

The aim of this study is twofold:

1. Modify existing codes to detect SWR and parameters including SWR duration, rate and long (>100ms) of SWR.
2. Identify SWR in the LFP dataset (from paper III, Haugland 2021) and reveal if GH impacts the SWR rate during immobility before, during or after exploration of a novel environment.

Due to the lack of earlier studies concerning the effect of GH on hippocampal SWRs, this study is highly exploratory, and accordingly, no strong assumptions for expected effects are expressed. However, as described in above sections, GH is associated with memory performance, and is affecting the excitatory properties of neurons. Measurable properties of the SWR, occurrence rate and duration, are also associated with memory performance, and may be susceptible for alterations following manipulations of the excitatory properties of the hippocampal neurons.

This study aims to see if GH may affect relevant properties of SWR that have been associated with memory performance by manipulating local GH expression and activity in dorsal hippocampus of rats and analysing the LFP from CA1.

The first hypothesis states that GH-overexpression will induce SWRs of longer duration compared to the control- and the aGH group. This will be tested by assessing the median SWR duration in addition to the fraction of long duration events (>100ms). The second hypothesis proposes that the number of ripples between the groups are different, and SWR rate in the antagonist GH group are proposed to occur less frequent compared to control and GH group. This will be tested by calculating the rate of occurrence expressed as SWR-event per second. Finally, the third hypothesis states that the GH group will exhibit larger fraction of SWR event of more than 100ms.

However, as there are several challenges concerning the detection of such SWR events, a supplementary focus involves optimizing the SWR detection in the LFP samples that have been recorded by the research group in Neurobiology in Tromsø.

5 METHODS

The data used in the current project are provided by the research group in Neurobiology in Tromsø (UiT). Both the preparation of the study design and the conduction of data collection was performed by Kamilla Haugland as a part of her PhD project. The experimental trials were carried out in animal labs in both Tromsø (University of Tromsø) and Bergen (University of Bergen). The main focus of her study was the investigation of GH on place cell remapping, and for a full description of the experimental design and methodology regarding the data collection provided by her (Haugland, 2021). The current study will use parts of this data to examine the detection of SWR and the possible effects of GH on SWR properties. Results from the remapping study indicated increased global remapping of place cells in GH-treated animals in the novel environment, changing their place field location. Therefore, data for the current study includes recordings from exploration in novel environment, pot sessions after novel environment, and from the first pot session for comparison.

5.1 DESIGN

To assess the possible effects of growth hormone on hippocampal sharp wave-ripples, the local field potential from CA1 was recorded in rats freely exploring a novel environment and during rest in pot. The LFP was obtained from one channel of the implanted tetrodes with low amounts of noise, recorded with AXONA Dacq system. Pre-processing of the raw LFP signal for further analysis and detection of SWR events was conducted in MATLAB R2021a (2021). Statistical analysis was conducted using IBM SPSS Statistics for Windows, Version 26.0 (2019). Visualisation of the signal for creating figures for the thesis were performed using Jupyter Notebook Python 3.

Young adult Long Evans rats (N=10), approximately 8 weeks old received recombinant adeno-associated viruses(rAAVs) in dorsal hippocampus, all rAAVs contained sequences expressing green fluorescent protein (GFP), in addition to either expressing GH (GH, n=2), antagonising GH (aGH, n=5) or GFP only (control, n=5). All rAAVs contained sequences GFP to visually verify the transfections. Antagonising GH was achieved using mutated GH to block GH receptor activity. After the transfection the animals recovered in their respective home cages. At the same time as they received the rAAVs, they got implanted microdrives for later measurement of neural activity.

After the recovery period following implantation of microdrives and injection with virus, the animals habituated to the environment used in the experimental setting while the tetrodes simultaneously were lowered gradually. Accomplished habituation was determined as the animals showed good behaviour, expressed as covering all parts of the box, in addition to detection of place cells.

In the experimental setting, the rats were freely foraging in either familiar or a novel environment condition. The familiar environment consisted of a squared arena, 100x100cm and 50cm high walls with a cue card on the centre of one of the walls. The novel environment consisted of a circular arena in either 90cm or 100cm in diameter in the same room.

The experimental setting consisted of two main alterations of different trial sequences the animals progressed through. One, only involving the familiar environment until they showed well separable place cells, and in the other, the novel environment was introduced as one of the trials. In the first version, the rats first spent 5 minutes in the pot. Then, they spent 2x10 minutes in the familiar box, with a 10-minute break in between, ending the session with 5 min in the pot. The other experimental alteration was similar; however, the second exploration setting was in the novel environment. In some experiments the rats were allowed to rest in the pot for 4-40 minutes in between the novel and the last familiar environment. For the current project, only recordings from periods in the first pot session, during exploration in novel environment, and pot sessions after previous exploration in novel environment were included for further analysing. The progression through the trials in each experimental alteration is presented below, with the initial time spent in parentheses.

Version 1

Pot session (5) – familiar box (10) – pot (10) – familiar box (10) – pot (5)

Version 2

Pot session (5) – familiar box (10) – novel environment (10) – familiar box (10) – pot (5)

Post-mortem assessments of the animals showed successful increase of GH in GH group and antagonising GH in aGH group compared to control, quantified by RT-qPCR.

Experimental protocol followed the European Community Council Directive 2010/63, The Norwegian Regulation on Animal Research, and were approved by Norwegian Animal Research Authority before initiation.

No statistical tests were conducted to predetermine the sample size. Unfortunately, the sample sizes in the current study are slightly lower than comparable other studies, and the duration of recording sessions used here are shorter than similar studies.

5.2 MATERIALS

5.2.1 Viral vectors

To manipulate the amount of hippocampal GH, three recombinant adeno-associated viruses (rAAVs) ½ chimeric pseudotypes were used. Either overexpressing GH and GFP, aGH and GFP, or GFP only, provided by Ki Ann Goosens, MIT. Three cassettes were synthesised, one containing the coding region of rat GH gene, another containing the rat GH gene with substitution of a single amino acid at position 120 (rGH-G120R), both containing internal ribosomal entry sites (IRES) and GFP. One last cassette containing IRES and GFP only.

5.2.2 Surgeries

All surgeries were done under analgesia and deep isoflourane gas anaesthesia to relieve the animals of any pain or discomfort. The head was fixated, and the skin were then cut open to allow for drilling of holes at appropriate positions at each hemisphere. The animals were randomly assigned to either of the viruses. Using a sterile 2µL Hamilton Syringe (Hamilton Company) and a pressure pump, the animal was injected with the virus. In the dorsal hippocampal CA1, the animals received four injections of 0.4 µL of virus solution (0.2µL/min) in each hemisphere, at anteriorposterior (AP), mediolateral (ML) and dorsoventral (DV) coordinates from dura: AP -3.0mm, ML +/- 1.2 mm, DV 2.3 mm; AP-3.5mm, ML +- 2.2mm, DV 2.2mm; AP-4.0, ML +- 2.4mm, DV2.4mm; AP -4.4mm, ML +-3.5mm, DV 2.8mm. After injection of virus, one to two microdrives were implanted in each hemisphere, using the coordinates, AP 3.5mm, ML +- 3.3 mm and DV 1.5mm. Finally, the skin was sutured, and the animals recovered in their home cages with a warm water bottle, receiving analgesics.

5.2.3 Data collection

The local field potential (LFP) was recorded using tetrodes attached to microdrives connected to an Axona data acquisition system (Axona Ltd). The signal was digitised using a pre-amplifier at 24-bit resolution, sampled at 4800Hz, and lowpass-filtered at a 500Hz cutoff.

A camera recorded the position of a light emitting diode (LED) positioned at the head stage of the Microdrive. The data from the position of the LED during the session were used to determine the speed of the animal during the recording.

The recording sessions used for SWR analysis was from the pot sessions before exploration, during exploration in novel environment consisting of a circular box enclosure instead of a familiar square box, and pot sessions after exploration. The lengths varied between approximately 5-10 minutes.

5.2.4 Data analysis

The LFP recordings were imported in Matlab using pre-written scripts. The position data were imported to Matlab using scripts from mTintCore (function: 'read_pos_file.m', written by Daniel Manson https://wiki.ucl.ac.uk/display/Hippo/mTintCore). Both preprocessing of the raw LFP and SWR detection was carried out in Matlab using scripts and functions from Buzcode repository (available at https://github.com/buzsakilab/buzcode) implemented externally by the Lab Nevrobiologi at UiT.

Some of the visualisations(for example fig x) were produced in Jupyter Notebook Python 3 using a combination of scripts from Hagen, E., Chambers, A.R., Einevoll, G.T. et al. (2020) and my own code implementation. In these visualisations, bandpassing (150-250Hz) were done using a 6^{th} order Butterworth filter applied to the LFP using 'scipy.signal.filtfilt'. From the bandpassed signal an envelope was computed a Hilbert transform smoothed with a Gaussian (4ms SD). Spectrogram was computed using complex Morlet wavelets (see Hagen, E., Chambers, A.R., Einevoll, G.T. et al. (2020) for full description of computation of wavelet spectrogram).

5.2.5 Calculations of dependent variables

As one of the aims of the project was to optimize SWR detection, I will describe the steps of detection in further detail in the results section.

The SWR detection was carried out on all recording sessions, producing output values for further statistical analysis. Parameters extracted to be included in the analyses was, SWR rate (SWR/second), proportion of long-duration SWRs (>100ms) and for duration, I used a measure of central tendency using the median duration of all detected SWR events for each recording because of the skewed distribution of SWR duration (Buzsáki, 2015).

5.2.6 Statistical analysis

Group comparison was carried out in IBM SPSS. The number of observations were different between the groups, and in some of the groups, observations was very few (<5). Because significance tests of normality, i.e. Shapiro-Wilk test, lacks power to detect possible violations of the normality assumption when the sample is small, and the power of regular parametric statistical methods is reduced with small sample sizes, group comparison were carried out using the non-parametric Kruskal Wallis test which does not assume normality and are therefore considered the better option (Field, 2013). The statistical analysis was conducted with the recording as the experimental unit; thus, one animal contributes to multiple recordings. Complications relating to the use of multiple data observations from the same entity will be discussed in the discussion section. Significance level was set to 0.05, and confidence interval-level 95%.

Due to SWR occurrence rate and length indicated to be affected by previous experiences, especially recent cognitive load, the comparisons were carried out using sessions with as little differences of previous experience as possible. Therefore, comparisons are done between recordings from similar recording setting. First, the first pot session before experiment, second during exploration in novel environment of circle arena, and last, the final pot session after experiment. The last category was further subdivided in pot session directly after exploration in novel environment of circle arena, and pot session after the entire experiment, which also included exploration in novel environment.

6 RESULTS

As one of the purposes of the current study was optimalization of SWR detection, the presentation of results will include a further description of the steps of the signal processing preceding the detection and exploration of how the code scripts performed in detecting SWR events. The subsequent statistical analysis utilizes the detected SWR events, calculating relevant properties related to both age and memory, generated by the scripts in conducting statistical comparisons between the GH manipulated groups to see if GH may exert any effects on the mentioned SWR properties.

6.1 SWR PRE-PROCESSING AND DETECTION

6.1.1 Pre-processing

In vivo electrophysiological recordings are prone to be influenced by several factors other than neural activity. Consequently, certain denoising procedures were conducted to reduce the artifacts and increase the signal-to-noise ratio.

The first denoising step was conducted already at the recording stage, where Axona Dacq were set to apply a notch-filter at the power line frequency around 50Hz.

Excessive gain during the recording, or otherwise large signal amplitude, lead to clipping of the signal, seen as saturation in the recorded LFP (see Figure 9). This produces periods of complete stationarity where no oscillations occur, and accordingly, no SWR is available for detection. For removing periods where the signal was clipped, a filter was applied removing the data points if 15 successive values of either maximum or minimum signal value was found.

