MULTIFRACTAL COMPLEXITY OF HIPPOCAMPAL MEMORY PROCESSING

BY

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List of Abbreviations

CR – Choice Response
DSE/DSI – Depolarization-induced Suppression of Excitation/Inhibition
DMS – Delayed Match-to-Sample
DNMS – Delayed Nonmatch-to-Sample
DWT – Discrete Wavelet Transform
FCT – Functional Cell Type
ISI – Interspike Interval
LNP – Last Nosepoke
LRTC – Long-Range Temporal Correlations
LTD – Long-Term Depression
LTP – Long-Term Potentiation
MF-DFA – Multifractal Detrended Fluctuation Analysis
SR – Sample Response
THC – delta-9-tetrahydrocannabinol
WLMA – Wavelet Leaders-based Multifractal Analysis
ABSTRACT

The hippocampus has long been associated with learning and memory occurring as reverberating activity patterns within ensembles of hippocampal neurons. Fractals, omnipresent in biological and physical systems, often manifest when systems are iterative or reverberatory, and consequently are detectable in spike trains of hippocampal neurons. This dissertation focuses on strengthening the association of neurophysiological forms of learning, memory and plasticity with fractal and multifractal complexity of interspike interval sequences recorded during numerous cognitive and behavioral conditions.

Chapter 2 provides methodological descriptions of three different forms of multifractal analysis relevant for results described in this dissertation.

Multifractal analysis of hippocampal neural spike trains recorded during the delayed nonmatch-to-sample task (DNMS) analyzed in Chapter 3 showed that memory processing neurons (“functional cell types”) exhibited greater long-range temporal correlations (LRTCs) and stronger multifractality than neurons without memory-related properties. By revealing increased multifractal complexity of unidentified neurons, this analysis promoted identification of additional functional cell types. Tetrahydrocannabinol (THC), the primary psychoactive component of Cannabis known to impair short-term memory, selectively reduced multifractal complexity of memory processing neurons.

Chapter 4 investigated whether LRTCs and multifractal complexity of hippocampal spike trains were endogenously-occurring phenomena or features of active memory processing. By recording from the same neurons before, during and after the DNMS task, the analysis showed that optimal memory processing was characterized by augmented multifractality and reduced LRTCs. THC was further shown to actively
suppress hippocampal network transitions normally supporting transfer from memory processing to resting states.

Changes in multifractal complexity associated with cognitive flexibility were examined in Chapter 5 using a reversal learning paradigm. Increased LRTCs and multifractality during the rule transition phase demonstrated that interweaving fractal dynamics are a property of flexible memory processing, supporting the notion of fractal synaptic plasticity features. The presence of strong LRTCs during the delay phase suggested that repetitive interspike interval sequences support working memory maintenance.

Taken together, the evidence supports the finding that LRTCs and multifractal complexity are markers of synaptic plasticity and neuronal computation. Estimation of the multifractal singularity spectrum can facilitate detection of pathological, cognitive and physiological states with implications for disease prevention and neuronal prosthetics.
CHAPTER 1: INTRODUCTION

Fractals, first described by Benoit Mandelbrot (1982), are naturally occurring phenomena characterized by infinitely complex, self-similar patterns across multiple spatial and/or temporal scales. Non-linear feedback and reiteration between multiple processes generate variability that often results in the appearance of complex fractal structures (Mandelbrot, 1982). Physical and physiological systems sometimes exhibit multiple interacting and interweaving fractal patterns that produce multifractal complexity. Brain function, specifically mnemonic processing, is known to rely on non-linear interactions and computations. The non-linearity of hippocampal memory function was analyzed to predict neuronal activity patterns in order to derive electrical stimulation protocols that enhance memory performance in rats (Berger et al., 2012; Hampson et al., 2012; Deadwyler et al., 2013) and nonhuman primates (Hampson et al., 2013). This dissertation focuses on integrating literature and research across multiple disciplines, including neuroscience, physiology, psychology and physics, in order to create a coherent description of the fractal and multifractal properties of hippocampal learning and memory. By recording hippocampal spike trains during operant conditioning working memory paradigms, I showed that long-range temporal correlations, an indication of monofractal character, are a feature of memory transmission. Additionally, multifractal complexity was revealed to coincide with effective memory processing in the absence of a memory-impairing cannabinoid, THC, and to reflect task complexity throughout reversal learning. Fractal and multifractal aspects of hippocampal interspike interval sequences may reflect multiple forms of synaptic plasticity occurring throughout the studied learning and memory paradigms.
1.1. Hippocampal Learning and Memory

1.1.1. Learning and memory

An enriched understanding of learning can be generated by integrating psychological, neurophysiological and fractal (physical) perspectives. Psychologically, learning is the acquisition of knowledge, skills, understandings or behaviors that may be integrated and synthesized with previous information via experience or study. Learning can occur after experiences or studying arising as goal-driven method to shape behavior over time. Performance is guided by using current and prior error information as feedback to elicit learning. Neurophysiologically, learning is hypothesized to occur via strengthening synapses with long-term potentiation (Bliss and Collingridge, 1993) or weakening them with long-term depression (Linden, 1994). Learning mechanisms that facilitate long-term behavioral consistency by continuously integrating single trial accuracy information may generate fractal long range correlations in involved time series (Wong and Shelhamer, 2013). In this fractal (physical) view, learning occurs via (nonlinear) feedback to generate memory reflected as the evolution of fractal patterns.

Memory is the ability to mentally store, maintain and retrieve information. Memory can be decomposed into three stages from an information processing perspective: 1) encoding or acquisition (receiving, processing and combining incoming information); 2) maintenance or storage (constructing a record of the acquired information); and 3) recall or retrieval (bringing back stored information). When categorizing based on content, memories can be classified as either declarative (explicit) and procedural (implicit) forms. Declarative memory involves events, people facts or figures that can be explicitly stated or explained while procedural memory is derived from motor programming.
Memories can also be classified based on their duration, where short-term memory transiently encodes moment-to-moment information and long-term memory archives information. Working memory is used to manipulate and transform information for very short durations and is distinct because it will be quickly forgotten. Short-term memory refers to ongoing properties of memory used to influence and alter behavior in ways that may be transferred to long-term storage. Rehearsal of information in short-term memory is required for storage and long-term retention. Short-term memory is estimated to have a capacity of about seven items or “chunks” of information. Long-term memory refers to a set of rules or ideas instilled in a more permanent manner. These memories can be recalled and used to influence ongoing behavior. An unlimited amount of long-term memories can be stored for an infinite amount of time.

From a dynamical systems perspective, long-range (temporal) correlations, also known as simply “long-term memory” in this literature, may arise when multiple timescales of memory interact and interweave in one signal. Two timescales could be derived from long-term and short-term (or working) theories of memory: a slow process that gradually learns and retains learned information and a fast process that learns and forgets rapidly (Wong and Shelhamer, 2013). Beyond these two examples, multiple long-term time scales could be derived from different events (i.e., days, months or years) and from different short-term processes (i.e., visual, auditory or physical). Long-range temporal correlations result when memory persists in a signal through time and imply “rich information content, high flexibility and purposeful variability” (Slifkin and Newell, 1999; Riley and Turvey, 2002; Wong and Shelhamer, 2013). Fractal dynamics operate through reiteration to enable robust and quick responses from an underlying system. Multifractal complexity emerges from the interaction of multiple fractal processes across spatiotemporal scales.
1.1.2. Hippocampal Anatomy and Connections

The hippocampus is located in the medial temporal lobe underneath the cerebral cortex. The name “hippocampus” originated from Latin because its physical appearance resembled a seahorse. The term hippocampal formation is used for the dentate gyrus (DG), the Cornu Ammonis (CA) fields CA1-4 and the subiculum (Figure 1.1). CA1 and CA3 are the largest and most readily recognized. Hippocampal information flows from DG to CA3 to CA1 and to the subiculum, with additional inputs arriving at all stages and outputs from the last two areas. CA2 is a very small region of the hippocampus and its presence is typically ignored in descriptions of hippocampal function. CA4 is considered a part of the DG and often called the hilus. The pyramidal (principal) cells of CA1, CA2 and CA3 release glutamate, the primary excitatory neurotransmitter of the nervous system, upon activation. Numerous subtypes of inhibitory interneurons surround the principal cells to prevent over-excitation. These interneurons, also referred to as basket cells, are involved in generating multiple hippocampal rhythms, such as theta, gamma and fast wave ripples, and coordinating spike timing. The principal cells of the CA3 region exhibit strong recurrent connections, while the interneurons of CA1 provide recurrent inhibition to the primarily feedforward (parallel) excitatory output within this region.
The entorhinal cortex (EC), located in the parahippocampal gyrus, is a major input to the hippocampal formation. Sensory information is initially received in primary sensory cortices (i.e., auditory cortex, visual cortex, somatosensory cortex and olfactory bulb) and gets further processed in primary (i.e. unimodal) and secondary (i.e. polymodal) association areas before arriving in the perirhinal cortex and being transmitted to the EC. The main hippocampal input arrives from the EC via the perforant path. Layer II of EC projects to the DG and CA3, while layer III of the EC sends inputs to CA1 and the subiculum. Perforant path input to the DG arrives at the granule cell layer (i.e., excitatory neurons of the DG) before being sent to CA3 via the Mossy Fibers. CA3 combines input from EC layer II and DG with its extensive recurrent connections and contacts CA1 via the Shaffer collaterals. CA1 synthesizes information from EC layer III, CA3 and thalamus before projecting to the subiculum. CA1 and the subiculum send information via the fimbria/fornix and cingulum bundle, the main output pathways of the hippocampus.
Additional hippocampal inputs arrive from numerous other subcortical areas, including the amygdala, the thalamus, the medial septum and the diagonal band of Broca, the ventral tegmental area, the supramammillary and retromammillary regions, the lateral preoptic and lateral hypothalamic areas, the raphe nuclei, locus coeruleus and many others (Heath and Harper, 1974). The presence of unique functional roles due to hippocampal interactions with each of these areas is widely accepted and the topic of much current research.

1.1.3. General Hippocampal Function

Psychologically, hippocampal function is typically summarized as supporting two major roles: spatial correlates of a cognitive map and working memory processes. The cognitive map theory proposes that the hippocampus acts as a ‘locale’ system that organizes the encoding and representation of observed stimuli within a spatial framework (O’Keefe and Nadel, 1978). It was proposed that the locale systems primarily stores and uses landmarks to support spatial navigation. Distinct neuronal classifications were derived to illustrate the cognitive map idea including place cells with place fields and head direction cells representing view and movement. More recently, the discovery of hippocampal “time cells” were proposed to encoding ordering of events and facilitate temporally organized recollection and memory processing (Eichenbaum, 2014). Neurophysiologically, spatial and temporal processing by patterning of hippocampal action potentials complement each other to generate accurate timing behavior required for spatial navigation and memory processing (Mauk & Buanomano, 2004; Atakin). The combination of spatial and temporal (alternatively termed nonspatial) neuronal correlations within the hippocampus would support a multidimensional representation.
The multidimensionality of hippocampal neurons would also serve an essential role in learning and memory. The hippocampus is primarily involved in forming and recalling memories when required to achieve short-term goals (Kumaran, 2008). Hippocampal processing is important for the consolidation of information from short-term memory to long-term memory. Long-term memories are believed to be stored in cortical regions as sparsely connected neuronal ensembles, also referred to as “memory engrams.” Evidence for this specified roles originated after studying the famous patient H.M. who suffered from intractable epilepsy (Scoville and Milner, 1957; Augustinack et al., 2014). In an attempt to stop his seizures, his hippocampus and surrounding regions were surgically removed. After the surgery, H.M. could no longer form new declarative memories but was able to recognize people and objects and remember events that occurred prior to the surgery. H.M.’s case demonstrated that the medial temporal lobe, and specifically the hippocampus, is not needed for perception but is required for transferring short-term memories into long-term memories. Since his long-term memory storage was unaffected, this surgery demonstrated that these memories were likely storage in other cortical areas outside the hippocampus.

1.1.4. Neurophysiological Properties of Neuronal Ensemble Formation

These two psychological descriptions, navigation and learning/memory, of hippocampal activity can be described neurophysiologically using the idea of cell assemblies, neuronal ensembles and neuronal avalanches. The idea of neuronal ensemble formation is based on synaptic plasticity, which began with Hebb’s theory about how dynamics and structure of neuronal circuits were shaped by repeated activity pattern sequences. He postulated: “Let us assume that the persistence or repetition of a reverberatory activity (or “trace”) tends to induce lasting cellular changes that add to its stability…. When an axon of cell A is near enough to excite a cell B and repeatedly or
persistently takes part in firing it, some growth process of metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased” (Hebb, 1949). He proposed that sequences of activity repeatedly propagated throughout a network of synaptically connected neurons elicited synapse strengthening that stabilized activity patterns and elicit formation of a cell assembly – also known as a neuronal ensemble.

A neurophysiological basis of Hebb’s theory came through the discovery of long-term potentiation (LTP), a persistent strengthening of synapses based on recent activity patterns (Bliss and Lomo, 1973; Lømo, 2003). LTP is necessary for the auto-associative reactivation of previous activity patterns inherent to hippocampal interactions. LTP results from calcium influx through NMDA receptors in the postsynaptic cell, which leads to a cascade of (molecular) events involving protein phosphorylation (CaMKII, CREB, PKA) and increased AMPA receptor expression to facilitate more postsynaptic glutamate activity (Larson and Vanderklish, 1997). LTP leads to increased firing rate and increased long-range temporal correlations (Lamanna et al., 2015). Blockade of LTP, by preventing NMDA receptor activation or knocking out molecular signaling pathways (such as CamKII, CREB or ERK) impaired spatial learning and memory (Morris et al., 1986; Holscher, 1999). Long-term depression (LTD) is the counterpart to LTP because it involves weakening of synapses after prolonged, repetitive low frequency (0.5-3 Hz) stimulation (Bear and Malenka, 1994; Malenka and Bear, 2004). A balance between LTP and LTD is necessary to maintain optimal neurophysiological function. Behavioral and electrophysiological evidence support the notion that LTP and LTD regulate synaptic strength as neurophysiological mechanisms of learning (Malenka and Bear, 2004).