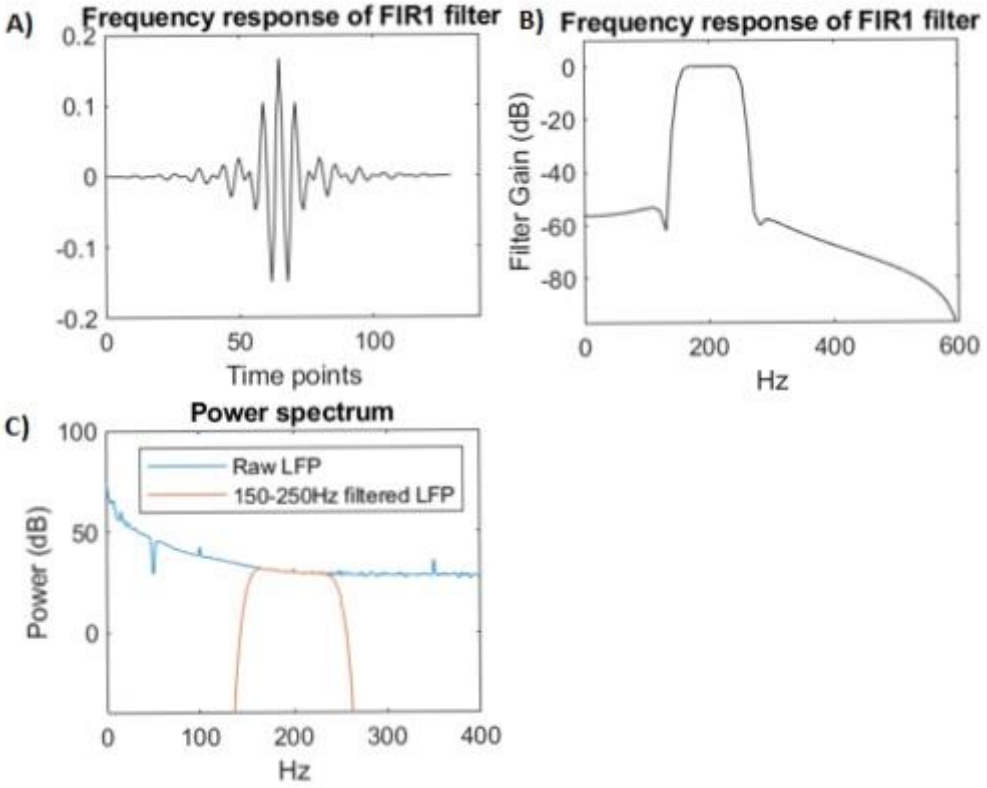
After removal of the saturated periods, prominent sharp fluctuations are often preceding and/or following the saturation. These large deflections in the raw signal can cause ripple effects after applying the bandpass filter, occasionally leading to detection of a false positive. Thus, to reduce the potential reduction of SWR detection accuracy, a noise filter was applied. This noise filter removed datapoints exceeding 25SD of the squared signal. Supplementing this noise filter, downsampling of the signal also contributes to reduce the amount of high frequency noise. Thus, the signal was downsampled with a factor of 4 using the built-in Matlab function for downsampling (‘downsample.m’), resulting in a sampling frequency of 1200Hz. The resulting sample frequency is sufficient for detecting oscillations within the ripple-band as the upper ripple-passband frequency (250Hz) still is lower than the resulting Nyquist frequency (600Hz).

Following the denoising steps, and preparing for SWR detection, the denoised and downsampled signal was bandpass filtered between 150-250Hz. Selecting a suitable filter is of importance, ensuring a sufficient signal-to-noise ratio, and for errors not to propagate through the subsequent analyses. I wanted a filter with as little attenuation at the cutoffs as possible while still avoid ripple effects. Therefore, I selected a Fir1 filter kernel (Fig x). After visual evaluations, I ended up using a 128^{th} order Fir1 filter, applied to the raw LFP using the Buzcode function ‘bz_Filter.m’. Visual assessment of the filter properties in the power

spectrum supports its suitability for filtering out unwanted frequencies while preserving the frequencies in the ripple-band (Figure 7).

Figure 7.

Evaluation of Fir1-flter Parameters



Note. A) 128th-order Fir1 filter kernel. B) Visualisation of the frequency response of the filter kernel by showing its power spectrum. C) Power spectrum of both the raw LFP and 150-250Hz bandpass-filtered signal used for further SWR detection.

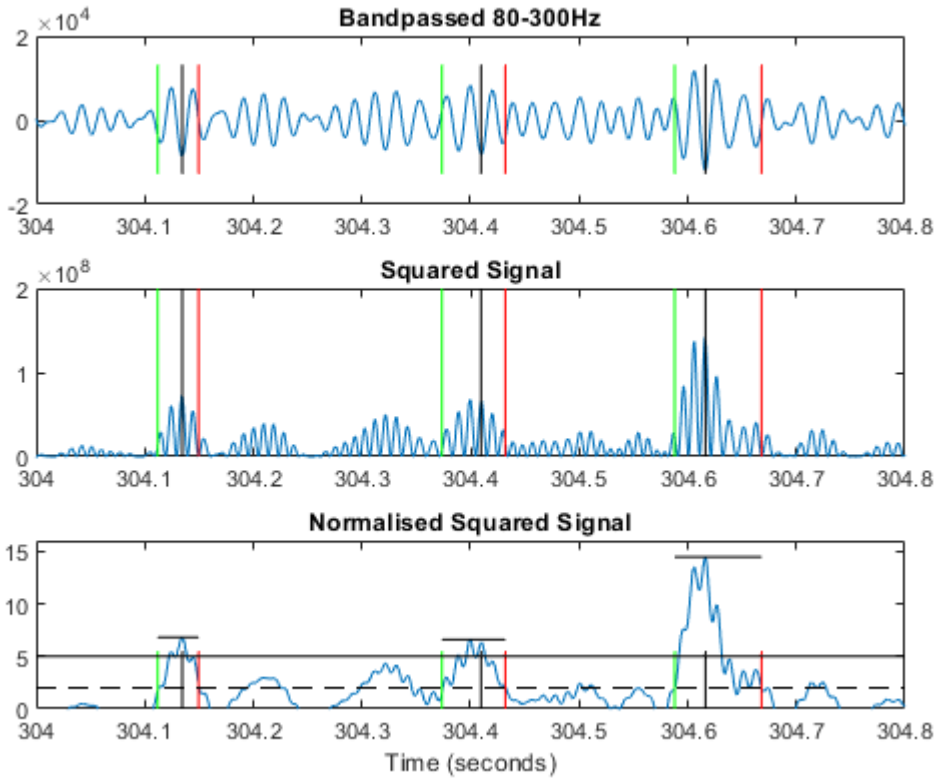
6.1.2 Detection

The detection of SWR event is carried out using the Buzcode function 'bz_FindRipples.m'. This detector is a modified version of the original algorithm developed by Csiscvari et al. (1999), and is widely used for SWR detection. SWR events are detected when the normalised squared signal (NSS) of the bandpass-filtered signal exceeds 5SD from the mean NSS (see fig x). The start and stop thresholds are set to 2SD determining the start time as the time point where the NSS falls below 2SD before the event power peak (>5SD) and stop time following the event peak. The event duration is calculated by subtracting the

end time from the start time. Events with duration >250ms and <15ms are discarded, and events occurring within 30ms interval are merged (Tingley & Buzsáki, 2020). A speed threshold was also included, rejecting SWR events if occurring when speed of the animal was >5cm/s.

Figure 8.

Results of the Sharp Wave Ripple Detector

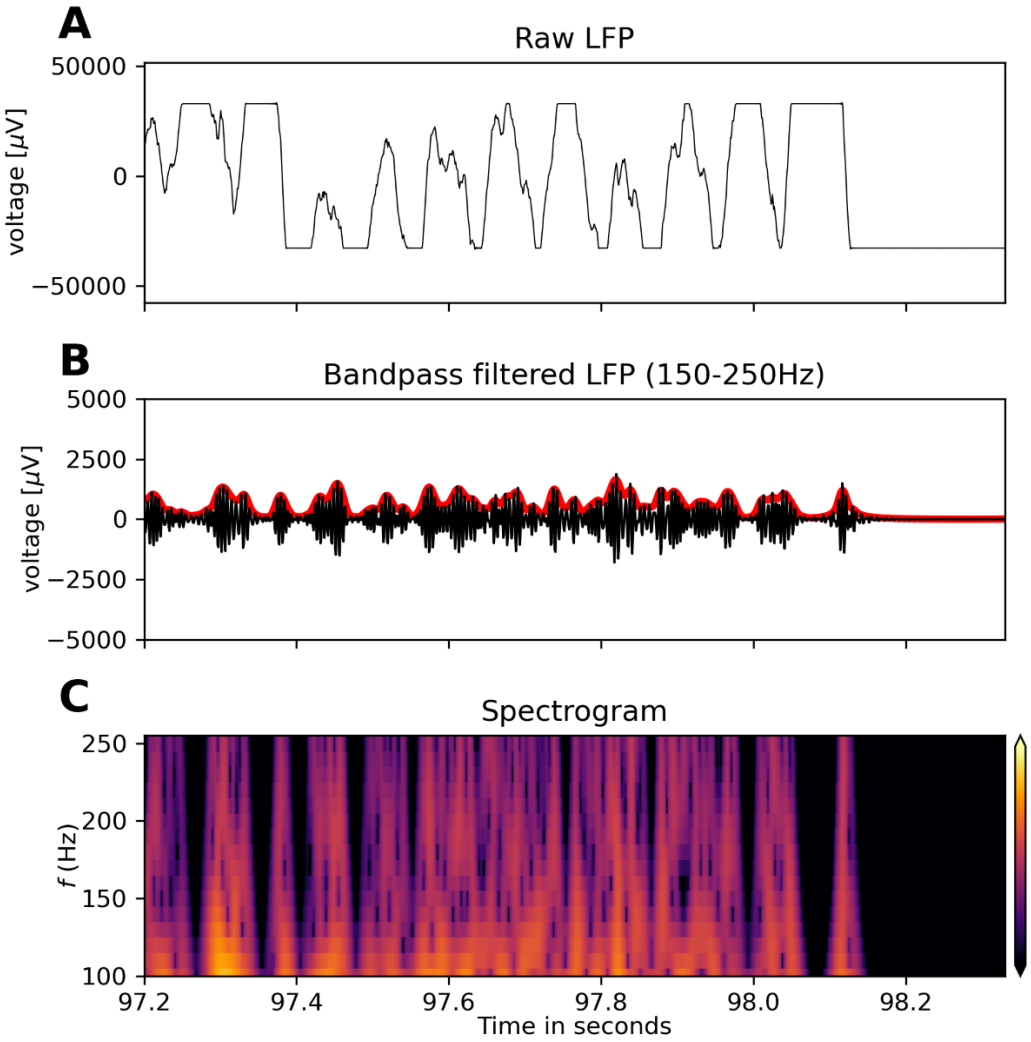


Note. Example of the SWR detection. The bandpassed signal were squared (middle plot) and normalised (lower plot). The timeseries plots are showing three detected SWR events, green vertical line indicating start (exceeding 2SD form the baseline of the normalised squared signal), red vertical line indicating stop, and black lines indicating SWR peak (peak normalised square signal). In the lower plot, the solid black line indicates the threshold for SWR detection (5SD), and stippled black line indicated threshold for start and stop time (2SD).

Although downsampling reduces high fluctuation noise in the raw signal, there are still incidences of noise in the form of large deflections in the raw LFP that are detected as a SWR (see Figure 9 and Figure 15). Therefore, a method for detection and rejection of these false positives is included by setting a criterion of minimum 6 peaks in the raw signal of each SWR.

Figure 9.

Local Field Potential from the Current Dataset.

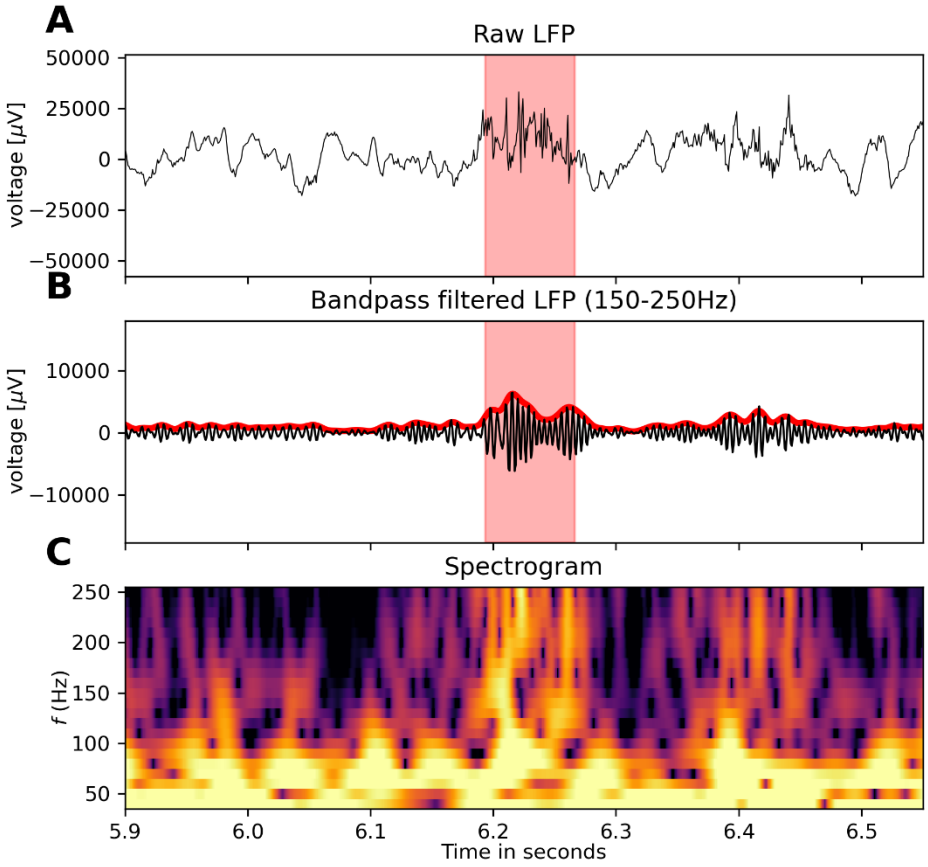


Note. Example of (A) noisy data of large fluctuations shown in raw LFP,(B) bandpass filtered signal (1250-250Hz) and (C) wavelet spectrogram.