Long-term synaptic plasticity can modulate synaptic computation by inducing alterations in short-term plasticity (Leibold and Bendels, 2009). Short-term enhancement
or short-term depression are optimized for working and short-term memory because they occur on shorter timescales, typically lasting for only a few minutes (Zucker and Regehr, 2002). Multiple forms of short-term enhancement, such as facilitation, augmentation and post-tetanic potentiation, are normally explained by increased levels of presynaptic calcium remaining after action potential discharge. Synaptic facilitation normally results from increased probability of neurotransmitter release, as opposed to increased number of release sites, produced by elevated calcium levels via a variety of ion channels and molecular mechanisms (Stevens and Wesseling, 1999). Post-tetanic potentiation results from a burst of action potentials arriving in a small time window resulting in increased synaptic efficacy lasting for 30 seconds to several minutes. Short-term depression may also occur after periods of increased neuronal activity due to depletion of the readily releasable pool or neurotransmitter vesicles, inhibitory feedback due to presynaptic autoreceptors or activation of retrograde messengers, such as endocannabinoids. The exact form of short-term facilitation or depression elicited depends on precise action potential timing, calcium availability and receptor actions that are beyond the scope of this introduction but reviewed in-depth by Zucker and Regehr (2002). However, it is important to realize the existence of such forms of synaptic plasticity and to consider their possible roles in neuronal computation when interpreting the results presented in this dissertation. Multiple forms of short-term plasticity likely occur during working memory performance that influence the fractal and multifractal properties of hippocampal spike trains. Short-term plasticity can support a diversity of computations, while long-term plasticity represents a neurophysiological basis for learning and memory (Abbott and Regehr, 2004). These multiple forms of plasticity provide the essential balance for successful long-term and short-term forms of memory.
The auto-associative nature of the hippocampal network allows memories, stored as activity patterns via a combination of LTP and LTD, to be completely reactivated from a small sample of input. A “phase sequence” occurs when a chain of cell assemblies are sequentially activated (Harris, 2005). Successive steps in a serial computation performed by a macroscopic network are represented as the progression of assemblies in a phase sequence. Phase sequences can arise from either external sensory input or internal dynamics to promote complex computations, such as memory recall, thinking, planning and decision making (Sakurai, 1999; Buzsáki, 2010). Upon activation, cell assemblies may elicit dynamical propagation of activity in the form of neuronal avalanches. Neuronal avalanches, scale invariant spatiotemporal neuronal activity patterns, reflect rapid proliferation of local synchrony (Beggs and Plenz, 2003). The range of avalanche sizes follows a power-law form, indicating fractal organization where the relationship between pattern sizes is propagated on all scales (Plenz and Thiagarajan, 2007). Specific spatiotemporal avalanche patterns reoccurring over numerous hours in organotypic cultures may represent spontaneous activation of cell assemblies. Neuronal avalanches are a dynamic neurophysiological phenomenon resulting from activation of chains of cell assemblies.

Synaptic plasticity occurring during acquisition and storage of memories may elicit formation of a “memory engram” (Liu et al., 2014). The idea of the memory engram is notoriously difficult to study because it is involved distributed activity across large neuronal populations. However, optogenetics combined with local field potential recordings and/or large scale multiunit electrophysiology (Hampson et al., 2012) are beginning to provide important features of cell assemblies/ensembles and neuronal engrams. For example, a recent study optogenetically manipulated hippocampal ensembles activated fear conditioning to artificially elicit fear memory recall by driving
internally generated sequences (Ramirez et al., 2013). It is possible that memory engrams consist of groups of sequentially activated cell assemblies (neuronal ensembles) whose dynamics may unfold as neuronal avalanches with fractal distribution properties.

Hippocampal mechanisms of neuronal coding responsible for spatial navigation, learning and memory has focused on describing interactions within and between frequencies of local field potential oscillations. Specific network states memory functions have been assigned to numerous frequency bands, including theta (4-8 Hz), gamma (50-80 Hz) and sharp-wave ripple (125-250 Hz). Theta frequency stimulation induces LTP (Larson et al., 1986) and prefrontal-hippocampal theta interactions predict memory performance (Hyman et al., 2010, 2011). It has been hypothesized that theta and gamma oscillations interact to encode and recall discrete sequences of items (i.e., locations, memories, thoughts) into and from memory storage (Lisman, 2005; Lisman and Buzsáki, 2008). In the theta-gamma scheme, the amplitude of the gamma (fast) oscillation is modulated by theta (slow) frequency so that neurons representing sequential items fire in adjacent gamma cycles of a theta cycle. Since the wavelength of theta is longer than gamma, this is equivalent to saying that specific assemblies fire at specific theta phases. As animals move through place fields, theta phase precession occurs when the firing of hippocampal place cell ensembles representing the environment systematically discharge at earlier phases of the theta cycle coinciding with the animal’s movement (Skaggs et al., 1996; Lisman and Buzsáki, 2008). Theta phase precession may occur discretely with spike times locked to gamma oscillations. Strength of theta theta-gamma phase-amplitude coupling supports multi-item working memory (Axmacher et al., 2010), memory encoding (Bott et al., 2015), memory recall (Shirvalkar et al., 2010) and routes information flow through hippocampus (Colgin et al., 2009).
While memory encoding and memory recall are believed to be supported by theta-gamma coupling and theta phase precession, memory consolidation may occur during hippocampal sharp-wave ripples. Theta rhythm is typically recorded during ongoing activity, but sharp-wave ripples are very short-lived events occurring during sleep or periods of quiescent waking. Replay and reactivation of temporally compressed neuronal firing sequences during sharp-wave ripples may stabilize the memory trace of recent behavioral episodes (Sadowski et al., 2011). Taken together, interactions between local field potential frequency bands may support neuronal ensemble/assembly formation by modulating spike timing and synaptic plasticity processes.

1.1.5. Conceptual Representations of Hierarchies of Neuronal Ensembles

Learning requires integration of related ideas in order to form abstract concepts and influence behavior. Such concepts may be represented as sparse, abstract and explicit representations in neuronal ensembles comprised of “concept cells.” Concept cells were originally proposed as a description for identified neurons that fired for specific people, such as Jennifer Aniston and Luke Skywalker (Quiroga et al., 2005; Quiroga, 2012). Each of these neurons invariably responded to faces, audio recordings and written names, and thus to the concept of a particular person. It was hypothesized that concept cells are responsible for translating present awareness, generated by sensory inputs or internal recollection, into long-term memories stored in cortical areas (Quiroga, 2012). The sparse and invariant properties of concept cells would allow a small number of cells to represent unique concepts independently of specific features of single occurrences. If two simultaneously recalled concepts are related, a subset of the neurons may respond to both concepts.
The neurophysiological properties and anatomical arrangement of hippocampal “functional cell types” (FCTs) allow them to be understood as a form of concept cells (Hampson et al., 1999). FCTs are hippocampal neurons that respond to specific combinations of spatial and nonspatial components of the delayed nonmatch-to-sample (DNMS) task. Their anatomical arrangement suggests a hierarchical arrangement where position cells (i.e., cells encoding left vs. right levers) and phase cells (i.e., cells encoding sample vs. nonmatch task phases) interact in specific hippocampal location to form conjunctive cells that respond to combinations, such as left sample or right nonmatch. Spatial and nonspatial (i.e., task phase) information must be integrated for successful performance on the DNMS task. The nonspatial component could also be interpreted as a temporal requirement related to memory maintenance throughout the delay period and thus may share properties of proposed “time cells” (Eichenbaum, 2014). This hierarchical arrangement (Colwill and Rescorla, 1990; Rescorla, 1992) may arise during formation of the long-term memory of the (operant) DNMS task and its behavioral requirements.

Analyzing cognitive flexibility using paradigms such as reversal learning and set-shifting permits examination of alterations in neuronal hierarchies (Egerton et al., 2005). Rescorla generated experimental evidence that operant (instrumental) training, such as the DNMS task, required a hierarchical associative structure where the stimulus is associated with the response-outcome association instead of a simpler stimulus-outcome association (Colwill and Rescorla, 1990). Reversal learning requires learning a new strategy while simultaneously suppressing previously appropriate responses.
1.1.6. Mechanistic Proposals

Sparsely connected concept cell assemblies and spatiotemporally distinct ensemble firing patterns for specific combinations of DNMS task events could therefore arise through training and be modelled using unsupervised learning neural networks, attractor dynamics and/or self-organized criticality. Unsupervised neural networks can learn to recognize individual faces even when shown multiple different versions (Waydo and Koch, 2008). The model by Waydo and Koch supported a mechanism of concept cell, and similarly FCT, generation by representing information in sparsely connected networks of non-linearly coupled neurons. Attractor models of recurrent networks, such as those in CA3, have also been used to mechanistically describe associative memory storage and recall (Rennó-Costa et al., 2014). These models “attract” network firing patterns to a stored pattern, even from partial inputs. In attractor models, phase sequences of cell assembly activity patterns are modeled as transitions through state space.

Self-organized criticality (SOC) theory was originally developed to describe macroscopic, fractal behavior of dynamical systems (Bak et al., 1987), such as neuronal networks. Neural network models using principles of SOC are able to distinguish between a multitude of inputs by creating specific output representations, akin to those of unsupervised learning networks. SOC is proposed as a universal mechanism for emergence of complex phenomena in dynamical systems which have a critical point as an attractor (Chialvo, 2010). The macroscopic behavior of an SOC system allows it to fluctuate between states via phase/state transitions. A feedback loop, between a slow process that gains energy from external inputs and a fast process that dissipates energy as cascades or avalanches, is responsible for maintaining the critical regime. SOC models are used to study neuronal avalanches (Plenz and Thiagarajan, 2007) and
believed to generate multifractal complexity (Ihlen and Vereijken, 2010). I hypothesize that fluctuations between neurophysiological states detected in hippocampal ensembles of FCTs can be detected as alterations in long-range temporal correlations and multifractal complexity of hippocampal neurons recorded and analyzed for this dissertation.

1.2. The Cannabinoid System

1.2.1. Cannabis

Cannabis, also known as marijuana, is a plant that has been used in many different countries for thousands of years as both a recreational drug and a medicine. Cannabis contains over 450 different compounds, including classes such as phytocannabinoids (i.e., plant cannabinoids), terpenoids and flavanoids that synergistically contribute to its psychological effects, odor, taste, and appearance. Over 70 phytocannabinoids have been isolated (Fisar, 2009), and even more are continually being discovered (Radwan et al., 2015). Cannabinoids are believed to be primarily responsible for the psychoactive and medicinal properties of cannabis, but recent evidence also attributes some therapeutic effects to terpenoids (Russo, 2011). Terpenoids, found in nearly all plants, are responsible for the scent of cannabis. Flavonoids are ketone-containing compounds responsible for flower coloration or pigmentation. The “Entourage Effect” is commonly used to describe the synergistic effect detected in some cases where plants provide more desired effects than isolated drugs derived from them (Mechoulam and Ben-Shabat, 1999). Although medically valuable (Sanchez-Ramos, 2015), the Entourage Effect makes cannabis very difficult to study in a controlled experimental environment, and therefore, many studies have focused on isolated plant constituents.
Delta-9-Tetrahydrocannabinol (Δ⁹-THC or simply THC), first isolated in 1964 (Gaoni and Mechoulam, 1964) is the most widely studied phytocannabinoid. THC is the primary active component of Cannabis and its concentration largely determines its psychoactivity. Many other phytocannabinoids increase or decrease cannabis's potency by interacting with THC. Research into these interactions is still in its infancy, but new medicines (i.e., Sativex) have recently been developed by harnessing the therapeutic potential of THC combined with Cannabidiol, a different, non-psychoactive phytocannabinoid (Russo and Guy, 2006; Valdeolivas et al., 2012).

Cannabis is the most widely used illicit substance worldwide. Cannabis use typically begins in adolescence and surveys show that almost 50% of 18 year olds have tried it at least once (Johnston et al., 2015). Cannabis users reported positive effects including stress relief, pain relief, euphoria, relaxation, and high sociability. Negative effects, including short-term memory loss, nausea, vomiting, dizziness, headaches, anxiety, depression, paranoia, fear and hallucinations have been reported. Medical uses of cannabinoids include treatments for epilepsy, glaucoma, eating disorders, multiple sclerosis, and chronic pain, among many others (Alexander, 2015; Whiting et al., 2015). Cannabis related memory impairments occur at substantially lower doses than its other well-known effects, such as analgesia, hypothermia and motor disruption. Because perceived harm of regular marijuana use is lower than ever (Johnston et al., 2015), possibly due to medical and recreational legalization in multiple states, it is more important now than ever that researchers understand the effects of cannabis.

1.2.2. Endocannabinoid System

The endocannabinoid system is comprised of a few endogenous cannabinoids ("endocannabinoids"), their receptors, uptake mechanisms and hydrolyzing enzymes.
Two types of cannabinoid receptors, cannabinoid-type 1 (CB1R) and cannabinoid-type 2 (CB2R), have been extensively studied in mammals (Pertwee and Ross, 2002). The CB1R is predominantly expressed in the CNS, with especially large concentrations detected in the hippocampus, cerebral cortex, lateral caudate, substantia nigra pars reticulata, molecular layer of the cerebellum and the olfactory bulb (Herkenham et al., 1991). The CB1R is the most abundantly expressed neurotransmitter and/or hormone receptor in the mammalian brain. CB1Rs are also located in the immune and peripheral nervous systems at much lower levels. Activation of CB1Rs suppresses release of subsequent neurotransmitters, both excitatory and inhibitory, including glutamate, GABA, acetylcholine, dopamine, norepinephrine and serotonin (Pertwee and Ross, 2002). CB2Rs were originally thought to solely reside outside the CNS, specifically in immune tissues, but have now been found inside the brain (Morgan et al., 2009).

Anandamide and 2-Arachidonoylglycerol (2-AG) are the two most extensively studied endocannabinoids. Endocannabinoids are not stored within neurons like other neurotransmitters, but instead biosynthesis and release occurs “on demand” after stimulation. Endocannabinoids act as retrograde messengers: after being released from postsynaptic neurons, they diffuse back and activate CB1Rs on presynaptic neurons to reduce subsequent neurotransmitter release. Retrograde endocannabinoid signaling can elicit two forms of short-term synaptic plasticity called depolarization-induced suppression of inhibition (DSI) or excitation (DSE) depending on whether the releasing (presynaptic) neuron contains GABA or glutamate. Endocannabinoids mediate a form of LTD in in order to facilitate learning and memory processing through mechanisms of coincidence detection and input specificity (Heifets and Castillo, 2009). 2-AG is the primary endocannabinoid responsible for the transient effects of DSI/DSE, while Anandamide has been shown to elicit more sustained effects, such as endocannbinoid-
mediated LTD (Heifets and Castillo, 2009). Up to 13 endocannabinoids have been proposed to exist, but their functional roles and relationships to synaptic plasticity, learning and memory are poorly understood (Pertwee, 2015). Experimental evidence shows that activation of the endocannabinoid system generally impairs working memory and long-term memory formation, while its inhibition can augment these memory processes in some instances. On the other hand, memory retrieval may be unaffected by endocannabinoid activation. The effects of endocannabinoid system activation or inhibition can be mimicked by application of exogenous cannabinoids, such as THC, or cannabinoid antagonists, respectively.