After assessing the final output of the SWR detection there was decided that the detection was sufficient, although still exhibiting occurrences of false positives and possibly false negatives. Though, some possible events are ambiguous. As is visualised in Fig 10, the marked red area depicts the detection of an event and the assessment of the visualised bandpassed signal power (B) and elevated frequency power in the ripple band (C) signifies a true SWR. However, there is also seen increases in the bandpassed signal power and a tendency of increases in the ripple-band seen in the spectrogram, although both more moderate than the preceding. Implications revolving this will be discussed in the discussion section.

Figure 10.

Example of Detected Sharp Wave Ripple Event.



Note. A) Shows the recorded raw local field potential with the red marking indicating the SWR period. B) Presentation of the bandpass filtered signal 150-250Hz with an envelope (red line) using Hilbert transform smoothed with a Gaussian (4ms SD). C) Spectrogram indicating activity in the ripple band. (implemented by codes from Hagen et al. (2020)).

6.2 Investigation of GH on SWR

Assessments of GH effects on SWR properties were done on recording sessions from different environmental and behavioural settings. The properties of SWR included, median SWR duration, SWR per second and the fraction of SWR >100ms. Statistical analyses compared properties of SWR between the GH manipulation groups in four different recording settings, in pot session before exploration (aGH *n*=9, control *n*=3, GH *n*=7), in exploration of a novel environment consisting of a novel circle enclosure in a familiar room (aGH *n*=9, control *n*=9, GH *n*=4), pot session immediately after the exploration of novel environment (aGH *n*=6, control *n*=2, GH *n*=1), and pot session after completing all experimental trials (aGH *n*=4, control *n*=5, GH *n*=3). All statistical comparisons were carried out using

independent samples Kruskal Wallis test in IBM SPSS. Additional tables and figures are in Appendix A-C.

Table 1.

Animals Included in the Study

Animal ID	Group	Environment					
		First pot session		Exploration NE		Pot session after NE	
		Total duration	Number of SWRs	Total Duration	Number of SWRs	Total duration	Number of SWRs
3113	aGH	1130.996	68	1380.089	179	651.058	92
3213	aGH	297.858	45	599.066	168	1488.733	145
3211	aGH	893.715	26	2945.533	339	1486.773	45
3021	Control	482.936	24	-	-	171.398	1
3116	Control	196.901	4	1254.662	328	539.513	48
3207	Control	-	-	1188.647	225	-	-
3209	Control	-	-	592.351	0	-	-
3212	Control	-	-	1198.873	164	2110.106	73
3118	GH	1494.847	59	1547.523	246	598.148	36
3120	GH	-	-	550.373	48	545.134	42

Note. Overview of the animals providing the data used in the current study, showing their group and total duration (seconds) and number of SWRs in the different recording settings. Values in "pot session after NE" includes combined recordings from both pot sessions immediately after circle and the last box session.

6.2.1 Effects of GH manipulation on SWR duration

The first hypothesis stated the expectation of the GH group to exhibit longer SWR. Therefore, I compared the median SWR durations observed in each recording of the groups differentiated by different recording settings. Because SWRs show different properties in different states and are affected by previous experiences three separate statistical comparisons are conducted, one for each recording setting. First, I examined recordings from the first pot session before the animals started the experimental trials. The comparison of SWR median duration in pot session before exploration showed no significant difference between the groups, $H(2)=0.916$, $p=.632$.

Then, recordings from the exploration setting were examined. SWR are not only related to sleep, but are also indicated to be involved in planning and memory retrieval in awake state (Roumis & Frank, 2015). Therefore, I wanted to investigate whether GH manipulation may influence the SWRs in exploration of a novel environment. The second comparison of SWR median duration, during exploration in novel environment, showed no significant difference between the manipulated GH groups, $H(2)=0.141$, $p=.932$.

Earlier observations have indicated an effect of GH on hippocampal remapping of place fields in response to novel environments, thought to be involved in enhancing spatial memory and adaptively create new cognitive maps of the novel environment (Haugland, 2021). In addition to this, SWR has been indicated to have a role in stabilisation of newly formed place fields (Roux et al., 2017). Therefore, the next analysis examined whether increased GH would lead to increased SWR duration in pot session after exploration of the novel environment, including recordings from pot sessions immediately after exploration of the novel circle environment and recordings from the last pot session of the experimental trials where novel environment was included. The third comparison of SWR median duration, during pot session after exploration of novel environment, showed no significant difference between the groups, $H(2)=0.517$, $p=.772$.

Because earlier studies have found SWR increase in resting periods directly after learning, an additional analysis was conducted using only the pot session recordings immediately after exploration of the novel circle environment. Comparing SWR duration in pot session after exploration of novel circle environment showed no significant differences between the GH groups, $H(2)=0.622$, $p=.733$.

Table 2.

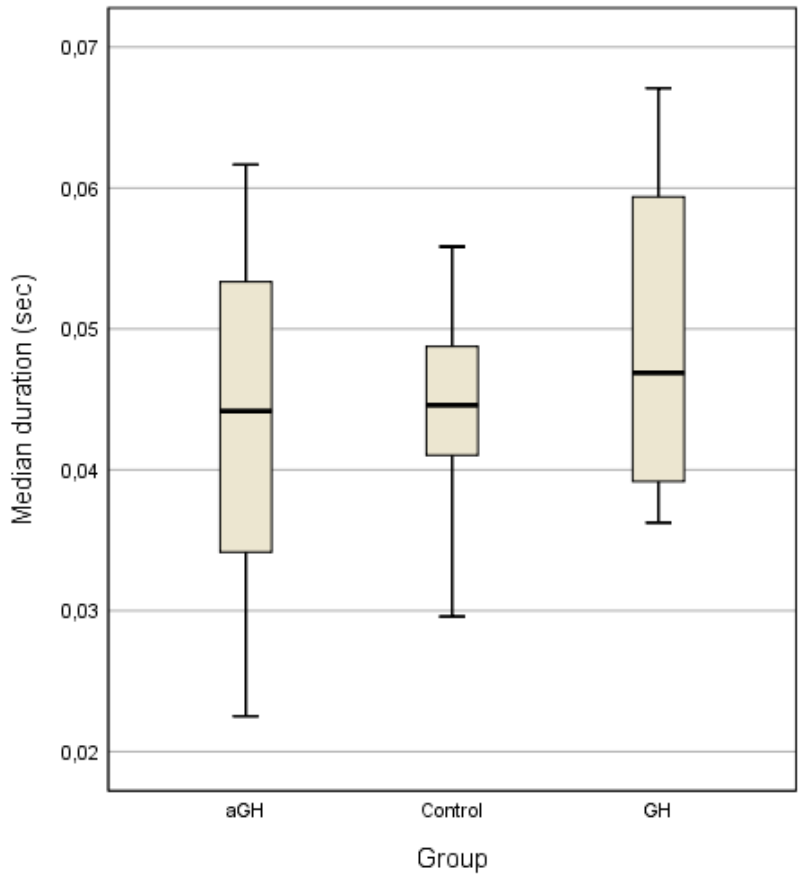
All Group Means of the Observed median Sharp Wave Ripple Durations.

Recording setting	aGH	Control	GH
Pot before	0.044 (0.015)	0.050 (0.009)	0.049 (0.010)
Exploration	0.044 (0.009)	0.049 (0.017)	0.047 (0.008)
Pot after circle only	0.043 (0.016)	0.052 (0.005)	0.036
Pot after last	0.044 (0.009)	0.041 (0.007)	0.054 (0.013)
Pot after (both)	0.044 (0.013)	0.044 (0.008)	0.049 (0.014)

Note. All values are depicted as mean (SD).

Figure 11.

Median Sharp Wave Duration in Pot Session after Novel Environment



Note. Boxplots include recordings of the different groups in pot session after exploration of novel environment.

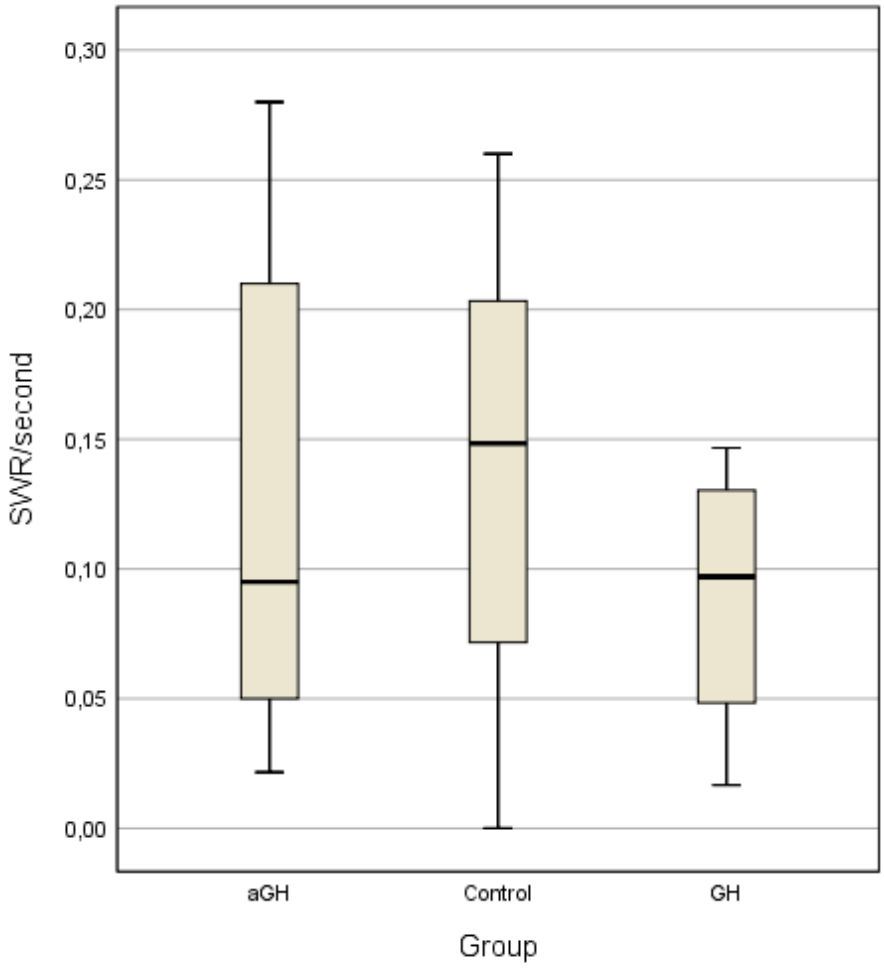
6.2.2 Effects of GH manipulation on SWR rate

The second hypothesis stated that aGH group will show lower SWR-rate. An earlier study has indicated aged animals exhibiting lower SWR rate during sleep compared to younger (Wiegand et al., 2016). It could thus be assumed that the effect of aGH resembles the age-related effects of lower SWR rate. Analysis of SWR rate was done on the same recordings as for analysis of SWR median duration and the statistical analysis follows the same structure. The first comparison of SWR rate was done on recordings in pot before exploration. There was found no significant differences between the groups, $H(2)=2.704$ $p=.259$.

Then for the second comparison, I asked if SWR rate would differ between the GH groups during exploration of the novel environment. Comparison of SWR rate during exploration in the novel environment showed the difference to be non-significant, $H(2)=1.765$, $p=.414$.

Figure 12.

Sharp Wave Ripple-rate in Awake Exploration of Novel Environment



Note. Boxplot showing GH, Control and GH values for SWR rate in SWR/second.

I also wanted to examine whether there was observed effects of GH manipulation in pot session recordings after exploration in novel environment. However, the third comparison of SWR rate, during pot session after exploration of novel environment, indicated no significant difference between the groups, $H(2)=0.357$, $p=.836$.

6.2.3 Effects of GH manipulation on long duration SWRs

The fraction of long duration SWRs are indicated to increase in novel environment and causally linked with better spatial memory (Fernández-Ruiz et al., 2019). Therefore, I set out to investigate whether GH manipulation would affect the fraction of long duration SWRs. As in the previous analyses, I examined the fraction of SWR >100ms in recordings during the first pot session, the awake exploration, and the subsequent resting period in pot. Testing the hypothesis stating that the GH-overexpression group are expected to exhibit a larger fraction of long duration SWRs.

Using independent samples Kruskal Wallis test, comparison of the groups in the first pot session showed no significant differences, H=0.643, p=.725. There was not found significant differences between the GH groups for fraction of long duration SWR (>100ms) during exploration of a novel environment, H=0.892, p=.640. Statistical comparison using independent samples Kruskal Wallis test showed no significant difference between the groups of fraction of SWR >100ms in pot session after exploration of a novel environment, $H(2)$=2.034, p=.362.

When separating the pot session after novel environment, and assessing the ones immediately after circle exploration and after the last box session separated, there was not found any significant group differences for either ($H(2)$=2.034, p=.362. and $H(2)$=2.072, p=.362., respectively).

No multiple comparisons were conducted, nor was effect sizes were calculated, due to the lack of finding of significant differences.

7 DISCUSSION

7.1 MAIN FINDINGS

The aim of the current study was to assess the detection of the important cognitive biomarker, the hippocampal sharp wave-ripple, in addition to investigate possible effects of growth hormone on SWR properties. The performance of the SWR detection was assessed by deliberate description of its implementation and detection output, and was found to be acceptable, although there was both cases of false positives and false negatives. While the effect of GH was assessed by manipulating the amount of hippocampal growth hormone, assigning rats to groups either overexpressing growth hormone, expressing a growth hormone-receptor antagonist, or the control group with no change in growth hormone. The neural activity of hippocampal region CA1 was recorded, measuring the local field potential from which the sharp wave ripples were detected and properties hypothesised to be affected by growth hormone were calculated. The analysis of group differences took into consideration the behavioural state of the rat during the recording, in addition to previous experiences and assumed cognitive load, due to its effect on the sharp wave ripple parameters examined. Thus, the statistical analyses were conducted on recordings in the same state and experimental setting. None of the statistical analyses conducted found statistically significant differences between the groups. However, this makes for an interesting discussion, where I will focus largely on methodological concerns related to the study and attempting to reflect on underlying causes of the results.

7.2 METHODOLOGICAL CONSIDERATIONS

7.2.1 Considerations of study design

To manipulate hippocampal GH, rAAVs was used to either promote GH overexpression or antagonist GH. However, with this method involves difficulties concerning the precise amount of virus taken up by the neurons, and the following amount of GH overexpressed (Haugland, 2021). Manipulation using viral infections also implies that there are different animals used in the different manipulation groups. Considering the low sample size, individual differences could therefore largely affect the results. Following this, by

separating the GH manipulation to different groups, it is not possible to establish a direct causal relationship between GH and SWR properties. An optimal method would consist of turning GH overexpression, and GH antagonist, on and off within each animal, reducing the possible confounding factors of individual differences and thereby be able to make causal links between the effect of GH levels on the measured SWR properties. Unfortunately, the current methods available restricts the possibilities for manipulation of GH directly in individual animals.

The experimental setup was not initially designed specifically for study of SWR. Originally, this study was designed to investigate GH effect on remapping of hippocampal neurons in different environments. Thus, not necessarily ideal for SWR assessment. As earlier studies have used tasks the animal needs to learn as part of the experiment, in contrast to the current study in which the animals were freely foraging, only searching for chocolate crumbles. Perhaps tasks of higher cognitive, or mnemonic, demand could be expected to trigger more and longer SWRs than just a novel environment, and potentially also lead to a greater effect of GH on SWR properties. For example, Eschenko et al. (2008) assessed SWRs in rats after learning an odour-reward association, and found elevated numbers of SWR occurrence and longer duration in sleep period following learning in rats that had learned the task. It would thus be interesting to see whether effects of GH on SWRs would be more pronounced in study designs involving tasks of higher cognitive demand, or, if there are effects of GH one memory task performance.

Studies have showed the occurrence of SWR are not evenly spaced through time, where some periods may exhibit high rates of occurrence whereas at other times, there may not be expressed a single SWR in periods lasting minutes (Tingley et al., 2021). This complicates our study somewhat, as the initial recordings have durations spanning from 5 to 10 minutes each. Further complicating the matter, profound amounts of data points were removed after removing saturated periods. Therefore, we would benefit from having longer duration recordings to increase the validity of the parameter concerning ripple rate. This serves as a reason for future studies to conduct longer duration recordings, if possible.

7.2.2 Statistical analyses

For the statistical analysis of SWR duration, a measure of central tendency were selected for each recording to avoid the challenges concerning clustered data (Moen et al.,

2016). Clustered data refers to the case of multiple observations from each experimental unit. In the current study, a possible solution for handling the multiple observations of SWR duration could be to pool all observations across the recordings of each group. However, this leads to concerns regarding the investigation of statistically significant differences between the groups as the multiple observations within each experimental unit are to some extent dependent on each other. Many statistical tests assume independence of variables, and therefore pooling all observations will reduce the power of the analysis. Another solution is to reduce the observations to a single summary statistic by determining a measure of central tendency for the observations. This is what was done in the current study. As the SWR event duration follows a skewed distribution (Fernández-Ruiz et al., 2019). Therefore, a median is used as the measure for central tendency.

Also, an obvious concern is our low sample size. Both the animals used, and recordings included and treated as the experimental unit were lower than comparable studies. The low sample sizes negatively affect the power of the statistical comparisons and the subsequent validity of the interpretation of the results. Both directly caused by the low sample size for the conducted statistical comparison, but also indirectly in that the more powerful parametric tests had necessarily be rejected.

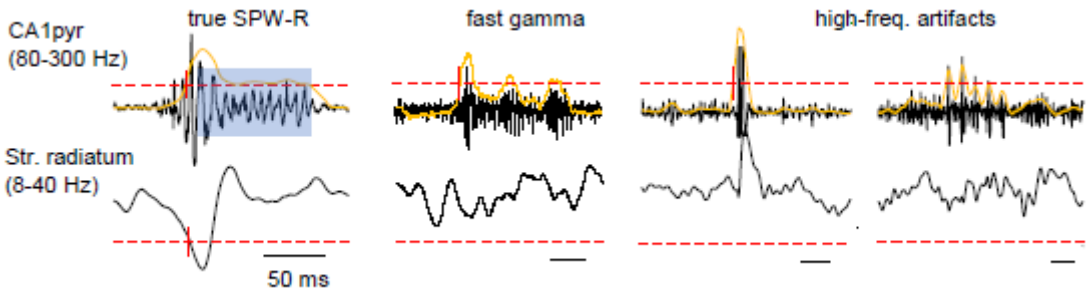
7.2.3 Sharp wave ripple detector

A prominent challenge for the SWR detection was to reduce the detection of false positives while at the same time correctly identifying all true SWR events present in the recording. Although there was applied methods aiming at increasing the detection precision, by executing denoising steps and appropriate filtering described in the methods and results sections, the current study would benefit from additional improvements to increase the precision of SWR detection. One such improvement would be to detect co-occurring sharp wave with the detected ripple, by including LFP channel from CA1 stratum radiatum or CA3, and only select the events where there was observed simultaneous sharp wave and ripple (Fernández-Ruiz et al., 2019). An additional EMG channel would also contribute to the reduction of false positive detection by controlling for muscle activity artifacts which could be present in the ripple band, causing a false positive. As Fig 13 illustrates, inclusion of detection of co-occurring sharp wave would help reject false positives caused by LFP activity in the fast gamma band, muscle activity and chewing artifacts. The figure illustrates four examples of LFP signals, all of which exceeding the threshold of event detection (red dashed line is the

threshold and yellow line the envelope of the LFP), however, only the leftmost are categorised as a true SWR event due to the co-occurring sharp wave in CA1 stratum radiatum.

Figure 13.

Local Field Potential from CA1 stratum pyramidale and CA1 stratum radiatum.

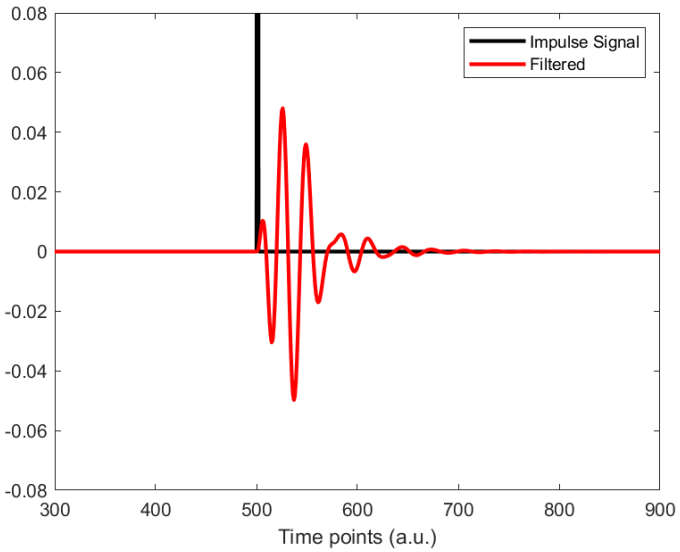


Note. The plots show four periods of the LFP, from Fernández-Ruiz et al. (2019) supplementary information. Horizontal dashed red lines indicate thresholds for both ripple detection (upper plots) and sharp wave detection (lower plots).

When applying the filter kernel to the LFP signal, large and high frequency noise causes unwanted ripple effects in the resulting, filtered signal (see Figure 9 and Figure 14). Both single large deflections and more high frequency fluctuations are observed, in varying amounts, in many of the recordings used in this study. In Figure 9, note the ripple-effects in the bandpassed signal corresponding to the large deflections in the raw LFP, potentially subject to detection of false positive.

Figure 14.

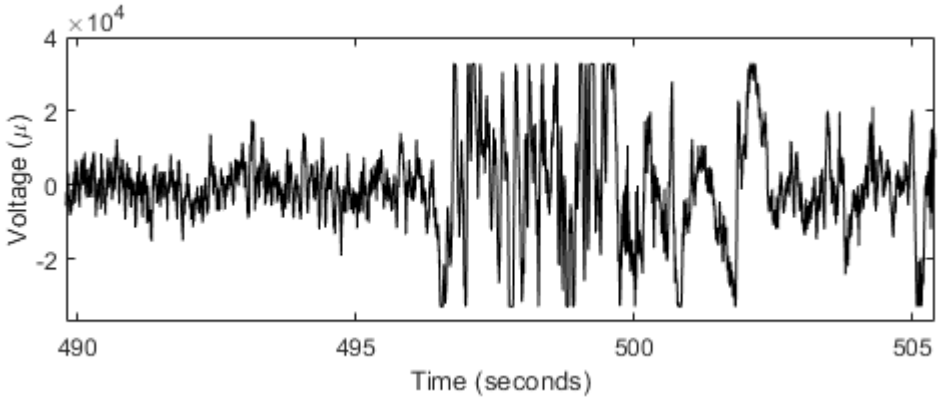
Simulated signal for visualising ripple artifact.



Note. Simulated signal (black line) and bandpass-filtered signal (red line). A visualisation of how a large deflection in the signal causes a ripple artifact in the filtered signal.

Figure 15.

Noise in raw Local Field Potential.



Note. Example of a period of large fluctuation noise from one of the recordings used in this study.

When examining the spectrogram in Figure 9, there are no visible large frequency peak in the ripple-band. Hence, in addition to include supplementary channels measuring and controlling for co-occurring sharp waves or EMG, the detection could also benefit from setting a minimum peak frequency of the event with short time Fourier transform (STFT), as was done by Wiegand et al. (2016), or using multitaper fast Fourier transform to check each event for having a spectral peak within the ripple band (Sullivan et al., 2011).

An intriguing emerging method of SWR detection utilizes deep Recurrent Neural Networks (RNN) with Long Short-Term memory (LSTM) layers, omitting the error prone methods, more prevalent today, that determines SWRs based on somewhat arbitrary defined

thresholds of deviations from the baseline of the power of the bandpassed signal (Buzsáki, 2015; Hagen et al., 2020). This novel method were found to exhibit better accuracy than conventional methods (Hagen et al., 2020). The detector used in the current study however are a modified version of the implementation of Csicsvari (1999) which first bandpasses the signal from which SWR events are detected based on multiples of standard deviations from the resulting normalised squared signal. The parameters for detection used in the current study were based on a combination of that used in other studies, in addition to a manual evaluation of the performance of the resulting detection. As have been explained in the introduction, SWR events exhibits variable amplitude. Consequently, there is no objectively defined standardised threshold for detection, and thus, using an arbitrary defined fixed threshold for detection, will struggle to correctly identify all true SWRs. The prevalent use of different fixed thresholds is therefore a concern, reducing external validity, making comparisons of results between studies to some extent inadequate. The mentioned automated detection of SWRs using LSTM-based RNNs is accessible as an open-source implementation, named RippleNet, and can, due to its independence of arbitrary defined fixed thresholds, contribute greatly to increase both reliability and validity of SWR research.

7.2.4 Qualitative issues

SWRs are in other studies not only found to increase in duration and occurrence rate in response to task learning but are also found to be affected by being exposed to a novel environment (Fernández-Ruiz et al., 2019). For the novel environment used in this study, only the shape of the box was altered, from a familiar square to a novel circle. In comparison, the study from Fernándes-Ruiz et al.(2019) used alterations of mazes, one familiar and one novel, when finding differences of both SWR duration and fraction of long SWR (>100ms) during awake immobility periods in the different mazed. They also compared and found significant differences of SWR duration between periods of performing a memory task and periods of open field exploration, with longer duration SWRs in the memory task setting. It would be interesting to see if there is an interaction between environmental setting and GH when assessing the SWR duration and rate. By designing an experiment to allow the individual animal to progress through the experiment, first in a familiar environment, before being exposed to a novel, cognitive demanding environment, and investigate whether GH manipulation may reduce the rate and duration of SWRs, as GH deficiency are associated with reduced memory abilities, maybe through reduction of SWR duration and rate. And

preferentially, on beforehand, conduct preliminary tests determining an appropriate sample size allowing for the use of a robust parametric repeated measures ANOVA.

The initial inspiration for assessing possible GH effects on SWR were based on the shared involvement both GH and SWR exhibits in memory processes, described in more detail in the introduction. For example, a previous study indicated increased global remapping in response to novel environment in rats with elevated GH levels, in addition to observing improvement of spatial memory (Haugland, 2021). Remapping is proposed as a mechanism for storing separate memories in orthogonal place maps in response to new experiences, and SWRs are indicated to stabilize the place fields, and thus place maps, after exploration of novel environment, through reactivation (Colgin et al., 2008; Roux et al., 2017; van de Ven et al., 2016). Causal relationships has been established between the SWR properties the current study was focusing on and memory, see for example (Fernández-Ruiz et al., 2019; Girardeau et al., 2009). At the foundation of the hypotheses stated were the indicated influence of GH on key biological mechanisms underlying memory (Nyberg & Hallberg, 2013). As GH was indicated to upregulate ion-channels in hippocampal neurons increasing their excitatory properties, it is reasonable to speculate that by increasing GH, subsequent SWR, would be affected. By increasing the potential for excitatory drive in hippocampal neurons, I suspected the excitatory events of SWRs to consequently, exhibit altered properties reflected in longer activity durations and increased sensitivity for triggering of the event.