1.2.3. Cannabinoid Effects on Memory

Impaired learning and memory function is one of the most frequently reported cognitive effects of cannabinoids (Ranganathan and D'Souza, 2006). In humans, cannabis was found to impair recall and recognition memory if smoked prior to memorization of word lists in one study (Abel, 1971) while recognition memory was spared in another (Miller and Cornett, 1978). However, if smoked after memorization, it had no effect on accuracy of delayed recall (Abel, 1971). Therefore, cannabinoids impair memory encoding but do not weaken recall of previously learned information, suggesting cannabinoid regulation of information transfer from short-term to long-term memory (Ranganathan and D'Souza, 2006). THC impaired verbal memory, text learning, associative learning and reaction time in another study (Block et al., 1992). THC was found to increase false positive error rates in recognition memory tasks and was associated with both external and internal intrusion errors. The increased intrusion errors may be related to increased irrelevant mental activity or “mind wandering” associated with augmented default mode network activity under THC (Bossong et al., 2013).
On a network level, cannabis shifts activity away from memory-related theta frequency activity towards default mode network recruitment (Bossong et al., 2013). Cannabis intake disrupts synchrony of theta oscillations and interferes with memory performance in humans (Ilan et al., 2004, 2005; Böcker et al., 2010). Cannabinoid receptor agonist CP5540 impaired spatial working memory performance while disrupting theta-frequency coordination between CA1 and prefrontal cortex (Kucewicz et al., 2011) and by decreasing hippocampal power of theta, gamma and sharp-wave ripples (Robbe et al., 2006).

By preferentially increasing excitation over inhibition (Lutz, 2004), cannabinoids could increase nonspecific excitatory firing leading to miscoding of relevant memory information (Deadwyler et al., 1996; Hampson and Deadwyler, 2000) and/or disrupt hippocampal network activity mediated by synchronized GABAergic neuronal populations (Puighermanal et al., 2012). CB1R activation shifts the excitation-inhibition balance to favor excitation (den Boon et al., 2014), possibly due to denser CB1R expression on GABAergic vs. glutamatergic neurons in the hippocampus (Kawamura et al., 2006). Exogenous cannabinoid agonists, such as THC and WIN 55,212-2, inhibit both LTP and LTD in hippocampal neurons through a CB1R-mediated mechanism (Collins et al., 1995; Davies et al., 2002; Fan et al., 2010). THC disrupts trained behavioral responses, neuroplasticity and neurogenesis (Ranganathan and D'Souza, 2006). THC and WIN 55,212-2 impaired performance of the DMS/DNMS task by impairing memory encoding during the sample phase (Heyser et al., 1993; Hampson and Deadwyler, 2000; Deadwyler et al., 2007).

Cannabinoid receptor stimulation triggers activation of multiple intracellular signal transduction cascades (Puighermanal et al., 2012). CB1Rs are normally coupled to Gi/o proteins, though other couplings have been reported (Howlett, 2005). Gi/o activation
inhibits adenylyl cyclase activity to reduce production of cAMP and consequently
decrease PKA activity. CB1R activation produces phosphorylation and activation of
members of the mitogen-activated protein kinase family including ERK1/2, p38, and c-
Jun N-terminal kinase (Howlett, 2005), modulates the phosphatidylinositol-3-
kinase/protein kinase B (Akt) signaling pathway (Ozaita et al., 2007), excites the mTOR
pathway (Puighermanal et al., 2009) and reduces CREB phosphorylation (Fan et al.,
2010). All of these molecular pathways have been linked to the disrupted memory
function and impaired synaptic plasticity associated with CB1R activation as reviewed by
Puighermanal et al., (2012).

The memory impairing effects of CB1R agonists can be blocked or reversed with
CB1R inverse agonists/antagonists, such as SR141716A and AM-251 or by CB1R
deletion. Genetic deletion of CB1R increases hippocampal LTP (Bohme et al., 2000)
and improves object recognition performance (Reibaud et al., 1999). Some studies
(Terranova et al., 1996; Lichtman et al., 2002; Robinson et al., 2008), but not all (Davies
et al., 2002), have shown that the CB1R inverse agonists/antagonists actually enhanced
memory performance when administered alone (Ranganathan and D’Souza, 2006). It
was even shown that SR141716A produced improved performance under high cognitive
load, long delay conditions (Deadwyler et al., 2007), suggesting that other studies may
not have elicited memory enhancement due to a performance ceiling.

Extinction and reversal learning may be mediated by endocannabinoid transmission.
CB1R knock-out impairs extinction (Hajos et al., 2000; Marsicano et al., 2002) and
spatial reversal learning in the Morris watermaze (Varvel and Lichtman, 2002).
Increasing endocannabinoid (Anandamide) signalling improves extinction (Laricchiuta et
al., 2013). This evidence would suggest that cannabinoid agonists should also facilitate
extinction, and while this was shown in one study (Pamplona et al., 2006), others have
demonstrated CB1R-agonist mediated reversal learning impairment (Egerton et al., 2005; Wright et al., 2013). Reversal learning impairment may result from CB1R agonist-mediated reduction in memory encoding ability required for second rule learning after extinction. Nonetheless, the evidence shows that the endocannabinoid system facilitates extinction and possibly forgetting of information stored in long-term memory upon reconsolidation. Further study of this specific property could provide valuable treatment options for behavioral disorders, such as post-traumatic stress disorder (Malá et al., 2015).

1.3. Fractals

Fractals provide new collections of ideas and therefore new ways of viewing nature. New methodologies for analyzing experimental data and novel interpretations arise from this fresh viewpoint. Self-similarity is the essential characteristic of fractals: deeper magnifications reveal finer details similar to organization of the whole. Fractals are found everywhere, in physiological and physical systems alike. Fractal theories have been proposed to describe the formation of the universe (Palmer, 2009) and existence of life (Kurakin, 2011). In DNA, genes specify the rules that generate anatomical structures, such as the lungs, heart and nervous system, instead of specifying the structures directly (Bassingthwaighte et al., 1994; Moreno et al., 2011). Reiteration or repeated application of these basic rules may generate structures with self-similarity arising from its own construction. This reiteration would create the hierarchical structure of nearly all biological and physiological systems.

Fractal is a general term used to describe geometrical and/or statistical self-similarity. Geometrical fractals exhibit precise self-similarity where components on the smallest scales are exact replicates of those on the largest scales. However, many signals in nature do not follow such a strict definition but can be described as statistical
fractals. The Cantor set is a geometrical fractal that is construct by repeating a generator rule across scales (Figure 1.2). The Cantor set is generated by repeatedly removing the middle third of the line segment on each iteration. This process creates a self-similar set where each level is a 1/3 reduced copy of the previous level. Fractals do not occupy the same amount of space as defined by Euclidean dimensions $E$ where a dot has a dimension of 1, a line has a dimension of 2 and a cube has a dimension of three. Instead the fractal dimension (or Hausdorff dimension) provides a quantitative measurement of the space-filling properties of an object by following a simple relationship:

$$D(h) = \log N / \log F$$

where $N$ is the number of pieces and $F$ is the scale factor. The fractal dimension describes how many new self-similar pieces are detected at finer resolutions (Bassingthwaigte et al., 1994). The fractal dimension of the Cantor Set is $D(h) = \log(2)/\log(3) = 0.63$. When understood from the second row (Figure 2.2), this relationship arises from the presence of 2 copies ($N = 2$) of the original that are each shrunk by a factor of 3 ($F = 3$). The same fractal dimension can be calculated from any iteration level. For example, the third row contains 4 copies, each of which was shrunk by a factor of 9 and leads to the same fractal dimension of 0.63.
Figure 1.2. Cantor Set is a prototypical example of self-similarity. The Cantor set is generated by removing the middle third of each segment at each iteration.

The Hurst exponent $H$ is a monofractal measure commonly used to describe smoothness by quantifying long-range temporal correlations (Hurst, 1951). The Hurst exponent ranges from 0 to 1. A low Hurst exponent signifies roughness and weak long-range temporal correlations, while a high Hurst exponent is detected in smooth signals to indicate strong long-range temporal correlations. The Hurst exponent can be directly related to the fractal dimension after taking into account the Euclidean dimension:

$$H = E + 1 - D(h)$$

Since both the Euclidean and fractal dimensions of a line are 2, the Hurst exponent is 1. A flat surface or a straight line is a perfectly smooth signal. A slightly roughened signal would extend slightly into the third Euclidean dimension and might have a fractal dimension of 2.1, equating to a Hurst exponent of 0.9. Anytime the Hurst exponent is less than one, the measured object intrudes into the higher dimension (Bassingthwaigte et al., 1994).

The definition of the Cantor set can be modified as an example of multifractal structure by accounting for density in a different way (Feder, 1988). Instead of removing the middle third at each iteration, imagine that each segment is cut in half and smashed...
together so that each segments’ length becomes 1/3 of the original while the mass of each segment is reduced to 0.5 of its original mass (Figure `1.3). This process causes the density of each segment to increase by 3/2 of the original (because density = mass / length = 0.5 / (1/3) = 3/2). After reiteration and mass conservation (total mass always equal to one), it is found that the mass becomes heterogenously distributed into small areas with high density:

![Figure 1.3. Triadic Cantor set. A bar is divided in half and hammered together to increase density upon each iteration. The height of the bar in each generation is proportional to its density. The Hurst exponent is the same as the original Cantor set, but this one exhibits multifractality.](image)

Multifractals can be generated by multiplicative processes that generate heterogenous distributions of variability. The binomial multiplicative cascade is an archetypical example of multifractality that works by distributing its mass between right and left sides in unequal ratios. By implementing a simple rule, a systematic unevenness is introduced across iterations. In this example of the binomial cascade, two different ratios were used to exemplify how differential degrees of long-range correlations and multifractality arise from variable levels of unequality (Figure 1.4). Beginning with a distribution of 100%, each sample was split exactly in the middle of the original to create two smaller samples with variable proportions. In the left example
(blue lines), the right side is always given 2/3 of the distribution leaving the left with the remaining 1/3. In the right example (orange lines), the right side is always given 3/4 of the distribution while 1/4 remains on the left side. This unequal shift is illustrated in the top two panels of figure 1.4. For the second iteration, each of the segments created from the first iteration are now split in half using the same distribution ratios (Figure 1.4; level 2). The third level comes from splitting all four of the segments created on the second level (Figure 1.4; level 3). This same redistribution is repeated for a set number of iterations. After 11 iterations, the binomial multiplicative cascade is generated (Figure 1.4; level 11).

Multifractal analysis of the resulting binomial cascade generates estimates of the Hurst exponent and the multifractal singularity spectrum. The log-log plots (Figure 1.4, bottom left) indicate that using a distribution ratio closer to 1/2 (i.e., 1/3) produces stronger long-range correlations than using a ratio closer to zero (i.e., 1/4). A more even distribution creates stronger correlations because successive intervals are more similar, while the more uneven distribution has large fluctuations and hence greater multifractality. The magnitude of multifractal complexity is summarized by the width of the singularity spectrum, which plots the fractal dimension $D(h)$ against the Holder exponent. Multifractal analysis is comprehensively described in chapter 2, but this example of the binomial cascade is introduced here to illustrate how propagation of a simple bias through successive perturbations is a mechanism for generation of multifractal complexity. Therefore, a simple underlying rule can generate multifractal complexity. In the context of neuronal firing patterns, multifractality generated by intermittency could be conceptualized as a combination of burstiness and variations in the burstiness structure over time (Zheng et al., 2005).
Figure 1.4 Binomial multiplicative cascade propagates bias to generate multifractal complexity. Binomial cascade is created using a ratio of 2/3-to-1/3 (blue, left example) and a ratio of 1/4-to-3/4 (orange, right example). At each level, the line is cut in have and the distribution is shift according to the predefined ratios. After 11 iterations, the repetitive structure of the binomial cascade becomes apparent (Note difference in y-axis maximum). Less inequality (blue) generates stronger long-range temporal correlations (bottom left, larger Hurst exponent) and weaker multifractality (bottom right) compared to greater inequality (orange).
1.3.1. Fractal Properties of Memory

Scale invariance is a feature of fractals that implies an underlying mechanism generates activity across all levels of processing. In the spatial domain, fractals are self-similar geometric objects with repeating patterns across many scales. In time series, scale invariance may arise from coarse-scale long-term and higher frequency fine-scale fluctuations interacting across a hierarchy of temporal and spatial scales (Werner, 2009). Fractal analysis can describe and quantify spatial and/or temporal correlations and suggest mechanisms for their generation (Bassingthwaighte et al., 1994). The presence of scaling (scale invariance) in physiological time series signifies long-term memory (i.e., long-range temporal correlations or long-range dependence) indicating that previous activity influences ongoing and future temporal fluctuations. Long-range temporal correlations (LRTCs) confer computational advantages such as “rich information content, high flexibility, and purposeful variability” (Wong and Shelhamer, 2012). Learning typically requires a balance between single trial accuracy (short-term component) and long-term performance based on previously learned rules. Therefore, long-range temporal correlations may arise in time series when short-term and long-term goals are integrated to generate behavior. Such a balance is reminiscent of interactions between long-term and short-term synaptic plasticity and suggests that when LRTCs are detected in spike trains, they may reflect a neurophysiological basis of memory processing (Lamanna et al., 2015).

Multifractal complexity arises when numerous, simultaneously occurring different fractal patterns overlap and interact. It is derived from the interaction of various substructures and subfunctions distributed over several spatiotemporal scales (Ihlen and Vereijken, 2010). Hierarchical structures can develop through such interactions and facilitate mnemonic function. If a novel association is formed, it can be integrated with
related features as long as memories exist for such items. Otherwise, a new hierarchy may form. Multiple hierarchies form over time and may interact with each other when items are involved in separate hierarchies. Participation in multiple hierarchies could be understood as involvement in several neuronal ensembles/assemblies and detected as dynamical transmission within different temporal segments of neuronal avalanches.

When multiple hierarchies develop, the involved neuronal networks might exhibit multifractal dynamics when activated by both memories simultaneously vs. activation by a single memory network. In this conceptualization, each (mono)fractal component carries one memory, and when both memory streams are activated together, multifractality may arise.

Multifractals can form through a process of reiteration. When a simple phenomena is learned, LTP could occur and elicit LRTCs in a population of neurons (i.e., a neuronal ensemble/assembly) (Lamanna et al., 2015). If a new, related rule is learned on top of this, LTP may occur again to elicit LRTCs in an overlapping population. When this process is reiterated, neuronal ensembles become segregated and some neurons become associated with multiple memory traces. I hypothesize that neurons involved in multiple memory traces would exhibit greater multifractal complexity compared to neurons involved in less complex processing. In the rest of the section, I will propose two examples to help explain the connections between psychological, neurophysiological and physical (fractal) conceptions of learning and memory that is a primary focus of this dissertation.