Finally, the CA1 are not considered the initiating region of the SWRs (Schlingloff et al., 2014). CA3 are shown to both innervate CA1 and be involved in initiating the SWRs by exhibiting gradual excitatory build-up preceding the CA1 ripple (Buzsáki, 1989). Therefore, the neuromodulatory effects of GH manipulations may be greater when applied in upstream areas involved in SWR initiation, i.e., the CA3. By shifting the excitatory drive of the recurrent, and already highly excitable, CA3 there may be triggered more SWRs reflected as increases in SWR rate.

7.3 CONCLUSION AND FURTHER OUTLOOK

The analysis of potential effects of hippocampal GH on SWR properties involved in memory failed to find statistically significant differences between groups of differing amounts of GH. After investigating the SWR properties between the groups using recordings from different behavioural states, there was found no tendencies of differences either. Various feasible reasons for this have been

discussed, among them, aspects concerning detection of events, although the small sample size may be considered the definitive issue.

Considering SWR detection, the limitations of the use of arbitrary defined fixed threshold in detection were described and examined. Hopefully, the discussion provides a rationale for the contribution the development of more objective and valid methods of SWR detection, as is initiated with the RippleNet, may carry (Hagen et al., 2020). This may also contribute to increasing the external validity of SWR detection, providing improved possibilities for sensible comparison of results between different studies. The development of increasingly accurate methods of SWR detection are of great importance for propelling the neuroscientific study further. As the introduction hopefully established, SWR is an immensely intriguing neural activity pattern involved in multiple cognitive processes essential for both humans and animals.

Simultaneously, increasing our knowledge of age-related fluctuations of hormonal influence on the neurobiological foundation of important cognitive functions remains an important part of neuroscientific research, both for clinical purposes and as a way of gaining knowledge of the workings of the brain and nervous system.

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9 APPENDIX – TABLES AND FIGURES FROM THE STATISTICAL ANALYSIS

For all boxplots, extreme outliers are marked with an asterisk (below 3*interquartile range (IQR) for the 1st quartile, or above 3*IQR for the 3rd quartile) and regular outliers (below 1.5*interquartile range (IQR) for the 1st quartile, or above 1.5*IQR for the 3rd quartile) are marked with a circle.

9.1 APPENDIX A – TABLE AND FIGURES FROM MEDIAN SWR FROM ALL RECORDINGS

Table 1.

Showing the median SWR duration in recording sessions in all settings in each group with mean and SD for each group in the last row.

	First pot session			Exploration			Last pot session		
	aGH	Control	GH	aGH	Control	GH	aGH	Control	GH
	0.070	0.059	0.040	0.045	0.045	0.047	0.053	0.041	0.052
	0.043	0.049	0.048	0.063	0.041	0.045	0.035	0.030	0.067
	0.044	0.042	0.037	0.048	0.042	0.038	0.057	0.041	0.036
	0.039		0.043	0.038	0.041	0.058	0.023	0.049	0.042
	0.045		0.058	0.036	0.048		0.031	0.056	
	0.062		0.054	0.048	0.043		0.062	0.048	
	0.041		0.064	0.031	0.045		0.051	0.045	
	0.022			0.048	0.090		0.038		
	0.027			0.043			0.034		
							0.053		
Mean (SD)	0.044 (0.015)	0.050 (0.009)	0.049 (0.010)	0.044 (0.009)	0.049 (0.017)	0.047 (0.008)	0.044 (0.013)	0.044 (0.008)	0.049 (0.014)

Figure 1.