I will relate my proposal to an example using the reversal learning paradigm in DNMS/DMS task. In the beginning, rats must learn a set of basic rules that govern how the entire apparatus works. They must first learn how to press both levers to receive rewards. Next, they learn how to nosepoke between two lever presses before receiving
a reward. Learning these basic rules is a prerequisite for learning how to press either the opposite or same lever (during the choice phase) as in the sample phase. They then can learn that only a nonmatch (or match) response on the second lever press will lead to a reward. A delay is added to increase cognitive load and require extended periods of working memory maintenance. The final version of the task requires integration of procedural rules (i.e., how to lever press and nosepoke), long-term memory (i.e., nonmatch or match rule), short-term memory and working memory on a trial-by-trial basis. Each trial requires encoding during the sample phase, maintenance during the delay and retrieval during the choice phase. Therefore, acquisition of the DNMS/DMS task may neurophysiologically arise through a hierarchical arrangement both within the hippocampal network and between the hippocampus and other brain regions. If the rule is reversed (i.e., from match to nonmatch), the associated hierarchical long-term memory must be altered in order to generate an adaptive behavior. The original rule is likely extinguished not forgotten, and therefore short-term memory processing may be necessary to actively suppress the former rule. Synaptic rewiring of neuronal ensembles with LTP and/or LTD may be detected as alterations in the neurophysiological hierarchical structure and consequently the fractal and multifractal features of involved spike trains.

I will now outline another example based on human memory. Let’s say that you park your car in the same parking lot each day, and every day your hippocampus encodes this location before you exit your vehicle to enter the building. At the end of the day, you usually exit the building and return to your car without trouble. However, on some days when you are distracted with an abundance of work, instead you return to a spot where you parked yesterday, or last week. When you are actively remembering where you parked, neuronal transmission through cell assemblies may produce LRTCs
in spike trains representing the previously stored location. Multifractal complexity may help these ensembles sort between where you parked today vs. yesterday (and all other days), but when the mind becomes distracted it is possible that the incorrect neuronal ensemble becomes activated. Multifractal complexity might be exhibited in hippocampal neurons during the retrieval of these memories and even more robustly if the same neuron in connected to multiple ensembles. Multifractal dynamics may be a way to guide network activity and support mechanisms of pattern completion and pattern separation through specific channels of heterogenous variability. By understanding neuronal ensembles/assemblies through a fractal lens, new interpretations can be constructed from novel perspectives.

1.3.2. Mechanisms of Fractals Generation

Synaptic plasticity is a structural form of memory that is essential for developing long-range correlations and multifractal complexity in neuronal populations (Nikulin and Brismar, 2004). Network oscillations regulate synaptic plasticity by co-activating groups of neurons. Neuronal ensembles/assemblies may form and be maintained through auto-associative interactions established by synaptic plasticity. Ionic currents are responsible for information transmission and synaptic plasticity at the cellular level. Synaptic plasticity is a common feature of these scale-free mechanisms suggesting that detection of fractal and multifractal complexity in neuronal systems reflects such plasticity.

"Instead of searching for ad-hoc laws for the brain, under the pretense that biology is special, most probably a good understanding of universal laws might provide a breakthrough since brains must share some of the fundamental laws of nature" (Chialvo, 2010). Throughout his review, Chialvo calls for replacing connectivist ideas by adopting a collectivist viewpoint to understand, describe and analyze brain dynamics in the field of
neuroscience. Nearly all macroscopic phenomena emerges through the collective dynamics of interacting microscopic components (Chialvo, 2010). Emergence refers to the “unexpected collective spatiotemporal patterns exhibited by large complex systems.” Complex systems consist of large conglomerates of elements that interact with nonlinear dynamics (Chialvo, 2010). 1/f noise has diverse origins and is considered an indicator of complex systems (Pu et al., 2013). In self-organizing critical (SOC) systems, sustained short-range interactions eventually elicit emergence of spatiotemporal long-range correlations (Chialvo, 2010), an essential component of fractal structure. SOC theory was developed to explain the ubiquitous appearance of power-law behavior (Bak et al., 1987) and it has been hypothesized that critical dynamics within the brain will generate fractal dynamics in its interacting elements. By incorporating fractal analyses and semantics, a collectivist vantage point may promote insight and richer theoretical descriptions.

Self-organized criticality (SOC) is an emergent property generated by propagation of local activity, typically in the form of an avalanche or cascade. Self-organized systems, such as neural networks, are thought to optimize two important components of neural computation: information transmission may be optimized in a critical regime while information storage may be optimized in the presence of metastable dynamics (Beggs and Plenz, 2003, 2004). Metastability in the brain facilitates integration and coordination of functional networks thought to ultimately generate behavior and cognition. Experimental evidence for the simultaneous existence of critical and metastable dynamics was demonstrated in in vitro cultured hippocampal neuronal networks (Pu et al., 2013). These networks exhibited macroscopic power-law scaling of avalanche size distributions while individual interspike interval sequences showed long-range temporal correlations, detected as average Hurst exponents of 0.71 (Pu et al., 2013). In
developing networks operating under criticality, self-organized interactions with long-range correlations may promote emergence of metastable activity patterns, also known as metastable state transitions (Pu et al., 2013). In other words, associative connections may spontaneously arise between groups of co-activated neurons eliciting neuronal assembly formation that is dynamically expressed as power-law neuronal avalanche distributions and fractal properties of individual spike trains.

Balance is a key concept in self-organized criticality. Models show that excitation-inhibition balance can arise naturally in sparsely connected neuronal populations where single neurons exhibit heterogenous nonlinear dynamics (van Vreeswijk and Sompolinsky, 1996), such as the hippocampus (Wixted et al., 2014). Memory performance of the self-organizing recurrent network model relies on a balance between external input and internally generated reservoir activity (Lazar et al., 2009). A similar associative memory network model found that an interaction between memory retrieval via attractor dynamics and optimal exploration of state space during critical regimes promoted flexibility and adequate memory formation simultaneously (Uhlig et al., 2013). Fractal temporal dynamics in a self-organizing neural network model emerged during peak optimization performance (Kwok and Smith, 2005). The fractal properties gave the network “the flexibility to escape local minima in the short term while maintaining its optimization objective in the long term” (Kwok and Smith, 2005). Criticality can promote meta-stable state switching to enable flexible short-term memory processing while permitting persistent focus on long term objectives. The fact that long-range temporal correlations arose when this interplay was optimized provides support for their role in flexible memory processing.

Neural network models can provide mechanistic descriptions of learning and memory. A Cantor coding model of hippocampal function suggested dynamical
mechanisms of category specificity during episodic memory formation (Tsuda and Kuroda, 2001; Yamaguti et al., 2011). In the model, CA3 sent discrete spatiotemporal conductance patterns to CA1 that resulted in self-similar hierarchical clustering of CA1 outputs corresponding to specific input sequences. Model predictions were partially validated experimentally by showing that spatiotemporal stimulation of Shaffer collateral synapses generated stable, self-similar clusters of CA1 neuronal activity (Fukushima et al., 2007). These theoretical and experimental results suggest that hippocampal processing leads to fractal hierarchies that may be the basis of related concepts, such as memory engrams, neuronal ensembles, assemblies and avalanches.

Maintaining appropriate function of physiological networks is a primary goal of medicine. Homeostatic control was proposed to perform such control by negative feedback that occurs quickly on short time scales. On the other hand, allometric control integrates long-term memory, manifested as inverse power-laws in time, with short term goals of interacting subnetworks (West, 2010). Allometric control enables adaptability and flexibility in order to meet the variable requirements of an ever-changing environment. The feedback and reiteration requirements of allometric control processes could generate fractal dynamics. Allometric control could alternatively be described by neurophysiologists using the terminology of synaptic plasticity, which allows the nervous system to adapt to constantly evolving environmental demands based on neuronal structures imprinted via previous experience. If fractal fluctuations are an indication of allometric control in properly working physiological systems, then their detection could aid in identification and classification of physiological, cognitive and pathological states.
1.3.3. Monofractal and Multifractal Complexity: from Behavior to Physiology

Behavior, the ultimate interface between neuronal dynamics and the environment (Chialvo, 2010), also exhibits fractal and multifractal dynamics. Fractal (1/f) scaling may be a universal property of human physiology and behavior (Van Orden et al., 2005) due to its presence in gait dynamics, breath sizes, reaction times, heart beat intervals, neurophysiological recordings across multiple scales from EEG/MEG/LFP to interspike intervals to ionic currents and across many other areas (Stanley and Meakin, 1988). The theory of interaction-dominant dynamics was originally used to explain how self-organizing properties of the brain generate behavioral response series with fractal scaling properties (Van Orden et al., 2003, 2005). The interaction-dominant theory was extended by Ihlen and Vereijken (2010) to incorporate the intermittent and irregular properties of multifractal features generated by coordination across spatiotemporal scales. The anti-thesis to interactive dynamics is component-dominant theory which states that scale-dependent functions can be decomposed into simple independently operating pieces or components. It has been proposed that 1/f dynamics can be caused by either component-dominant (Wagenmakers et al., 2005) or interaction-dominant dynamics (Van Orden et al., 2005) and therefore multifractal analysis is necessary to distinguish between these theories. Although the advantage of multifractal complexity is emphasized here, important results have nonetheless been detected by quantifying monofractal and multifractal dynamics within the nervous system.

Experimental evidence for the utility of multifractal analysis in response interval analysis was first presented by Ihlen and Vereijken (2010). Response intervals from a human reaction time task requiring subjects to respond as quickly as possible following visual stimulus presentation exhibited significantly multifractal fluctuations (Ihlen and Vereijken, 2010). However, when subjects were instead instructed to respond 1 second
after the stimulus, their response series were not multifractal but exhibited stronger monofractal long-range correlations than the reaction time task (Ihlen and Vereijken, 2010). It was hypothesized that multifractal features of reaction times manifested as intermittent periods of fast vs. slow response times and represented waxing and waning of attention and task commitment. The monofractal structure detected during interval estimation would arise from a hypothesized increase in memory workload and consequently increased attention. Since participants were asked to respond in 1 second intervals, accurate estimates would generate a smooth time series of responses with similar values throughout. The coordination between multiple time scales manifested as attentional fluctuations and detected as multifractal complexity was shown during the reaction time task, but suppression of these interactions lead to reduced multifractality in the interval estimation task.

Alterations in fractal scaling might indicate breaking and reforming of associations governing cognitive performance (Stephen et al., 2012). Monofractal and multifractal properties of executive control were assessed in a card sorting task requiring flexible rule switching by comparing explicit vs. induction conditions (Anastas et al., 2011; Stephen et al., 2012). In this task, cards were sorted based on three dimensions: animal, color and accessory; the sorting rule was either given (explicit condition) or determined by trial and error (induction condition). Executive control was defined as stability in selective attention to the current sorting rule and adaptability during rule changes (Stephen et al., 2012). Results showed that the Hurst exponent immediately increased after each rule change before gradually falling in the explicit condition, but in the induction condition, the Hurst exponent followed a “negative parabolic trajectory” by starting low and increasing during rule induction before behavior settled into a stable pattern of rule application coinciding with a reduced Hurst exponent (Anastas et al.,
In a subsequent study, stronger long-range temporal correlations in the absence of multifractality corresponded to reduced rule switching ability, but stronger multifractality weakened this relationship. Taken together these experiments suggest that multifractal fluctuations support flexible rule-switching ability by modulating the influence of long-range temporal correlations (Anastas et al., 2011; Stephen et al., 2012; Kelty-Stephen et al., 2015). The results presented in chapter 5 are consistent with these studies and provide novel consideration for the role of multifractal complexity in reversal learning.

Quantification of interactivity using multifractal complexity was extended by examining human interactions. Navigation in a virtual environment was performed by one subject paired with another human or artificial agent (Bedia et al., 2014). By analyzing multifractal complexity of a collective variable (the derivative of their virtual separation), results indicated that human-human interactions elicited stronger long-range temporal correlations and greater multifractal complexity than human-computer interactions (Bedia et al., 2014). Interestingly, individual human movement series did not exhibit such complexity alone, suggesting that fractal structure emerged during genuine social interactions in a shared space between both subjects could not be reduced to individual components (Bedia et al., 2014). Another study recorded EEG from six human team members performing a submarine piloting and navigation task detected emergence of multifractal complexity during teamwork periods (Likens et al., 2014). These studies provide behavioral and neurophysiological support for the utility of multifractal complexity as an indicator for human interactivity.

Monofractal and multifractal analyses have been used to detect physiological, cognitive and pathological status to improve diagnosis criteria in numerous studies. The loss of variability indicates a loss of physiological control. Healthy human heart beat
intervals were shown to exhibit multifractal complexity and a reduction in such complexity was detected in patients with congestive heart failure (Ivanov et al., 1999). Interestingly, beta-blocker administration restored multifractal complexity in congestive heart failure patients (Chiu et al., 2007) but decreased multifractality in healthy patients (Amaral et al., 2001). The heartbeat intervals from the most aerobically fit males and females exhibiter stronger long-range correlations and greater multifractality than less fit participants (Lewis and McNarry, 2013). Another study showed that the multifractal nature of human cerebral blow flow was severely decreased in chronic migrainers (West et al., 2003). These results indicate that multifractal complexity of human blood flow dynamics is a promising indicator of physiological health.

Detection of mono- and multifractals indicate interactions among multiple spatiotemporal scales and evidence for these interactions comes from all levels of neuroscience research encompassing behavior, network level (i.e., fMRI, EEG, MEG), LFP, interspike intervals, and ion channel kinetics. Fractal characteristics at the network level reflect the emergent properties of neuronal subsystems, while fractal properties of the neurons indicate nonlinear interactions of ionic channels (Segev et al., 2002). The same notion could alternatively (but equivalently) be conceptualized in the reverse direction since scale invariance reflects reciprocal interactions among and between all spatiotemporal scales. Fractal dynamics have been detected throughout all levels of the nervous system and important results and implications will be presented in a top-down manner. At the conceptual core of this presentation is the ability to detect state changes in a scale-free manner and emphasize the ubiquitous appearance of fractal complexity in the nervous system.

Multifractal analysis has also facilitated detection of physiological and pathological states in neurophysiological and hemodynamic (fMRI BOLD) time series. While it is
difficult to generalize results from fMRI studies with regards to directional changes in long-range temporal correlations and multifractality due to heterogenous comparisons including type of behavioral task, brain region assessed and recording time (i.e., before, during or after task), the utility of the multifractal technique is clear: Changes in monofractal and/or multifractal complexity of BOLD signals could differentiate periods of task or rest conditions (Wink et al., 2008; He, 2011; Ciuciu et al., 2012), age, cholinergic blockage (Suckling et al., 2008), influence of ethanol consumption on Default Mode Network activity (Weber et al., 2014), and classification of diseases, such as Alzheimer’s (Warsi et al., 2012; Ni et al., 2015) and autism (Lai et al., 2010).

Long-range temporal correlations and multifractal complexity of global network oscillations, measured by EEG or MEG, enable robust neuronal information processing (Linkenkaer-Hansen et al., 2001). Alterations in LRTCs differently reflect changes in arousal state (Nikulin and Brismar, 2004) and external stimulus application (Linkenkaer-Hansen et al., 2004). General tendencies indicated that LRTCs are inversely proportional to arousal state since the deepest sleep states exhibited strongest LRTCs while wakefulness showed the weakest LRTCs (Nikulin and Brismar, 2004; Weiss et al., 2009; Zorick and Mandelkern, 2013). Multifractal complexity of human EEG signals was better than the Hurst exponent and spectral analysis for indicating sleep stages (Weiss et al., 2011). Allometric regulation of EEG fractal and multifractal dynamics evolved during natural development (Polonnikov et al., 2003) and influenced attention and learning capacity (West, 2001). Including multifractal measures improved EEG-based Brain-Computer Interface signal classification performance (Brody et al., 2012), schizophrenia diagnosis (Slezin et al., 2007) and seizure prediction (Dutta et al., 2014; Gadhoumi et al., 2015; Zhang et al., 2015). The stability of LRTCs over numerous
recording days supports their utility as a reliable metric of malfunction in the dynamics of network fluctuations (Nikulin and Brismar, 2004).

Propagation of local field potentials as neuronal avalanches optimize information transmission and storage in cortical cultures, as suggested by operation in a self-organized critical state (Beggs and Plenz, 2003, 2004). Multiple different neuronal avalanche activity patterns coexisted in cortical cultures, suggesting a possible role as a memory substrate or memory engram (Beggs and Plenz, 2004). Long-term stability (up to 10 hours) and rich diversity of neuronal avalanche patterns suggest that they might represent dynamical propagation of activity through neuronal assemblies (Plenz and Thiagarajan, 2007). In cultures, microelectrode arrays can sample the entire network, but avalanches are more difficult to detect in vivo because such recordings can only obtain very small samples in localized areas. However, deliberately undersampling a probabilistic excitable cellular automaton model in a critical state yielded log-normal avalanche size distributions similar to in vivo data, while sampling all neurons yielded the expected power-law avalanche distribution (Ribeiro et al., 2010). A different study succeeded in detecting in vivo neuronal avalanches in cortical motor regions in nonhuman primates to indicate the universality of this phenomenon (Petermann et al., 2009).

The detection of fractal neuronal firing properties nullified the commonly held belief that spike trains were Poisson point processes (Werner, 2010). As longer samples are acquired from fractal action potential sequences, the variance-to-mean ratio exhibits a power-law increase instead of becoming closer to unity and therefore follows a Levy distribution. Noxious and non-noxious stimuli differentially altered multifractal properties of wide dynamic range neuron spike trains in normal vs. injured rats (Biella et al., 1999). The central circadian pacemaker, the suprachiasmatic nucleus, who required neuronal
network interactions to generate fractal patterns, may regulate multifractal dynamics of heart rate variability (Ivanov et al., 1999; Hu et al., 2012). Sympathetic neurons exhibiting fractal properties were hypothesized to contribute to the fractal component of heart rate variability (Lewis et al., 2001; Das et al., 2003; Gebber et al., 2006). It was also shown that ‘smoothing’ of the fractal features occurred as information was transmitted from two consecutive brainstem areas, suggesting that LRTCs become stronger with feedforward processing (Gebber et al., 2006). Fractal spike trains were also detected in retinal ganglion, lateral geniculate, auditory, but not vestibular neurons (Teich, 1989; Teich et al., 1990, 1997). Spike trains from human hippocampal neurons with strong LRTCs showed stronger average cross-correlations than neurons without LRTCs suggesting that temporal correlations (i.e., LRTCs) are spatially transferred throughout interconnected assemblies (Bhattacharya et al., 2005). Differential fractal and multifractal indices between basal ganglia regions and normal vs. pathological populations could improve deep brain stimulation accuracy and Parkinson’s disease treatment (Rodríguez et al., 2003; Zheng et al., 2005).

Unmasking of fractal spike train properties via LTP may facilitate neuronal information transfer by allowing the presynaptic neurons to inform postsynaptic neurons of its neurophysiological state change (Lamanna et al., 2015). LTP induction increased mini EPSP frequency and enhanced fractal (power-law) behavior of miniature activity recorded from hippocampal CA3 and CA1 cultures using whole-cell patch clamp (Lamanna et al., 2015). Importantly, fractal behavior was not detected in spontaneous activity prior to LTP induction and was blocked, along with increased miniature frequency, using BAPTA to prevent intracellular calcium accumulation (Lamanna et al., 2015). LTP coincided with increased vesicle fusion rate that would transfer to the neuronal action potential series and up to the network level. This finding (Lamanna et
al., 2015) also confirmed the prediction of Lowen et al. (1997) that synaptic potentiation could be induced by neuronal activity with long-range temporal correlations (Lowen et al., 1997). LTP is a neurophysiological phenomenon that alters the fractal nature of spike trains and is therefore a candidate for the appearance and function of long-range temporal correlations detected and analyzed in this dissertation.

The confirmation that LTP coincided with increased LRTCs complements evidence of fractal miniature EPSP occurrence as well as channel openings and closings. Open and close times of hippocampal voltage-dependent potassium channels exhibit fractal fluctuations (Liebovitch and Sullivan, 1987). By computing the kinetic rate constant of protein conformation changes, it was shown that the fractal distribution of energy barriers between open and closed conformational states generated fractal ion channel kinetics (Liebovitch and Sullivan, 1987; Bassingthwaighte et al., 1994). A modification of the Hodgkin-Huxley model showed that a self-similar quasi-fractal chain of conformational states in ion channel behavior lead to fractal firing patterns (Lowen et al., 1997), similar to those observed in spontaneous release (Lowen et al., 1999; Takeda et al., 1999).

The cited studies are a small sample of the evidence for fractal and multifractal properties across all levels of the nervous system. The essential result demonstrated that LRTCs are a neurophysiological manifestation of LTP (Lamanna et al., 2015). LTP increases interactivity between neurons that may lead to enhanced multifractal complexity during the formation of neuronal ensembles and dynamical neuronal avalanches. Additionally, strong experimental support for the utility of multifractal analysis as a biomarker for physiological, pathological and cognitive states was presented.
1.4. Conclusions

A brief summary of learning, memory, hippocampal function, cannabinoids, fractal and multifractal literature have been presented in an attempt to integrate numerous diverse fields of science. The main conceptual message is that memory, in both neurophysiological and fractal senses, arises through allometric adjustments of behavior through alterations in synaptic plasticity reflected as long-range temporal correlations and multifractal complexity. Synaptic plasticity enables learning in neuronal systems and the presence of memory, quantified with fractal and multifractal complexity, in neuronal spike trains is indicative of such plasticity occurring during neuronal ensemble formation and activation. The results presented in subsequent chapters of this dissertation emphasize the relationships between synaptic plasticity and the mono/multifractal properties of spike trains.
1.5. References


CHAPTER 2: MULTIFRACTAL ANALYSIS METHODS

Multifractal analysis may either be done from a global perspective by measuring how the variability of the signal changes across multiple scales or from a local perspective by measuring the variability of the signal at each point in time (Struzik, 2000). Multifractal Detrended Fluctuation Analysis (Kantelhardt et al., 2002; Ihlen, 2012) and Wavelet Leaders-based Multifractal Analysis (Jaffard, 2004; Lashermes et al., 2005; Jaffard et al., 2007; Wendt and Abry, 2007; Wendt et al., 2007; Ciuciu et al., 2012) are considered optimal methods for extracting multifractal features from a global perspective in the time and wavelet domains, respectively. Local variability can be quantified from a local perspective by computing the local Holder exponents directly from a time series (Ihlen, 2012; Trujillo et al., 2012).

These three methods are described in more detail below using mathematical equations in *italics*, Matlab code in *Courier New* font, and illustrations of the methodologies using two interspike interval sequences from the DNMS task, one from a vehicle session and the other from a THC session. The same neuron is used in all examples.

2.1. The Local Hölder Exponent

The local variability in the signal $X(t)$ may be defined by the local Hölder exponent $h$, which is the largest $h$: $h(t_0) = \sup \{ h : X \in C^h(t_0) \}$ that satisfies the equation:

$$|X(t) - P_{t_0}(t)| \leq C |t - t_0|^h$$

(1)
where $C$ is a positive constant and $P$ is an $n$th degree polynomial with $n$ less than $h$ (Struzik, 2000). In practice, determination of the local Holder exponent involves a sliding window calculation around each data point in the time series. First, the interspike interval series ($signal$) is converted from the noise-like signal into the walk-like signal by removing the mean an integrating (Fig. 2.1). Next, scales ($scale$) are defined as odd integers to enable symmetrical sliding window sizes around and including each data point. For scale 7, the Hölder envelope (right side of equation 1) is fit to three adjacent data points in each direction while 8 data points are used for scale = 17. The double loop structure calculates the root mean square ($RMS$) residual between a polynomial fit and the data series for each data point in the time series (inner loop) across all defined scales (outer loop). This yields a 6 cell $RMS$ variable where each cell contains a sequence of residual values for the entire time series for all 6 scales.

```
Text box 1. Local Holder Exponent (Step 1 of 2)
X=cumsum(signal-mean(signal));
scale = [7,9,11,13,15,17];
halfmax=floor(max(scale)/2);
Time_index=halfmax+1:length(X)-halfmax;

% Loop over scales
for ns=1:length(scale)
    halfseg=floor(scale(ns)/2);
    % Loop over all segments with sliding window
    for v=halfmax+1:length(X)-halfmax;
        % Get indices for each segment
        Index=v-halfseg:v+halfseg;
        % fit data to an mth order polynimal polynomial
        C=polyfit(Index,X(Index),m);
        fit=polyval(C,Index);
        % calculate Residual for each segment
        RMS{ns}(v)=sqrt(mean((X(Index)-fit).^2));
    end
end
```
Converting ISI Noise to Random Walk

**Figure 2.1. From ISI to Walk.** Converting the noise-like interspike interval (ISI) time series (blue) to the random walk time series is done by removing the mean and integrating the signal. This conversion is the first step in local Holder exponent determination and multifractal detrended fluctuation analysis. For illustration purposes, the original ISI signal was multiplied by 5.

The local Holder exponent is calculated from the root mean square deviation for each data point (Text box 2). The center of the local RMS spread is determined through regression and saved as RegFit. The loop for each scale calculates the residual fluctuation resRMS of log2(RMS{ns}) around the regression line (RegFit). The convergence of the local RMS (Fig. 2.2, bottom left) is used to estimate the local Holder exponents $H_t$ as the slope of the line from local RMS in log-coordinates to the endpoint of the regression line (RegFit) at the largest scale $\text{maxL}$. The local Holder exponents for scales 7 and 17 are plotted below an ISI sequence of the vehicle neuron (Fig. 2.2). The largest ISIs coincide with the smallest local Holder exponents, which
indicate areas of randomness (i.e., $h$ near 0.5). In periods with multiple short ISIs in sequence, long-range temporal correlations are detected as large local Holder exponents near 1.

A histogram of local Holder exponents is computed and normalized to obtain the probability histogram ($Ph$) (Fig. 2.2, bottom middle). The fractal dimension ($Dh$) is obtained by a log transformation of the normalized probability distribution and the singularity spectrum is derived by plotting the fractal dimension ($Dh$) against the Holder exponents ($Htbin$) (Fig. 2.2, bottom right). By quantifying local variability of a signal using the local Hölder exponent, alterations in neuronal response dynamics can be visualized and readily linked to behavioral activity (e.g. Chapter 5). Matlab code for this analysis is available online (Ihlen, 2012).

```matlab
Text box 2. Local Holder Exponent (Step 2 of 2)
C = polyfit(log2(scale0),log2(Fq0),1);
Regfit = polyval(C,log2(scale));
Hq0=C(1);
maxL=length(Time_index);
for ns=1:length(scale);
    % derived from middle values from signal X
    RMSt=RMS{ns}(Time_index);
    % get residuals of root mean square
    resRMS=Regfit(ns)-log2(RMSt);
    % use to normalize local holder exponent
    logscale=log2(maxL)-log2(scale(ns));
    Ht(ns,:)=resRMS./logscale + Hq0;
end
Ht_row=Ht(:);
BinNumb= round(sqrt(length(Ht_row)));
[freq,Htbin]=hist(Ht_row,BinNumb);
Ph=freq./sum(freq);
Ph_norm=Ph./max(Ph);
Dh=1-(log(Ph_norm)./log(mean(diff(Htbin))));
```
Figure 2.2 The singularity spectrum is a histogram of local Holder exponents. (top) An interspike interval sequence from one neuron. (Middle) The local Holder exponent sequence derived from the ISI time series for scales 7 and 17. Computation using larger scales yields a smoother series because it requires extended periods of low variability for large Holder exponents to be obtained. (Bottom left) The RMS values obtained at each scale are illustrated. (Bottom center) A probability histogram of the local Holder exponents is obtained by transforming local RMS values. (Bottom right) The singularity spectrum is the probability histogram of Holder exponents normalized in log-coordinates.
2.2. Multifractal Detrended Fluctuation Analysis (MF DFA).

Multifractal Detrended Fluctuation Analysis estimates the multifractal singularity spectrum from a global perspective in the time domain. First, a neuronal spike train is converted to a sequence of interspike intervals (ISIs; Fig. 2.1, blue), represented as $x$, and commonly referred to as a noise-like time series (Ihlen, 2012). The ISIs are converted into a random walk-like time series $Y(i)$ by subtracting the mean and integrating the ISI signal $x$:

$$Y(i) = \sum_{k=1}^{i} [x_k - \langle x \rangle], \ i = 1, ..., N$$ (2)

The random walk $Y(i)$ is divided into $N_s$ non-overlapping segments of equal length $s$ during the outer for loop. The local quadratic trend $y_v$ (fit) is calculated for each of the $N_s$ segments $v$ by a least-square fit of the series to determine the local root mean square variation $F(RMS_{scale})$ for each segment $v$ during the inner for loop (Figure 2.3):

$$F^2(v, s) = \frac{1}{s} \sum_{i=1}^{s} \{ Y[(v-1)s + i] - y_v(i) \}^2$$ (3)

In this way, $F^2(v, s)$ is essentially the mean-square error difference between the fit $y_v$ (fit) and the walk-like ISI sequence $Y$. The regression fit can be performed using any polynomial order ($m$), and fits of orders $m = 1$ (Figure 2.3, top) and $m = 2$ (Figure 2.3, bottom) are shown as solid black lines around the walk-like signal $Y$. The distance between the solid black lines and the dashed black lines indicates the local fluctuation $RMS_{scale}[ns](v)$ (Figure 2.3). Computation of the local variability $RMS_{scale}$ at all defined scales $scale$ yields a reduced representation of the time series used to compute the overall RMS $F$ (Figure 2.4). Slow changing fluctuations will influence $F$ at large scales while rapid fluctuations are preferentially represented at smaller scales. In
the case of ISI sequences, this means that large ISIs (lower firing rates) influence the overall RMS (F) at smaller scales because they appear as large, rapid changes in amplitude. Conversely, short ISIs and bursting behavior will influence F at larger scales. Large and small scales are defined relative to the average amplitude of the signal removed in the first step of MFDFA to yield the walk-like time series (Y).