Pot before experiment

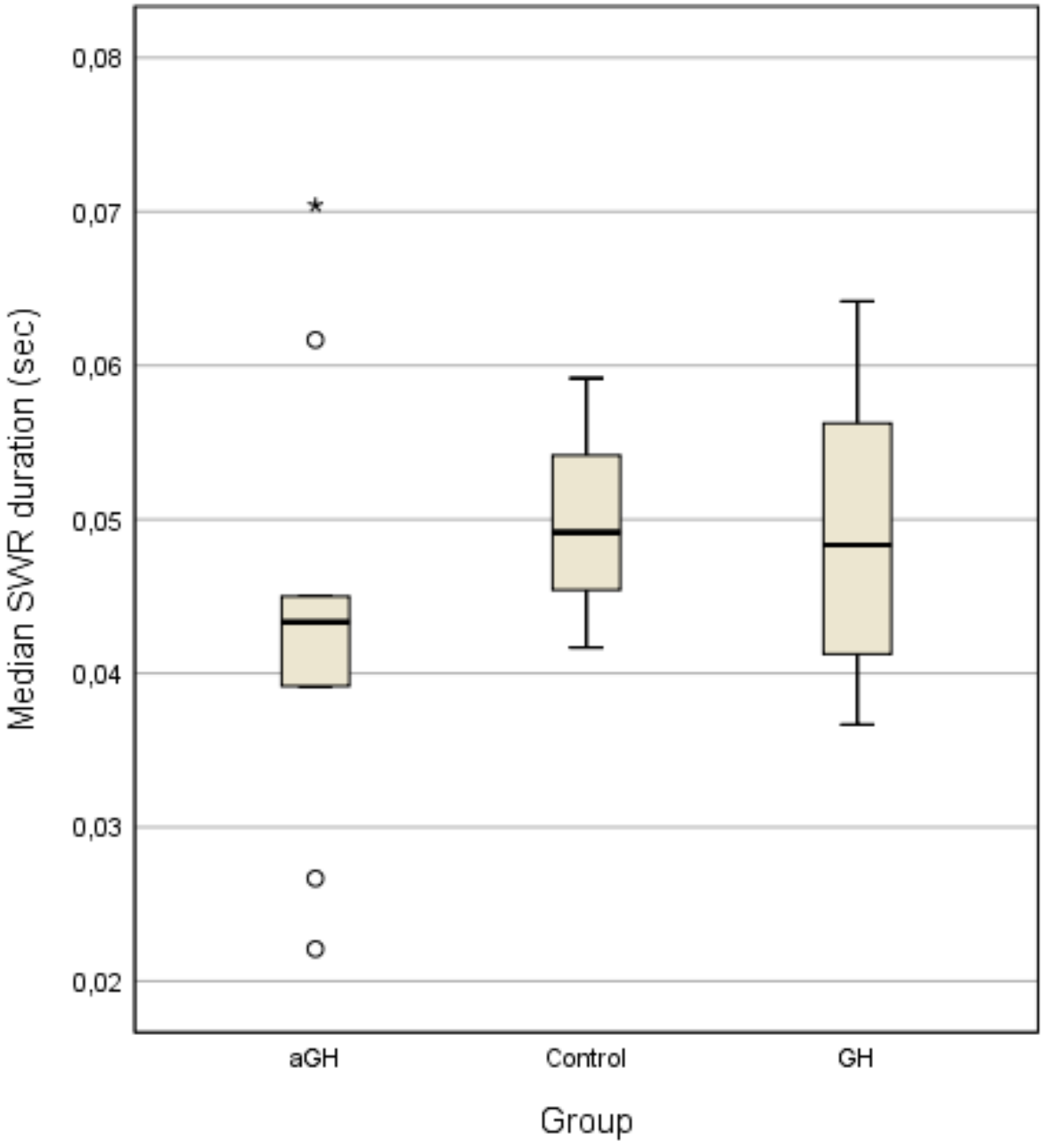


Figure 2

Exploration

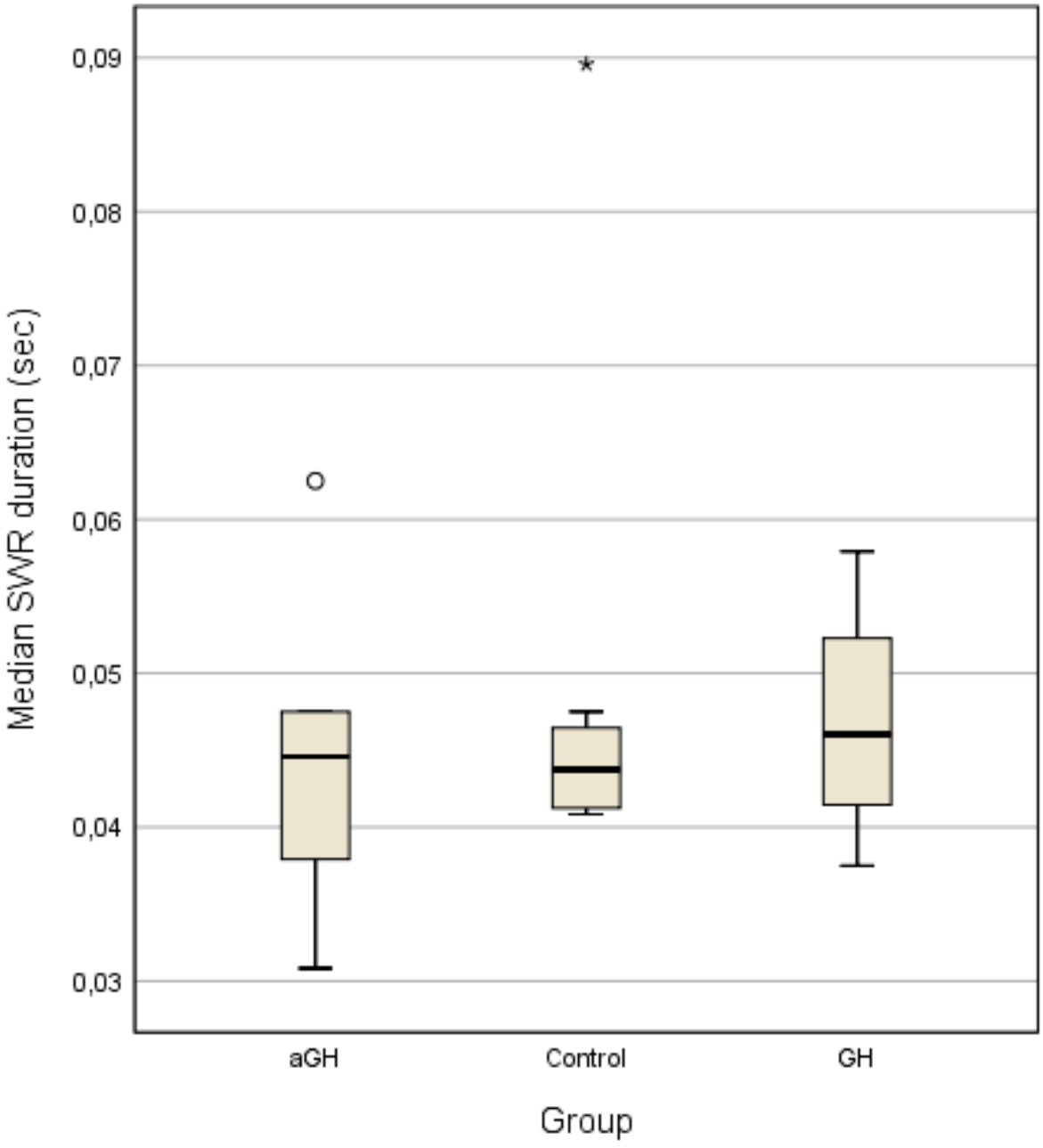


Figure 3

Pot immediately after circle

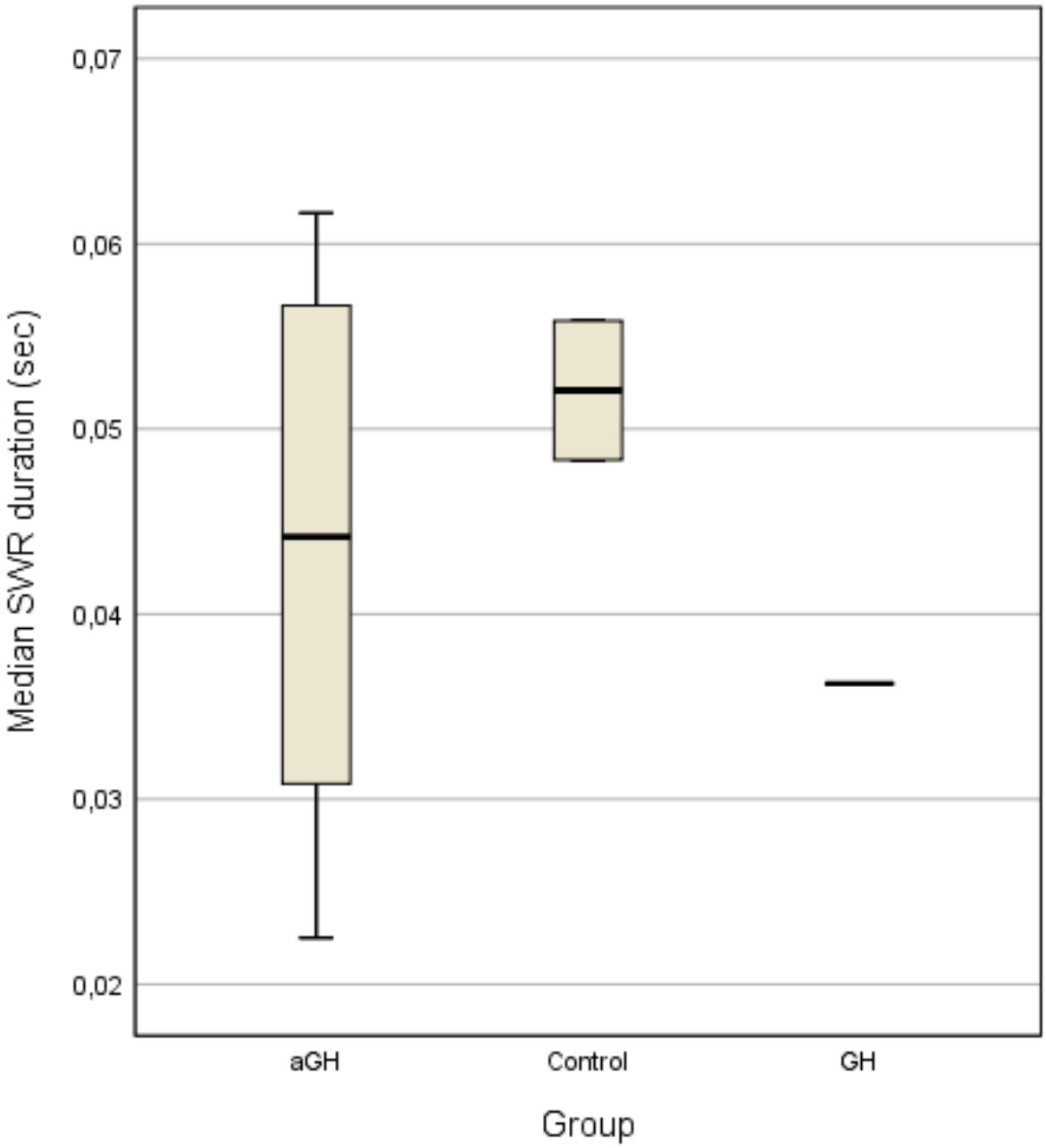
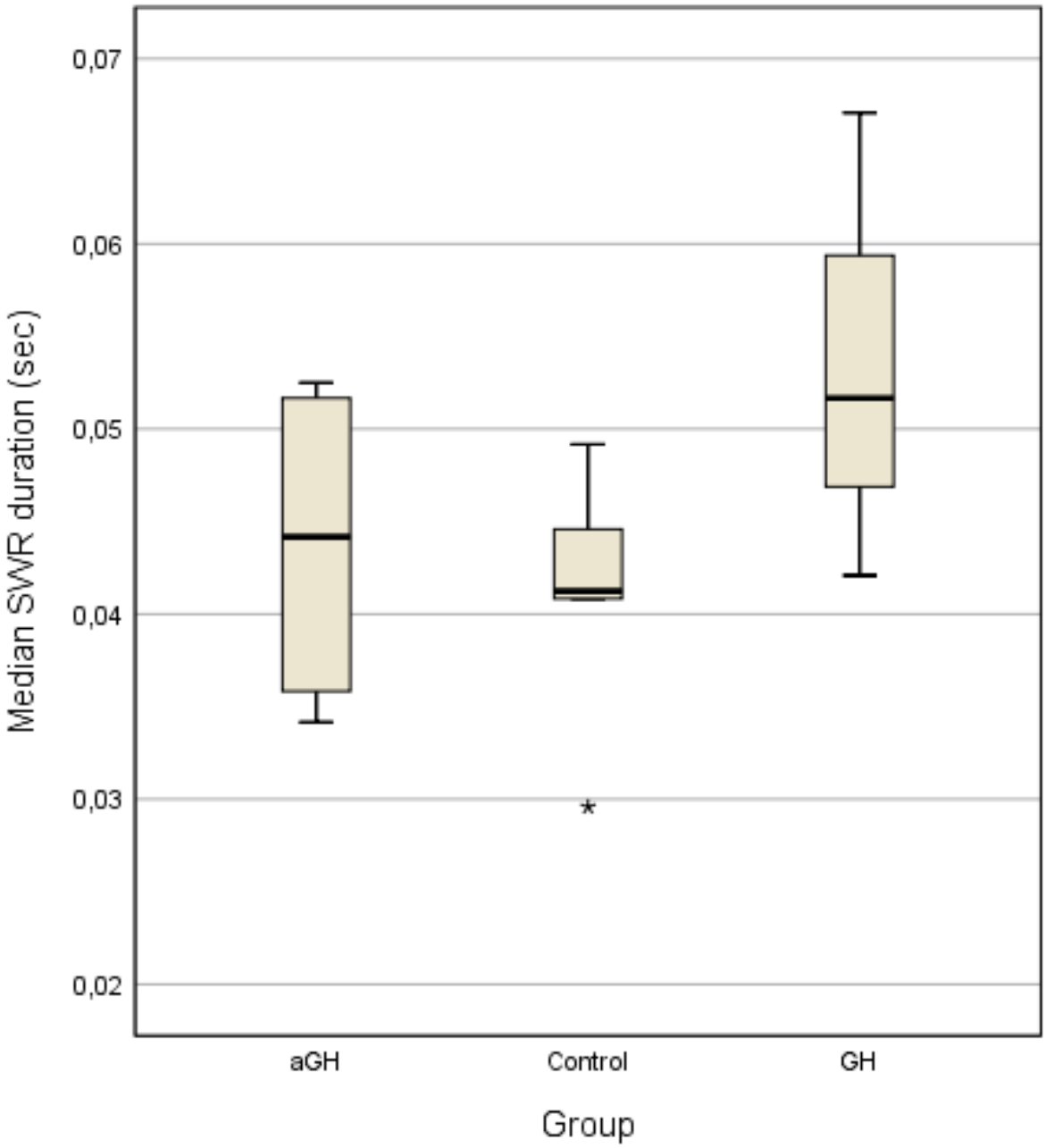


Figure 4

Pot after last box



9.2 APPENDIX B – TABLE AND FIGURES OF SWR RATE FROM ALL RECORDINGS

Table 2.

SWR rate all observations

	First pot session			Exploration			Last pot session		
	aGH	Control	GH	aGH	Control	GH	aGH	Control	GH
	0.150	0.013	0.081	0.280	0.260	0.182	0.123	0.093	0.123
	0.113	0.053	0.023	0.243	0.252	0.147	0.107	0.057	0.053
	0.067	0.027	0.023	0.210	0.203	0.114	0.097	0.055	0.045
	0.033		0.020	0.148	0.172	0.080	0.077	0.043	0.013
	0.027		0.013	0.095	0.148	0.017	0.050	0.040	
	0.027		0.010	0.055	0.147		0.042	0.019	
	0.023		0.010	0.050	0.072		0.042	0.003	
	0.013			0.040	0.013		0.030		
	0.010			0.022	0.000		0.018		
							0.017		
Mean (SD)	0.078 (0.049)	0.031 (0.020)	0.032 (0.025)	0.195 (0.097)	0.207 (0.095)	0.108 (0.063)	0.060 (0.038)	0.044 (0.029)	0.059 (0.046)