Text Box 3. Multifractal Detrended Fluctuation Analysis (1 of 2)

Y=cumsum(signal-mean(signal));  (Figure 2.1)
scale = [16,32,64,128,256];
q = -3:0.5:3;
m = 1;
for ns=1:length(scale) %scale is bin size
  % # of segments at this scale
  segments(ns)=floor(length(Y)/scale(ns));
  % Compute local RMS around a trend fit{v} for each time segment
  for v=1:segments(ns) % v is a time index
    % Indicies of nonoverlapping bins by scale
    Index=((((v-1)*scale(ns))+1):(v*scale(ns)));
    % fit a polynomial trend to each segment
    C=polyfit(Index,Y(Index),m);
    fit=polyval(C,Index);
    % local fluctuation (RMS) of residual variation of data from fit (Figure 2.3-2.4)
    RMS_scale{ns}(v)=sqrt(mean((Y(Index)-fit).^2));
  end
  F(ns)=sqrt(mean(RMS_scale{ns}.^2));
end

% Multifractal fluctuation function (Figure 2.6)
for nq=1:length(q),
  qRMS{nw,nq}=RMS_scale{nw}.^q(nq);
  Fq{nw,nq}=mean(qRMS{nw,nq}).^(1/q(nq));
end

Fq(q==0,nw)=exp(0.5*mean(log(RMS_scale{nw}.^2)));
Figure 2.3. Fit and local fluctuation (RMS) calculated for the first two segments at all scales. The local trend (fit), solid black line, is polynomial fit to the walk of the ISI, blue lines. The top row was calculated using a 1\textsuperscript{st} order polynomial fit ($m=1$) and the bottom row was calculated using a 2\textsuperscript{nd} order polynomial fit ($m=2$). The local variation (RMS\_scale) is signified as the dashed black lines around the regression fit. The local fluctuation (RMS\_scale) for the first two segments signifies the local variability of each segment and is the essential component of (monofractal) detrended fluctuation analysis.
Figure 2.4. Local fluctuation (RMS_scale) for all segment sizes. The local fluctuation, RMS_scale (colored lines), for the vehicle neuron exemplifies how long-range temporal correlations can be detected in ISI time series. The average RMS for each scale, F, is plotted as a black line. Note that F increases at larger scales.
Figure 2.5. Hurst Exponent is the slope of the regression line of overall RMS (F) versus scale. The slope of the regression line (RegLine) is the power-law exponent, or the Hurst exponent (Hurst). The example neuron recorded in the vehicle and THC conditions exhibited similar Hurst exponents and therefore similar long-range temporal correlations, as indicated by parallel lines. The overall RMS (F) for the vehicle is larger and this is an indication for increased variability in the vehicle neuron vs. THC neuron.

The Hurst exponent (Hurst, 1951) is the slope of the regression line (RegLine) between log2(scale) and log2(F) (Fig. 2.5). A greater slope yields a larger Hurst exponent and indicates increased LRTCs which define how fast the overall root mean square variation grows with increasing segment size (scale). Hurst exponents greater than 0.5 indicate the time series contains positive correlations (i.e., persistent structure), Hurst exponents ranging from 0 to 0.5 indicate negative correlations and uncorrelated Gaussian noise has a Hurst exponent equal to 0.5. Specifically, “long-range” refers to influences up to 256 data points away since this is the largest scale used in the analysis. Therefore, variability up to 256 ISIs in the past positive influences neuronal spike trains in this

```matlab
C=polyfit(log2(scale),log2(F),1);
Hurst=C(1);
RegLine=polyval(C,log2(scale));
```

Text Box 4. Monofractal Hurst Exponent (Figure 2.5)
example (Fig. 2.5). The example vehicle and THC spike trains showed similar Hurst exponents, but the larger values of F computed for the vehicle neuron indicate larger variability compared to the THC spike train (Fig. 2.5).

Visualization of the walk like ISI sequence at multiple q-order statistical moments permits observation of the structure of variability quantified by multifractal analysis (Fig. 2.6). Periods of low activity (i.e., clusters of short ISIs, faster firing rate) are amplified with negative moments (Fig. 2.6). Conversely, periods of high activity (i.e., clusters of longer ISIs, slower firing rate) are amplified with positive moments. The qth order fluctuation function \( F_q \) in Text Box 3 is determined by averaging over all segments \( v \) for each qth power (Fig. 2.6):

\[
F_q(s) = \left\{ \frac{1}{N_s} \sum_{v=1}^{N_s} [F^2(v,s)]^{q/2} \right\}^{1/q}
\]
Figure 2.6. Illustration of q-order RMS. (q_{RMS} in Text box 3). The vehicle neuron is in blue and the THC neuron is in green. Periods of low variability (small ISIs) are amplified by negative q values and periods of high variability (large ISIs) are amplified by positive q-values. Note that the vehicle neuron exhibits more periods of variability with larger magnitude compared to the THC neuron, ultimately revealed as greater multifractal complexity.
The scaling behavior of the fluctuation function is seen by analyzing log-log plots of $F_q(s)$ versus $s$ for each $q$th power (Fig. 2.7, top left). Standard (Monofractal) Detrended Fluctuation Analysis calculates the Hurst exponent from the slope of the power-law regression line between the overall root mean square variation $F$ across multiple scales, $s$, for a single statistical moment, $q = 2$. Multifractal analysis performs the same linear regression for a broad range of statistical moments $q$. The $q$-order fluctuation function $F_q(s)$ ($F_q$) increases as a power-law for large values of $s$ if the signal $x$ contains LRTCs (Fig. 2.7, top left):

$$F_q(s) \sim s^{H(q)}$$  \hspace{1cm} (5)

In multifractal signals, changes in LRTCs occur at different $q$-order statistical moments. These variations are visualized by comparing the $q$-order Hurst exponents (slopes of regression lines) on log-log plots of $F_q(s)$ versus scale $s$, where each line is computed from a different $q$th power (Fig. 2.7, top left). Our example shows more variable slopes in the vehicle condition (Fig. 2.7, blue) compared to the similar slopes in THC condition (Fig. 2.7, green), indicating greater multifractality during the vehicle condition. The $q$-order fluctuation function $F_q(s)$ is one way to visualize multifractal properties of variability, but generally the multifractal singularity spectrum (Fig. 2.7, bottom right) is constructed to illustrate the distinction between (mono)fractal and multifractal signals. The $q$-order Hurst exponent ($H_q$) is calculated in a similar manner as the monofractal Hurst exponent but now with multiple statistical orders $q$ (for loop in Text box 5). The $q$-order Hurst exponent $H(q)$ ($H_q$) is converted to the $q$-order mass exponent $\tau(q)$ ($\tau_q$) (Fig. 2.7, top middle to top right):

$$\tau(q) = qH(q) - 1$$  \hspace{1cm} (6)
Finally, a Legendre transform relates $\tau(q)$ to the fractal dimension $D(h)$ ($D_q$) and Holder exponent $h$ ($h_q$) (Fig. 2.7, top right and bottom):

$$h = t'(q) \text{ and } D(h) = qh - \tau(q)$$  \hspace{1cm} (7)

The singularity spectrum is a transformed histogram of the q-order Hurst exponents (Fig. 2.7). Negative q-values, representing areas of low variability, end up on the right side of the spectrum while positive q-values end up on the left side. This relationship is in accordance with the local method (Section 2.1) because low variability is always on the right and high variability on the left. The magnitude of multifractality is determined by the width of the singularity spectrum and consequently the range of Holder exponents $h$ covered by the ISI signal. The Hurst exponent is closely approximated by the Holder exponent at the apex of the singularity spectrum (where $D(h) = 1$). In our example, the singularity spectrum for the THC condition is narrower, and thus less multifractal than the singularity spectrum computed for the control condition (Fig. 2.7, bottom right).

**Text box 5. Multifractal Detrended Fluctuation Analysis (2 of 2)**

```matlab
for nq=1:length(q)
    C = polyfit(log2(scale),log2(Fq(nq,:)),1);
    Hq(nq) = C(1);
    qRegLine{nq} = polyval(C,log2(scale));
end

% q-order mass exponent
    tq = Hq.*q-1;

% Legendre Transform
    hq = diff(tq)./(q(2)-q(1));
    Dq = (q(1:end-1).*hq)-tq(1:end-1);
```
Figure 2.7. Estimation of the singularity spectrum from q-order fluctuation function. Vehicle neuron is represented in blue and THC neuron is shown in green in all panels. (Top left) The slopes of the q-order fluctuation function exhibit greater change in the vehicle neuron vs. THC neuron. (Top middle) The q-order Hurst exponent is the slope of the fluctuation function obtained for each q value. (Top right) The q-order mass exponent is obtained from Hq as in Text Box 5. (Bottom) The set of Holder exponents h_q and fractal dimensions D_q are obtained via a Legendre transform to yield the singularity spectrum.
2.3. Wavelet Leaders Multifractal Analysis (WLMA) and Log-Cumulants

The Matlab code used in this chapter was designed to exemplify computation of wavelet coefficients and wavelet leaders. The optimized WLMA algorithm released by Wendt et al., (2007) was used for all data analysis in this dissertation.

The singularity spectrum may also be calculated from a global perspective in the wavelet domain by estimating the scaling exponents $\zeta(q)$ of a signal:

$$E|X(at)|^q = |a|^\zeta(q) E|X(t)|^q$$

where $a$ is the scaling parameter and $q$ is the statistical moment. For monofractal processes, $\zeta(q)$ is constant. For multifractal processes, $\zeta(q)$ is a range of power-law exponents. WLMA computes the scaling exponents by measuring how the absolute value of the wavelet coefficients $d_x$ changes as a function of scale. The first step in WLMA is to transform the data to the wavelet domain with a Discrete Wavelet Transform (DWT) (Fig. 2.8):

$$d_x(j,k) = \int_R X(t) 2^{-j/2} \Psi_0(2^{-j}(t - 2^j k)) \, dt$$

where $\Psi_0$ is an appropriately chosen mother wavelet. A Daubechies wavelet with 4 vanishing moments was used (‘db4’) in this example and throughout the dissertation. The wavelet coefficients $d_x$ (coeff) are extracted from the DWT on a dyadic grid at scales equal to $2^j$ and time shifts equal to $2^j k$ (Fig. 2.8). In Text Box 6, the wavedec function performs wavelet decomposition across 8 scales (scaleMax) of the ISI signal (x) yielding the wavelet coefficients (C). The for-loop ($k = 1:scaleMax$) expands wavelet coefficients for visualization, allowing coeff to be plotted in Figure 2.8. The
wavelet leaders $d_L$ (leaders(j).value) are calculated from the wavelet coefficients for every point on the dyadic grid by finding the maximum wavelet coefficient among the immediately adjacent wavelet coefficients for the current scale (j) and all smaller scales (j-1) (Fig. 2.9).

$$d_L(j,k) = \max \{ dx(j',k') \}$$ \hspace{1cm} (10.1)

such that

$$(k-1)2^j \leq 2^j k' < (k+2)2^j$$ \hspace{1cm} (10.2)

---

**Text box 6. Wavelet Coefficients and Wavelet Leaders**

```matlab
scaleMax = 8;
[C l] = wavedec(x,scaleMax,'db4');
len = length(x);
coeff = zeros(scaleMax,len);
for k = 1:scaleMax
    d = detcoef(C,l,k);
    d = d(:)';
    d = d(ones(1,2^k),:);
    % Wavelet coefficients (Figure 2.8)
    coeff(k,:) = wkeep1(d(:)',len);
end

% Wavelet Leaders (Figure 2.9)
coef_temp = unique(coeff(1,:),'stable');
% Extract Wavelet Leaders from the dyadic grid
leaders(1).value = max([coef_temp(1:end-2);... 
    coef_temp(2:end-1);coef_temp(3:end)]);
for j = 2:scaleMax
    coef_temp = unique(coef(j,:),'stable');
    leaders(j).temp = max([coef_temp(2:end);... 
        leaders(j-1).value(1:2:end-1);leaders(j-1).value(2:2:end)]);
    leaders(j).value = max([leaders(j).temp(1:end-2);... 
        leaders(j).temp(2:end-1);leaders(j).temp(3:end)]);
end```

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Figure 2.8. Discrete Wavelet Transform. (Top) Absolute coefficients (coeff) obtained from the discrete wavelet transform were computed for the vehicle and THC neuron. (Bottom) Neuronal ISI sequences for each neuron. Note that the vertical axis is higher for the vehicle neuron.
**Figure 2.9. Extracting Wavelet Leaders from Dyadic Grid.** (Left) Wavelet Leaders (leaders(j).value) are the largest wavelet coefficients from each highlighted section of the dyadic grid. (Right) When plotted, Wavelet Leaders are a filtered representation of wavelet coefficients that help reduce redundancy and enable more efficient multifractal analysis.

If $n_j$ is the number of wavelet leaders $d_L(j,k)$ available at every scale $2^j$ for the time series $X(t)$, then the structure function $S(j,q)$ can be defined as:

$$S(j, q) = \frac{1}{n_j} \sum_{k=1}^{n_j} |d_L(j,k)|^q \quad (11.1)$$

where $k = 1,2,\ldots,n_j$. This function behaves as a power-law over analysis scale $2^j$ for a set range of scales and for a set range of statistical orders $q$. Statistical orders $q$ should contain both positive and negative numbers in order to properly estimate multifractality. As in MFDFA, positive $q$-values amplify periods of large variability while negative $q$-
values amplify areas of low variability (i.e., short ISIs). Therefore, positive $q$-values estimate areas with small (near random) local Hölder exponents $h$ and negative $q$-values estimate areas with higher local $h$ values (i.e., LRTCs). The scaling exponent $\zeta(q)$ (Fig. 2.10, left) may be calculated from the structure function $S(j,q)$ to quantify changes in variability as a function of scale $j$ and statistical orders $q$:

$$S(j,q) = F_q(2^j)^\zeta(q)$$  \hspace{1cm} (11.2)

where $F_q$ is a constant independent of $j$. The singularity spectrum (Fig. 2.10, right) can then be estimated directly from the scaling exponent using a Legendre transform (Wendt and Abry, 2007).

$$\zeta(q) = \inf_h \left( 1 + qh - D(h) \right)$$  \hspace{1cm} (12)

This leads to the basis of the Wavelet Leaders-based Multifractal Formalism:

$$D(h) = \inf_{q \neq 0} \left( 1 + qh - \zeta(q) \right)$$  \hspace{1cm} (13)

As with MFDFA, positive $q$-values are located on the left side and negative $q$-values are located on the right.
Figure 2.10. Scaling Function and Singularity Spectrum. WLMA also shows that the vehicle neuron is more multifractal than the THC neuron because it yields a wider singularity spectrum.

The scaling exponents may also be rewritten as second characteristic functions, a standard function expansion of the natural log of the time averaged wavelet leaders $C_p^j$. The power law expansion of the first two terms of $C_p^j$ are

$$C_1^j = \mathbb{E}[\ln[\bar{d}_L]] = c_1^0 + c_1 \ln 2^j$$  \hspace{1cm} (14)$$

$$C_2^j = \mathbb{E}[\ln[\bar{d}_L]^2] - (C_1^j)^2 = c_2^0 + c_2 \ln 2^j$$  \hspace{1cm} (15)$$

The log-cumulants $c_1$ and $c_2$ are calculated from the slope of $C_p^j$ versus scale and correspond to specific attributes of the multifractal singularity spectrum (Wendt and Abry, 2007; Wendt et al., 2007). The singularity spectrum of a one-dimensional time series may be approximated as a polynomial expansion around its maximum using the log-cumulants (Wendt et al., 2007; Ciuciu et al., 2012).

$$D(h) = 1 + \left(\frac{c_2}{2!}\right)^{h-c_1^2} + \left(-\frac{c_3}{3!}\right)^{h-c_1^3} + \ldots$$  \hspace{1cm} (16)$$

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\(c_1\) is a self-similarity parameter, which measures long-range temporal correlations (LRTCs) and it takes values very closely related to the global Hurst exponent \(H\) (Wendt et al., 2007). \(c_2\) is a function of the width of the multifractal spectrum and acts as a test of mono- vs. multifractal. Monofractal signals are self-affine with a narrow range of Hölder exponents uniformly distributed throughout the signal. Multifractal signals deviate from pure self-affinity by expressing a wide range of nonuniformly dispersed variability throughout distinct regions. \(c_2\) is always negative due to the inverted parabolic shape of the singularity spectrum. As \(c_2\) becomes more negative, it indicates that the signal is more multifractal. We mainly focus on \(c_1\) and \(c_2\) because they describe the main features of the singularity spectrum, location and width, and are the most robust to calculate.

The Wavelet Leaders code was obtained from Wendt's freely available, online MATLAB toolbox, the WLMA Toolbox (Wendt et al., 2007; \url{http://www.irit.fr/~Herwig.Wendt/software.html}).
2.4. References


CHAPTER 3: MULTIFRACTAL ANALYSIS OF INFORMATION PROCESSING IN HIPPOCAMPAL NEURAL ENSEMBLES DURING WORKING MEMORY UNDER Δ⁹-TETRAHYDROCANNABINOL ADMINISTRATION

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3.1. Abstract

Background: Multifractal analysis quantifies the time-scale-invariant properties in data by describing the structure of variability over time. By applying this analysis to hippocampal interspike interval sequences recorded during performance of a working memory task, a measure of long-range temporal correlations and multifractal dynamics can reveal single neuron correlates of information processing.

New Method: Wavelet Leaders-based Multifractal Analysis (WLMA) was applied to hippocampal interspike intervals recorded during a working memory task. WLMA can be used to identify neurons likely to exhibit information processing relevant to operation of brain-computer interfaces and nonlinear neuronal models.

Results: Neurons involved in memory processing ("Functional Cell Types" or FCTs) showed a greater degree of multifractal firing properties than neurons without task-relevant firing characteristics. In addition, previously unidentified FCTs were revealed because multifractal analysis suggested further functional classification. The cannabinoid-type 1 receptor partial agonist, tetrahydrocannabinol (THC), selectively reduced multifractal dynamics in FCT neurons compared to non-FCT neurons.

Comparison with Existing Methods: WLMA is an objective tool for quantifying the memory-correlated complexity represented by FCTs that reveals additional information compared to classification of FCTs using traditional z-scores to identify neuronal correlates of behavioral events.

Conclusion: Z-score-based FCT classification provides limited information about the dynamical range of neuronal activity characterized by WLMA. Increased complexity, as measured with multifractal analysis, may be a marker of functional involvement in memory processing. The level of multifractal attributes can be used to differentially emphasize neural signals to improve computational models and algorithms underlying brain-computer interfaces.
3.2. Introduction and Background

3.2.1. Memory Processing in Nonlinear Hippocampal Dynamics

Hippocampal neural ensembles are known to represent crucial information for successful memory performance in a variety of tasks (Berger et al., 2011, 2012; Deadwyler et al., 2013; Eichenbaum et al., 1996; Goonawardena et al., 2010; Hampson et al., 2013; O’Neill et al., 2013; Tayler et al., 2013). The delayed nonmatch-to-sample (DNMS) task requires spatial and nonspatial working memory processing across three timescales: encoding during the sample lever press, retention throughout a variable delay interval, and recall during the nonmatch decision phase (Deadwyler et al., 1996). By analyzing the neuronal discharge rate around these task events, the activity of a subset of task-relevant hippocampal principal neurons, known as functional cell types (FCTs), was shown to represent essential correlates of spatial working memory (Goonawardena et al., 2010; Hampson et al., 1999). FCTs can be identified using their mean firing rate response (calculated as a z-score) during combinations of key behaviorally relevant task events; however, this method involves subjective decisions and cannot completely characterize the full dynamic range of neuronal activity. Z-score calculations require a priori designated behavioral events, selection of a generalizable activity baseline (Hampson et al., 1999), and are prone to high degrees of intra- and inter-session variability. Z-score calculations may miss important features of neuronal information processing occurring around events which have not been identified prior to analysis. By comparing results of z-score-based FCT designation with measures of multifractal complexity, hypotheses concerning dynamic neuronal functionality can be strengthened.

The nonlinear multi-input, multi-output model (MIMO) is an effective predictor of hippocampal memory encoding (Hampson et al., 2012), and it has been successfully used as a neuroprosthetic to improve memory performance in both rats (Hampson et al.,
FCTs have been shown to significantly contribute to the MIMO hippocampal model (Hampson et al. 2012) suggesting that behavioral correlates of individual hippocampal neurons exhibit nonlinear and possibly fractal features in their spike trains. Hippocampal neuronal ensembles have previously been pre-screened using z-score based FCT identification, but since this method employs subjective decisions during computation, it is hypothesized that multifractal analysis could provide an objective measure of the information processing capacity of hippocampal neurons. This would improve both the speed and accuracy of the current MIMO model (Song et al., 2009) and facilitate translation into the human population. The nonlinearity and variability of hippocampal interspike interval sequences may carry a significant amount of memory processing information, and multifractal analysis can provide a quantitative measure of these dynamical features. Fractal analysis and 1/fα-power-law-distribution have previously been used to describe dynamics of neuronal avalanches (Beggs and Plens, 2003, 2004), EEG oscillations (Benayoun et al., 2010; Linkenkaer-Hansen et al., 2001, 2004), cell morphology (Bernard et al., 2001), and interspike intervals (Das et al., 2003; Lewis et al., 2001; Teich et al., 1990). Fractal analysis and 1/fα-power-law-distribution are able to quantify self-similarity of the whole data set but cannot describe local fluctuations in the data occurring over time. We therefore hypothesize that multifractal analysis, which can simultaneously quantify self-similarity and local fluctuations (Zilber et al., 2013), can be used to detect neural information processing relevant for memory performance in hippocampal spike trains based on the distribution of interspike interval variability throughout the DNMS task. The qualitative information provided by FCT classification concerning neuronal information representation can be complemented with multifractal analysis to promote richer interpretations of neurophysiological data.
3.2.2. Background: Fractal Nature of Time Series Data

Fractal analysis quantifies the irregularity, also known as singularities, in objects and signals by detecting correlations among these irregularities across multiple spatial and/or temporal scales. The term “fractal” was originally used to describe “self-similar” objects that contain identical repeating structures at an infinite set of smaller scales (Mandelbrot, 1982). Later the term fractal was expanded to include “self-affine” objects; i.e. objects with structure that is statistically equivalent, but not identical across an infinite set of smaller scales (Mandelbrot, 1985). Self-affinity describes a wider range of natural formations, such as those found in coastlines, clouds, mountain ranges and branching patterns of trees. More recently, fractal analyses have also been used to describe scale-invariant fluctuations occurring over multiple time scales in biological signals, turbulence, sun spot activity, and financial markets (for a review, see Kantelhardt, 2012). In scale-invariant structures, all measurement scales contribute equally to the dynamical activity patterns. Understanding these scaling relationships may provide important details about the underlying information processing mechanisms (Ciuciu et al., 2008).

The singularity spectrum is a succinct way of conveying the scale invariant self-similarity of the data along with local temporal variability. The singularity spectrum plots the local Hölder exponent $h$, a measure of local variability, versus the fractal (Hausdorff) dimension $D(h)$ of data points with the same local Hölder exponent. Monofractal signals are defined by a single scaling relationship which remains constant for the entire time series. Multifractal signals have regions of high variability interspersed with regions of low variability. As a result of this dispersed variability, multiple scaling relationships form throughout the entire time series. Based on results obtained with multifractal analysis, three representative hippocampal neurons recorded during a working memory task are shown in figure 3.1: a random (uncorrelated) signal, a monofractal signal, and a multifractal signal. The top graphs display the interspike interval sequences of each
neuron (section 3.3.4.5), while the bottom graph shows their singularity spectra. The most common local Hölder exponent occurs at the apex of the inverted parabola is also called the global Hurst exponent $H$ (Brazhe and Maksimov, 2006; Mandelbrot, 1982). It describes the global self-similarity of the data set and is analogous to the power law exponent obtained from fractal analysis in $1/f^\alpha$-power-law distributions. If $H$ falls between 0 and 0.5 the system is anti-persistent, $H$ equal to 0.5 corresponds to a random Gaussian process, and a persistent signal has an $H$ from 0.5 to 1. A persistent series is one containing long-term positive correlations (or long-range temporal correlations) and is often referred to as having “long term memory,” while anti-persistent structure indicates negative correlations over time. Since random signals not correlated, the uncorrelated data in figure 3.1 has a global Hurst exponent of 0.5. In this example (Fig. 3.1), the monofractal signal has long-range temporal correlations and a Hurst exponent of 0.65. In the context of neuronal activity, long-range correlations could be described by repeating series of activity patterns. For example, neuronal bursting will be followed by more bursting, while quiescence will most likely be followed by more quiescence (Hu et al., 2013). In a fractal context, “long-range” refers to correlations to multiple previous data points. For example, one ISI may be correlated with 5 of the immediately prior ISIs, and long-range temporal correlations would arise if numerous occurrences exist throughout a signal. The uncorrelated and monofractal signals should have non-existent spectra width, but the small range of local Hölder exponents is a result of analyzing real data sets of finite duration. The singularity spectrum of the multifractal signal is much wider than those of other signals because the heterogenous distribution of variability can only be quantified by a range of Hölder exponents.
**Figure 3.1. Comparison of singularity spectra for multiple types of time series.**

(Top) Three example interspike interval time sequences recorded from the hippocampus (Methods section 3.3.4.5).  (Bottom) The respective singularity spectra computed for each signal: uncorrelated signal (red), monofractal (green), and multifractal (blue).  The singularity spectrum quantifies the distribution of variability in time series.  The fractal (Hausdorff) dimension $D(h)$ on the vertical axis is plotted against the range of Hölder exponents $h$ on the horizontal axis (Methods sections 3.3.4.3-3.3.4.4).  The global Hurst exponent and consequently the peak position on the horizontal axis is 0.5 of the uncorrelated signal.  The monofractal and multifractal signals have global Hurst exponents greater than 0.5 indicating long-range temporal correlations in the signal.  The multifractal spectrum (blue) is much wider than the others because a larger range of Hölder exponents is needed to accurately describe the heterogenous distribution of variability.  The uncorrelated was selected based on results obtained using multifractal analysis because it has a global Hurst exponent of 0.5.
The complex neuronal interactions governing behavior, memory and cognition can be further understood by appreciating the implications of what “multifractality” means in a broader context. Interactivity, both between neurons and between an organism and its environment, is an important feature that can be empirically quantified using multifractal analysis. Recent research drawing from Gibsonian views of ecological psychology has suggested that multifractal properties arise from interactions from many signals across multiple scales (Kelty-Stephen et al., 2013), hence its name: “interaction-dominant theory.” Interaction-dominant theory views a system as resulting from the synergistic, interrelated activity of all parts and postulates that common patterns are extracted and reused throughout the learning process across all contexts (Dixon et al., 2012; Ihlen and Vereijken, 2013). A counter argument, component-dominant theory, states that variability is caused by particular sources and an underlying behavior arises from specific, localized system constituents (Ihlen and Vereijken, 2010, 2013). Earlier work suggested that interaction-dominant phenomena could be dissociated from component-dominant processes based on $1/f^\alpha$ power-law distributions and long-range temporal correlations (Kello et al., 2007; Van Orden et al., 2005), but it is now believed that only multifractal models can disentangle these theories (Ihlen and Vereijken, 2010). Multifractal cascade dynamic theory suggests that scale-invariance and fractal properties can come from “temporally correlated fluctuations of many different sizes” (Stephen et al., 2012). Multifractal dynamics suggests that a signal interacts with other signals, possibly from other local neurons or additional distant brain areas, and the amount of mutual interaction is quantified by the width of the singularity spectrum (Ihlen and Vereijken, 2010).