Figure 5

Pot before experiment

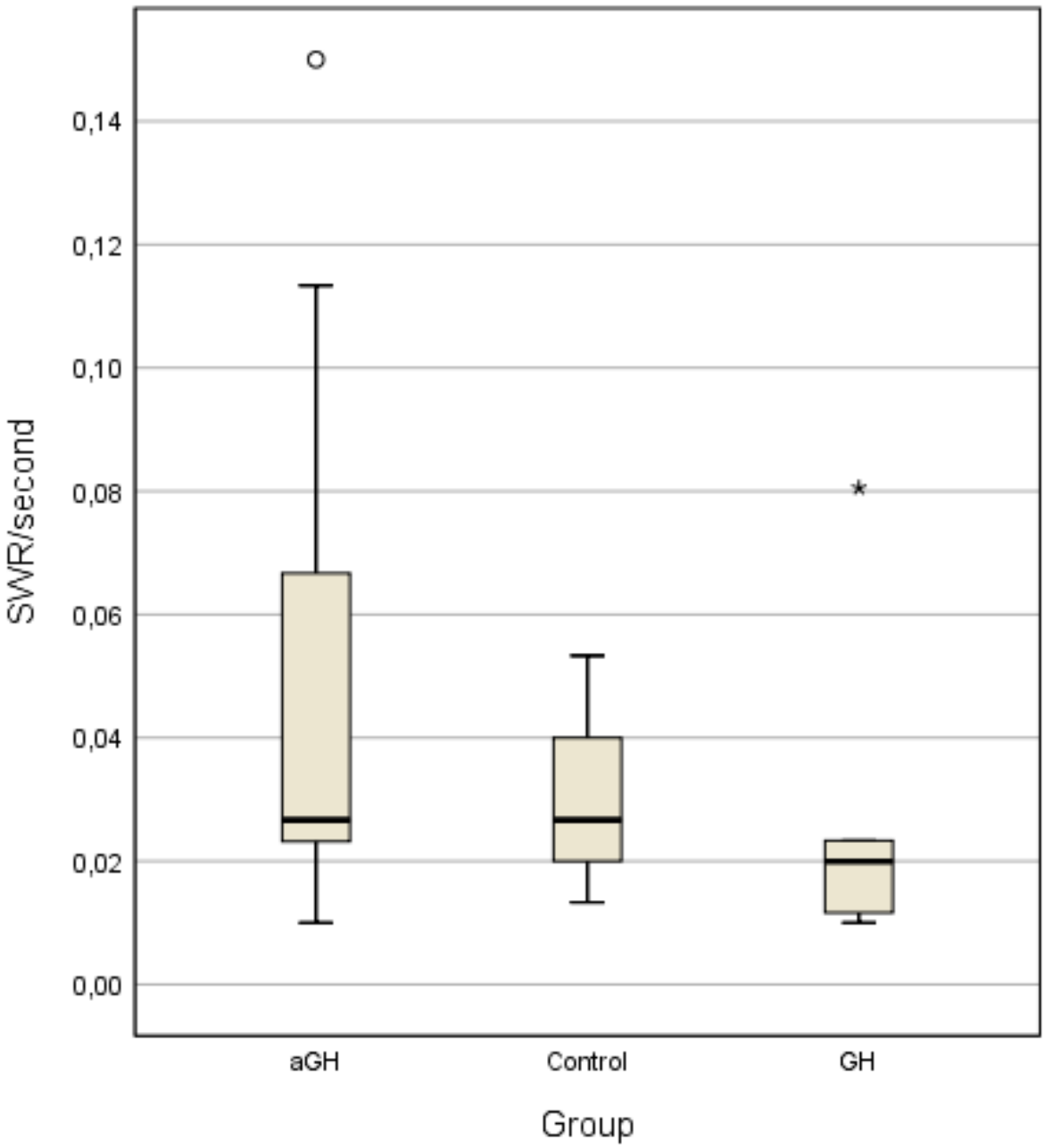


Figure 6

Exploration

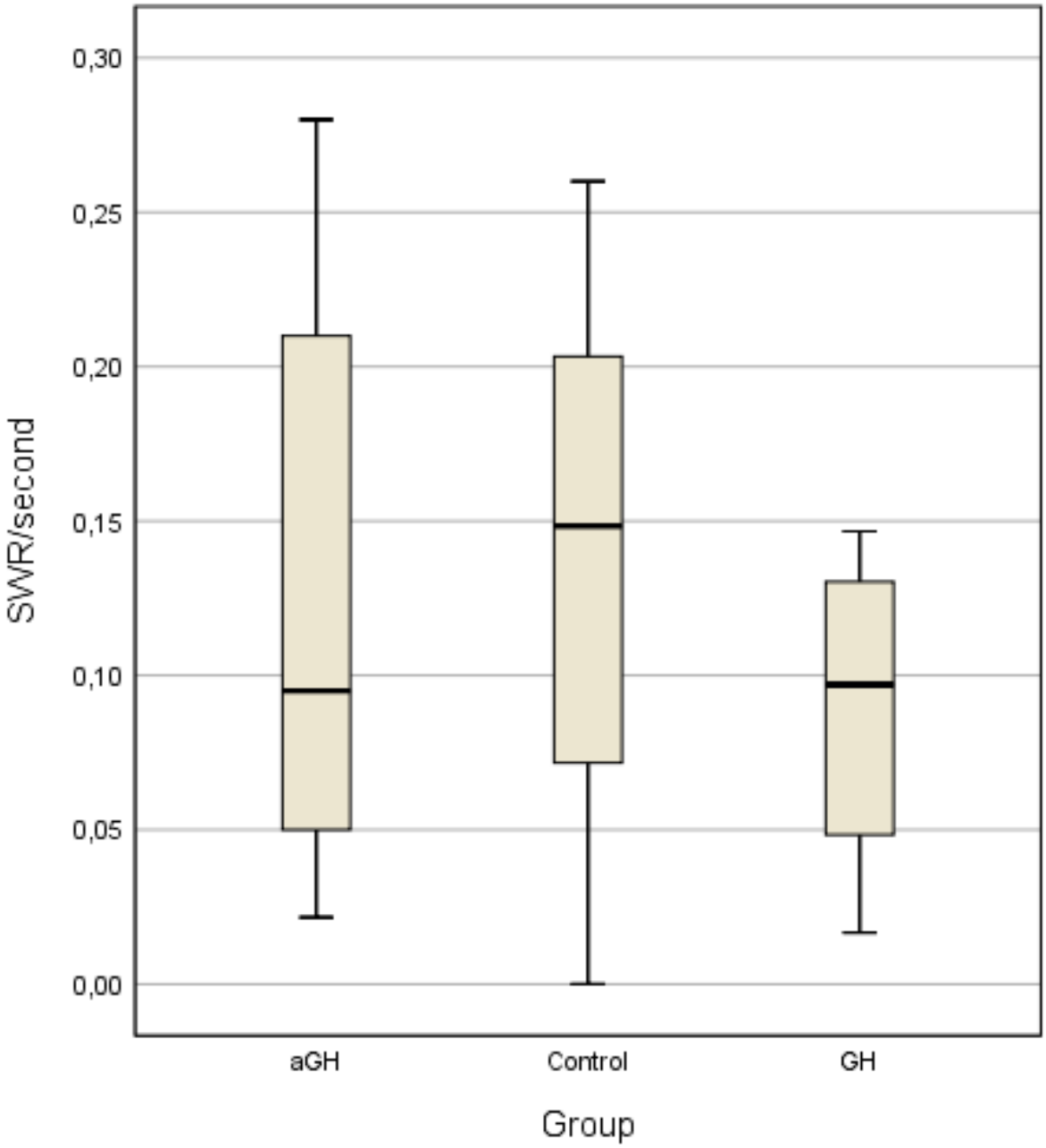


Figure 7

Pot last circle

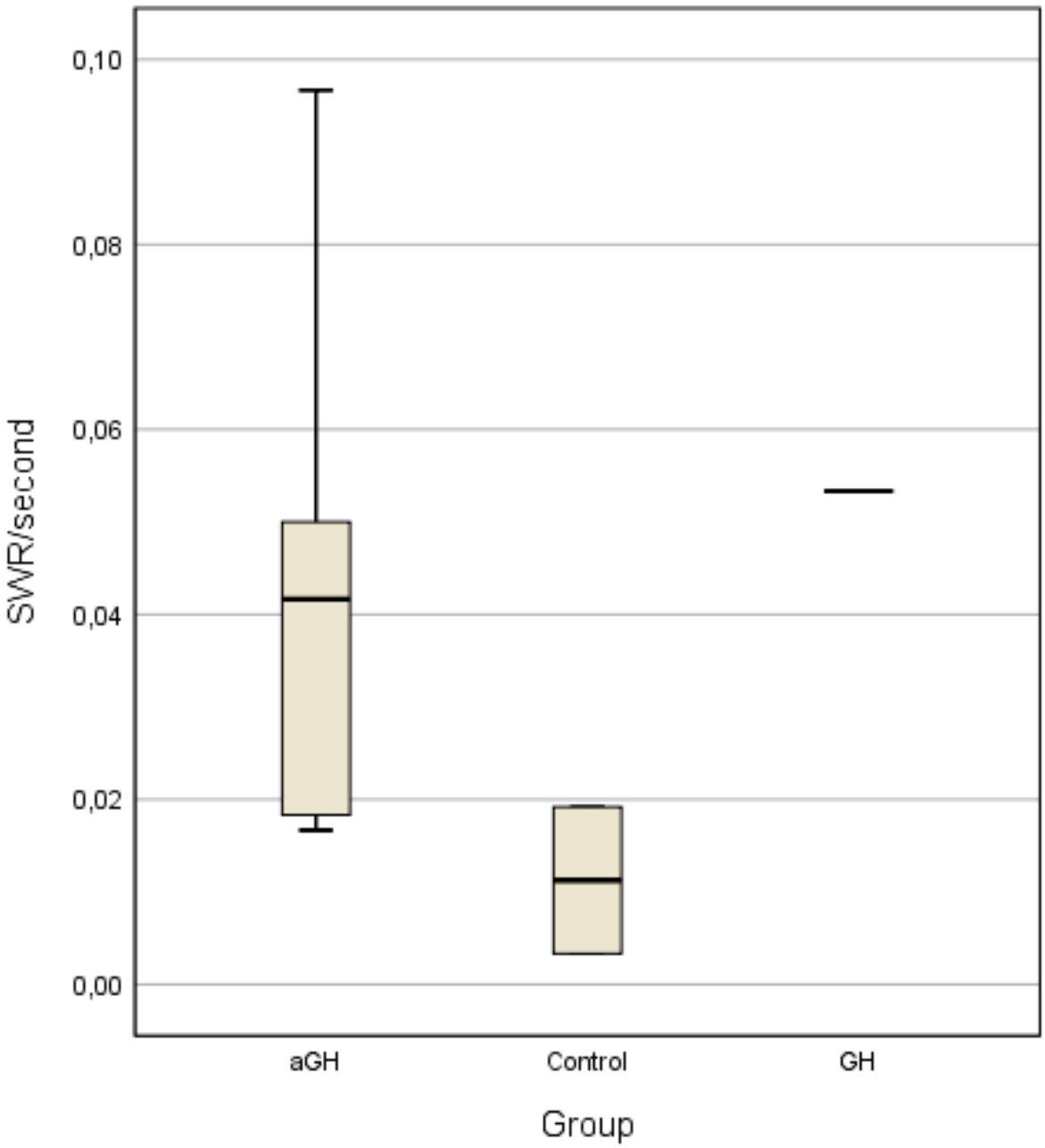
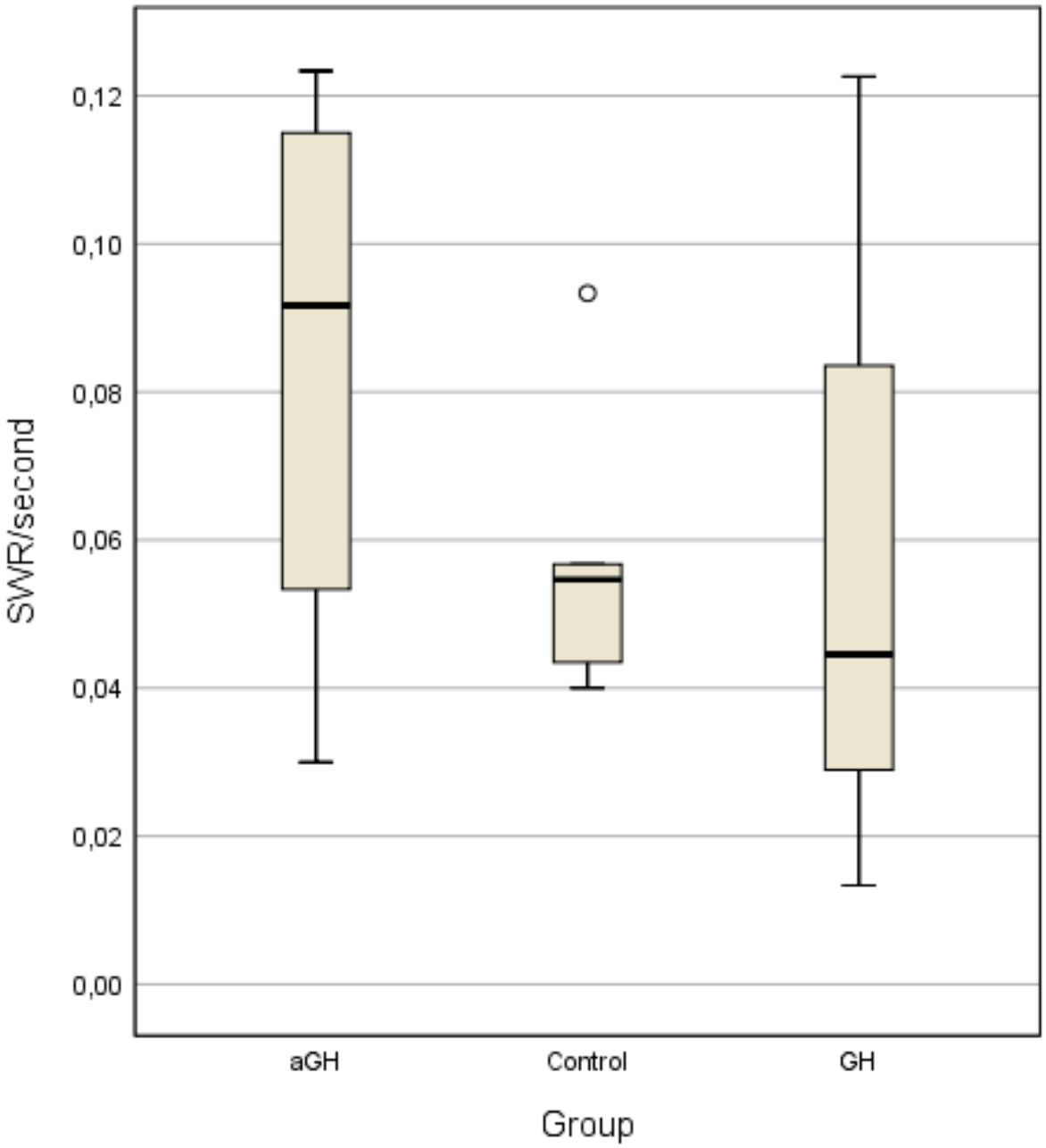


Figure x

Pot last box


SWR/second
0,12
0,10
0,08
0,06
0,04
0,02
0,00
aGH
Control
GH
Group

9.3 APPENDIX C – TABLES AND FIGURES OF FRACTION LONG DURATION FROM ALL RECORDINGS

Figure 8

Pot before experiment

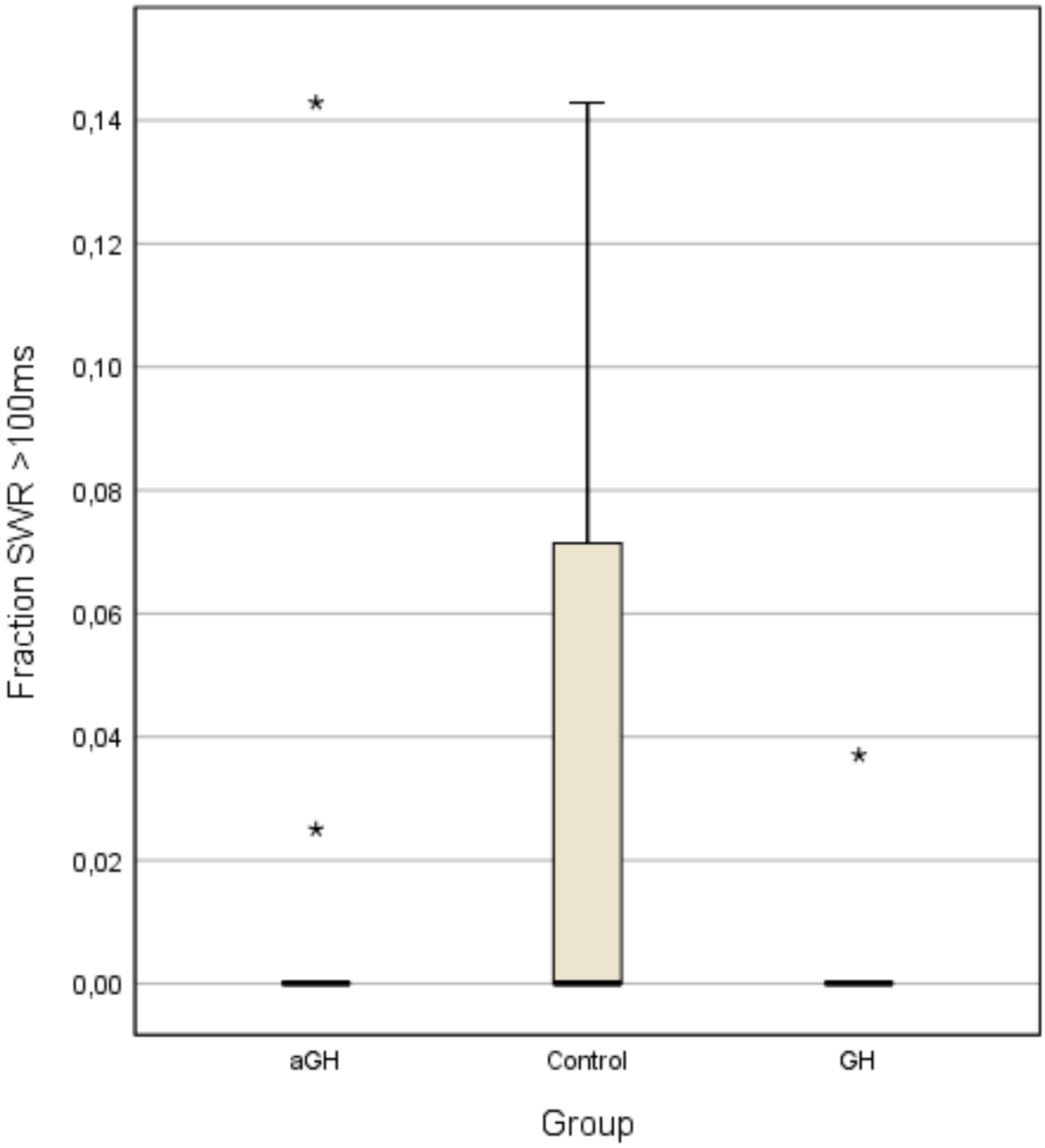


Figure 9

Exploration

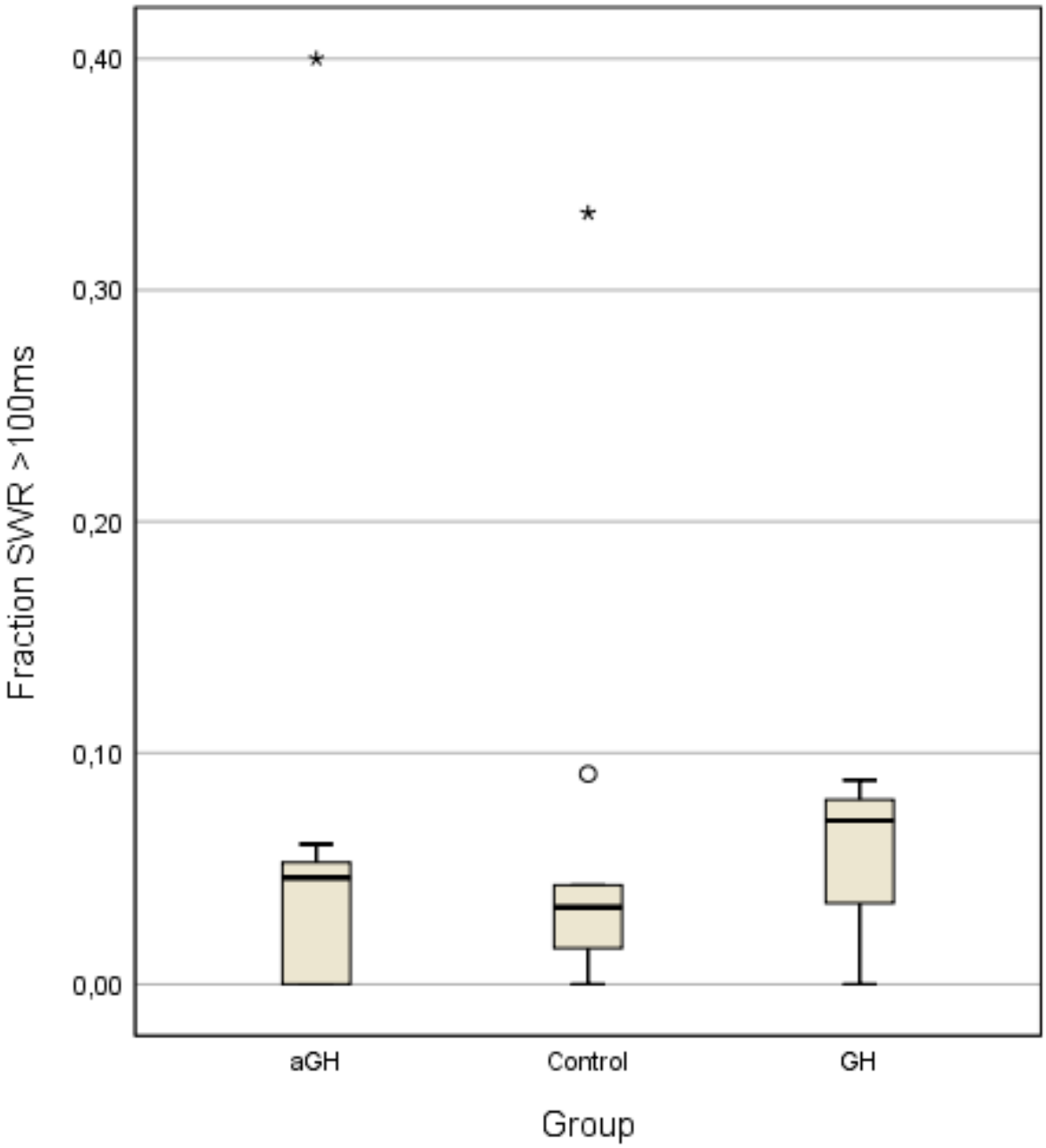


Figure 10

Pot last circle

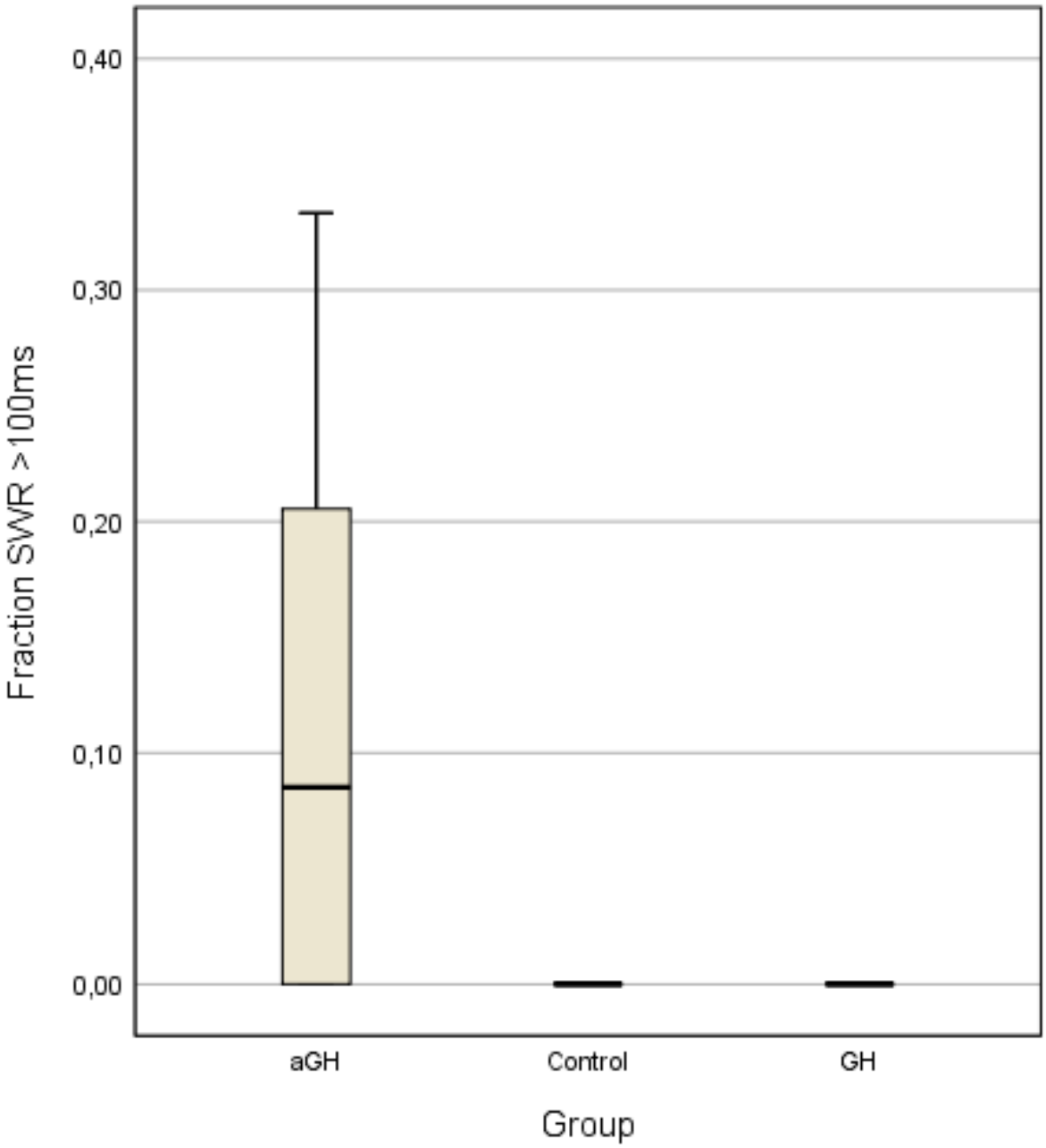


Figure 11

Pot last box

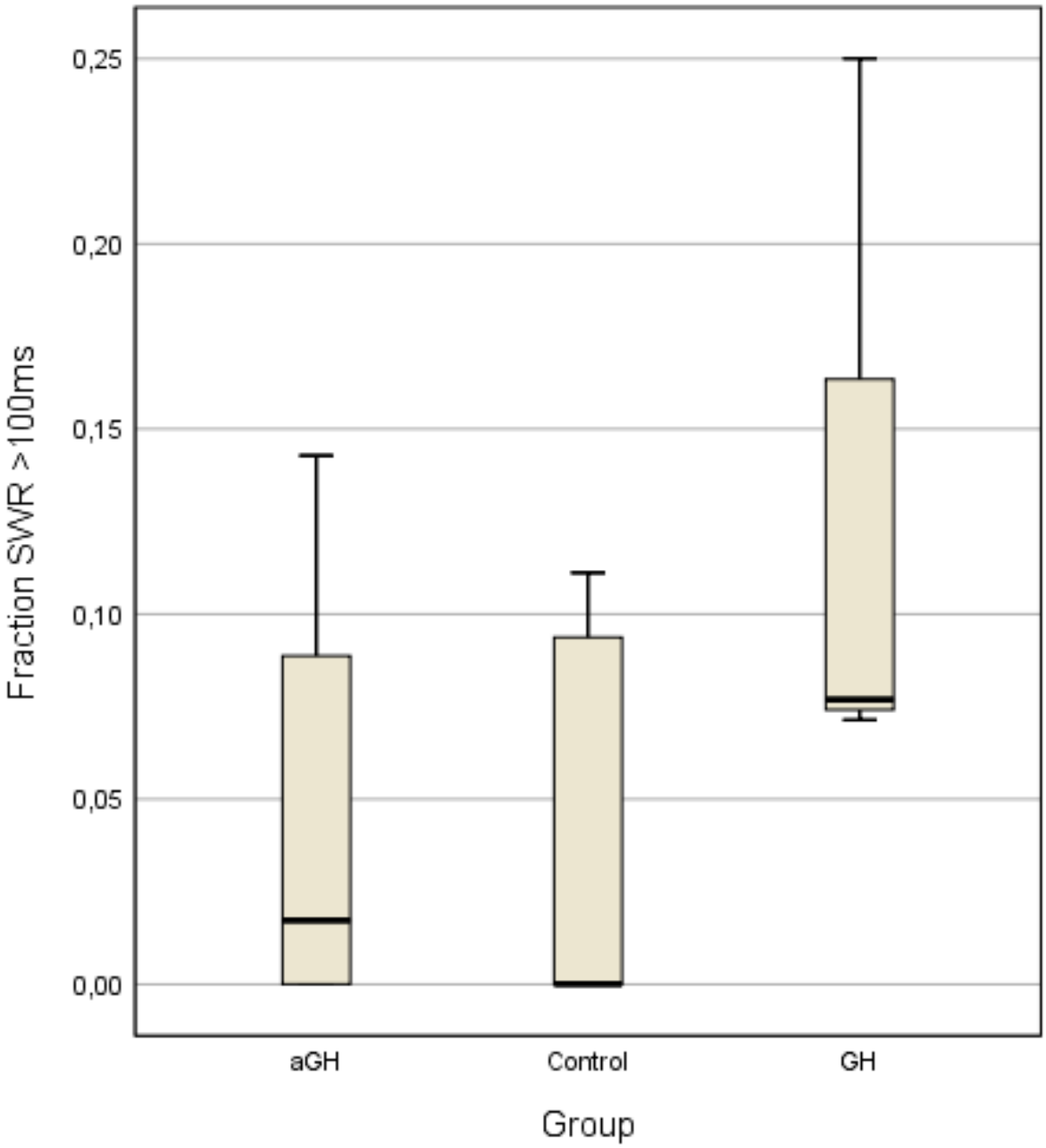


Table 3.

Pot session after exploration novel environment. After circle only

	SWR rate			Media duration			Fraction Long SWRs		
	aGH	Control	GH	aGH	Control	GH	aGH	Control	GH
	0.050	0.003	0.053	0.053	0.056	0.036	0.100	0	0
	0.097	0.019		0.035	0.048		0.070	0	
	0.042			0.057			0.205		
	0.042			0.023			0.000		
	0.018			0.031			0.000		
	0.017			0.062			0.333		
Mean (SD)	0.044 (0.029)	0.011 (0.011)		0.043 (0.016)	0.052 (0.005)		0.118 (0.130)	0 (0)	

Table 4.

Pot session after exploration novel environment. Last pot session only.

	SWR rate			Media duration			Fraction Long SWRs		
	aGH	Control	GH	aGH	Control	GH	aGH	Control	GH
	0.030	0.055	0.123	0.051	0.041	0.052	0.000	0.000	0.071
	0.123	0.093	0.013	0.038	0.030	0.067	0.034	0.000	0.250
	0.077	0.043	0.045	0.034	0.041	0.042	0.000	0.000	0.077
	0.107	0.057		0.053	0.049		0.143	0.094	
		0.040			0.045			0.111	
Mean	0.084	0.058	0.060	0.044	0.041	0.054	0.044	0.041	0.133
(SD)	(0.041)	(0.021)	(0.056)	(0.009)	(0.007)	(0.013)	(0.068)	0.056	0.102