Many techniques can compute the singularity spectrum, such as Wavelet Transform Modulus Maximus (WTMM; Arneodo et al., 1993), Adaptive Detrended Fluctuation Analysis (AFA; Kuznetsov et al., 2012; Riley et al., 2012), Arbitrary-Order Hilbert
Spectral Analysis (AOHSA; Huang et al., 2011), Empirical Mode Decomposition (EMD; Flandrin and Goncalves, 2004; Goncalves et al., 2007), Multifractal Detrended Fluctuation Analysis (MF DFA; Ihlen, 2012; Kantelhardt et al., 2002; Oświeimka et al., 2006), and Wavelet Leaders-based Multifractal Analysis (WLMA; Jaffard et al., 2007; Serrano and Figliola, 2009; Wendt et al., 2007; Wendt and Abry, 2007). WLMA has many advantages over the other methods: it is computationally efficient, it is able to deal with a broader range of singularity types, and it is able to estimate the Hölder exponents in regions of low variability. In the latter case, problems associated with estimating the Hölder exponents may be circumvented by estimating the singularity spectrum directly from the infinite series expansion of the log-cumulants of the scaling exponents. The first three parameters of this expansion \( c_1, c_2, \) and \( c_3 \) describe the singularity spectrum's peak, width, and asymmetry, respectively. The parameterization of the singularity spectrum with these three parameters provides a convenient and efficient means of comparing singularity spectrum along with classifying time signals (Ciuciu et al., 2008, 2012; Wendt et al., 2007).

3.2.3. Fractal Analyses of Hippocampal Neural Spike Trains

We used the WLMA to estimate the singularity spectrum from the interspike intervals (ISI) of rat hippocampal (CA3 and CA1) neurons recorded during the delayed nonmatch-to-sample (DNMS) task, a working memory task with spatial and nonspatial components (Deadwyler et al., 1996; Hampson et al., 1999). Neurons were tracked in animals under control conditions and also with delta-9-Tetrahydrocannabinol (THC) administration. The general idea of this paper is that the singularity spectra, a quantitative measure of multifractal properties, will be able to distinguish task-correlated from non-correlated neurons and differentiate between control and THC sessions. We tested this general idea with three specific hypotheses. Our first hypothesis is that functional cell types (i.e. FCTs or FCT neurons – as defined in Goonawardena et al., 2010; Hampson et al., 1999)
will have larger long-range temporal correlations than non-FCT neurons. Since FCTs are involved in memory processing, the long-range temporal correlations could arise from their similar and repeating firing patterns during specific behavioral events. Our second hypothesis is that FCTs will contain more multifractal dynamics, possibly due to abrupt, intermittent changes in neuronal activity coinciding with variable DNMS task requirements. THC, the main psychoactive constituent of cannabis (Gaoni and Mechoulam, 1964), disrupts memory function (Hampson and Deadwyler, 2000), sequential processing (Hampson et al., 1989), perception (Atakan et al., 2012), theta power (Ilan et al., 2004; Kucewicz et al., 2011), hippocampal neural spike-timing (Robbe et al., 2006), and hippocampal neural synchrony (Goonawardena et al., 2011). Therefore, THC can be used as a tool to differentiate the role of hippocampal mnemonic processing during the DNMS task under memory-impairing conditions. And finally, our third hypothesis is that THC administration will reduce multifractal dynamics of all recorded hippocampal principal cells.

3.3. Materials and Methods

3.3.1. Subjects, Training and Drug Administration

3.3.1.1. Animals

Subjects were Long-Evans rats (Harlan) aged 4-6 months (n=6) individually housed and allowed free access to food with water regulation to maintain 85% of ad libitum body weight during testing. All animal protocols were approved by the Wake Forest University Institutional Animal Care and Use Committee, in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care and the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023).
3.3.1.2. Apparatus

The behavioral testing apparatus for the delayed nonmatch to sample (DNMS) task is the same as reported in other studies (Hampson and Deadwyler, 2000; Hampson et al., 2012) and consisted of a 43 × 43 × 50 cm Plexiglas chamber with two retractable levers (left and right) positioned on either side of a water trough on the front panel. A nosepoke device (photocell) was mounted in the center of the wall opposite the levers with a cue light positioned immediately above the nosepoke device. A video camera was mounted on the ceiling and the entire chamber was housed inside a commercially built sound-attenuated cubicle.

3.3.1.3. Behavioral Training Procedure

The DNMS task consisted of three main phases: Sample, Delay and Nonmatch. The sample phase initiated the trial when either the left or right lever was extended (50% probability), requiring the animal to press it as the Sample Response (SR). The lever was then retracted and the Delay phase of the task initiated, as signaled by the illumination of a cue light over the nosepoke photocell device on the wall on the opposite side of the chamber (Fig. 3.2A). At least one nosepoke (NP) was required following the delay interval which varied randomly in duration (1-30 s) on each trial during the session. The Nonmatch phase began when the delay timed out, the photocell cue light turned off and both the left and right levers on the front panel were extended. Correct responses consisted of pressing the lever in the Nonmatch phase located in the spatial position opposite the SR (nonmatch response: NR). This produced a drop of water (0.4 ml) reward in the trough between the two levers. After the NR the levers were retracted for a 10.0 s intertrial interval (ITI) before the next Sample lever was presented to begin the next trial. A lever press at the same position as the SR (Match Response) constituted an “error” with no water delivery and turned off of the chamber house lights for 5.0s and
the next trial was presented 5.0 s later. Individual performance was assessed as % NRs (correct responses) with respect to the total number of trials (80-100) per daily (1 hr) sessions.

3.3.1.4. Drug preparation and administration.

Delta-9-THC was obtained from the National Institute on Drug Abuse as a 50 mg/ml solution in ethanol. Detergent vehicle was prepared from Pluronic F68 (Sigma, St. Louis, MO), 20 mg/ml in ethanol. Δ⁹-THC was added to the detergent-ethanol solution (0.5 ml of either THC), and then 2.0 ml of saline (0.9%) was slowly added to the ethanol-drug solution. The solution was stirred rapidly and placed under a steady stream of nitrogen gas to evaporate the ethanol (~10 min). This resulted in a detergent-drug suspension (12.5 mg/ml THC), which was sonicated and then diluted with saline to final injection concentrations (0.5–2.0 mg/ml THC). On drug administration days, animals were injected intraperitoneally with the drug-detergent solution (1 ml/kg) ~10 min before the start of the behavioral session. Our experience with these experiments has shown that performance after vehicle injection is not significantly different than no injection, and therefore was omitted during this series of experiments to minimize risk of infection to the animals. At least two no injection days were imposed between each drug-testing session. All drug solutions were mixed fresh each day.

3.3.2. Hippocampal Electrode Array Surgery

All surgical procedures conformed to National Institutes of Health and Association for Assessment and Accreditation of Laboratory Animal Care guidelines, and were performed in a rodent surgical facility approved by the Wake Forest University Institutional Animal Care and Use Committee. After being trained to criterion performance level in the DNMS task animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic frame. Craniotomies (5mm-
diameter) were performed bilaterally over the dorsal hippocampus to provide for implantation of 2 identical array electrodes (Neurolinc, New York, NY), each consisting of two rows of 8 stainless steel wires (diameter: 20 μm) positioned such that the geometric center of each electrode array was centered at co-ordinates 3.4 mm posterior to Bregma and 3.0 mm lateral (right or left) to midline (Paxinos and Watson, 1997). The array was designed such that the distance between two adjacent electrodes within a row was 200 μm and between rows was 400 μm to conform to the locations of the respective CA3 and CA1 cell layers. The longitudinal axis of the array of electrodes was angled 30° to the midline during implantation to conform to the orientation of the longitudinal axis of the hippocampus, with posterior electrode sites more lateral than anterior sites (Fig. 3.2C). The electrode array was lowered in 25-100 μm steps to a depth of 3.0 - 4.0 mm from the cortical surface for the longer electrodes positioned in the CA3 cell layer, leaving the shorter CA1 electrodes 1.2 mm higher with tips in the CA1 layer. Extracellular neuronal spike activity was monitored from all electrodes during surgery to maximize placement in the appropriate hippocampal cell layers. After placement of the array the cranium was sealed with bone wax and dental cement and the animals treated with buprenorphine (0.01–0.05 mg/kg) for pain relief over the next 4-6 hrs. The scalp wound was treated periodically with Neosporin antibiotic and systemic injections of penicillin G (300,000 U, intramuscular) were given to prevent infection. Animals were allowed to recover from surgery for at least 1 week before continuing behavioral testing (Berger et al., 2011).

3.3.3. Multineuron Recording of Hippocampal Ensembles

3.3.3.1. Electrophysiological Monitoring and Acquisition of Neuronal Data

Animals were connected by cable to the recording apparatus via a 32-channel headstage and harness attached to a 40-channel slip-ring commutator (Crist Instruments, Hagerstown, MD) to allow free movement in the behavioral testing.
chamber. Single neuron action potentials (spikes) were isolated by time-amplitude window discrimination and computer-identified individual waveform characteristics using a multi-neuron acquisition (MAP) processor (Plexon Inc., Dallas, TX, USA). Single neuron spikes were recorded daily and identified using waveform and firing characteristics within the task (perievent histograms) for each of the DNMS events (SR, LNP & NR). Only isolated spike waveforms exhibiting firing rates consistent with CA1 and CA3 principal cells (i.e. 0.5-10.0 Hz baseline firing rate) and stable behavioral correlates across sessions were employed for experimental manipulations and model development (Deadwyler et al., 2007; Berger et al., 2011). Hippocampal neuron ensembles used to analyze encoding of DNMS events consisted of 15-32 single neurons, each recorded from a separate identified electrode location on either of the bilateral arrays.

3.3.4. Hippocampal Neural Analysis

3.3.4.1. Identification of Functional Cell Types

Prior studies from this laboratory have identified hippocampal neurons recorded as above by “Functional Cell Types” (FCTs) described by different behavioral correlates of DNMS task-related events such as lever position and/or phase of the task (Hampson et al., 1999; Goonawardena et al., 2010). Individual neurons exhibit firing rate increases in response to Sample and Nonmatch responses (Fig. 3.2B); however, the defining characteristic an FCT in this context is that the neuron responds by increased firing rate only to a specific combination of events within the trial. Neural firing in response to each Sample (SR) or Nonmatch (NR) response event is analyzed by standard score ($Z = \frac{\text{peak firing rate} - \text{mean of the baseline firing rate}} {\text{std. dev of baseline firing rate}}$) using 100 ms bins. The baseline firing rate and standard deviation were computed 2.0-2.5 seconds prior to the SR, and the peak firing rate was computed ±1.2 seconds around the instant of SR or NR. Only neural firing during correct trials was used for z-score
calculations. Neurons were then categorized according to simple responses: position cells: Left-lever only, Right-lever only; phase cells: Sample-response only, Nonmatch-response only; conjunctive cells: Right Sample only, Left Sample only, Right Nonmatch only, Left Nonmatch only; or Trial-type cells: Right Sample + Left Nonmatch, Left Sample + Right Nonmatch (Hampson et al., 1999; Goonawardena et al., 2010). In addition, increased neuronal activity during the “last nosepoke” (LNP in Fig. 3.2A) was also analyzed which allowed for the creation of a new class of FCT: Nosepoke-Nonmatch cells.

Neurons were classified as FCTs based on the combination of z-scores calculated around sample, last nosepoke, and nonmatch responses. Average firing rate around DNMS events was computed for multiple days to create one overall average for each neuron for each event, and z-scores were calculated based on the overall averages; this was done separately for control and THC sessions. Neurons with z-scores greater than or equal to 3.19 (p < 0.001) in control sessions were considered to be FCTs.

Designations of FCT (e.g., Conjunctive or Trial-Type) were made by examining which combination of events (six total: sample, nosepoke and nonmatch for both right and left levers) showed significant increases in firing rate. The average z-score and z-score standard deviation were calculated using these six z-score values for each neuron. If neurons had more than three out of six possible significant peaks, only peaks above the “z-average half std. dev” (z-average half std. dev = average z-score + 0.5*(z-score std. dev.)) were considered for FCT group designation. Even when using this criterion, some neurons still exhibited a combination of three significant peaks and were designated as another FCT-group, “3 Peaks.” All neurons were recorded during at least two sessions (days) each under both control and THC conditions (drug conditions), with some being recorded during more than 20 total sessions.
Figure 3.2. Delayed nonmatch to sample (DNMS) task and hippocampal functional cell types. A. Diagram of different phases of DNMS task: 1) Sample lever presentation (SP) in one of two positions (left or right) and Sample lever response (SR) followed by 2) Delay interval of random durations during which delay timeout was contingent on a nosepoke (last nosepoke, LNP) into photocell mounted on opposite wall, followed by 3) simultaneous presentation of both levers (left and right) in Nonmatch phase in which 4) a Nonmatch Response (NR) on the lever opposite the spatial position to the prior SR produced, delivery of 0.2 ml of water to the trough between levers for the correct (Nonmatch) choice or 5) a response on the same lever as the SR shut off houselights for 5s indicating an incorrect (Match) choice and no reward. Timeline shows sequence of task phases: ITI – intertrial interval; SP – sample lever presentation; SR – sample response; Delay – delay interval; NPs – nosepokes during Delay; LNP – last nosepoke; NR – nonmatch position response; Reinf. – delivery of water (0.2 ml) reward. B. Examples of functional cell types of hippocampal neurons recorded on same bilateral arrays determined by correlated firing (± 1.5s) to Sample (SR) and Nonmatch (NR) task events (0.0s). Raster and peri-event histograms for 4 different types of FCTs, Left and Right Trial Types, Right Sample Conjunctive and Nonmatch Phase, are shown for each lever position (right or left) and each phase (sample or nonmatch) of the task (Hampson et al., 1999). C. Hippocampal recording array consisted of eight pairs of stainless steel 20 μm wires positioned longitudinally within each hippocampus at 200 μm intervals. For each pair one electrode was positioned in CA3 and the other in CA1 cell field in a line tangent to the longitudinal axis of the hippocampus. Arrays were implanted bilaterally in each hippocampus providing a total of 32 electrodes per animal.
3.3.4.2. Statistical Analysis of FCTs based on Multifractal Log-Cumulants

Neurons were divided into FCTs groups (as described in section 3.3.4.1) and repeated measures ANOVAs were performed with Statistical Analysis Systems (SAS) software (SAS Institute) using each neuron as the subject identifier, each session as a within-subjects effect, and FCT group as the group identifier. Since each session was used as the within-subjects effect, log-cumulants (details in section 3.3.4.4) from all sessions from all neurons recorded in at least two sessions of both control and THC (four sessions total) were used (section 3.3.4.1). The covariance structure used was compound symmetry. In total, two repeated measures ANOVAs were performed for both $c_1$ and $c_2$ as dependent variables while examining main effects of FCT group and drug condition (control or THC) and an interaction between the two. One set of repeated measures ANOVAs was performed with three “FCT-groups:” classic FCTs, new FCTs and non-FCTs (section 3.4.3-3.4.4). Another set of repeated measures ANOVAs was performed after breaking down FCTs into specific FCT-groups (section 3.3.4.1 and 3.3.5; position, phase, conjunctive, trial-type, nosepoke-nonmatch, and 3 peaks) and comparing a total of 7 “FCT-groups.” Posthoc tests were performed using Tukey-Kramer adjustments for multiple comparisons.

The set of same repeated measures ANOVA procedures were used to perform control analyses with mean firing rate (section 3.4.4-3.4.5) and ISI standard deviation (section 3.7) as dependent variables. Two repeated measures ANOVAs were performed for each dependent variable; one comparing classic FCTs, new FCTs and non-FCTs and the other analyzing all FCT-groups (7 total groups including non-FCTs).

When examining an effect of hippocampal location (section 3.4.6), no FCT-group designations were made and hippocampal location (CA3 or CA1) was used as the group identifier. Main effects of hippocampal location and hemisphere (left or right) were tested, as well as an interaction between them.
3.3.4.3. Calculation of Scaling Exponents and Multifractal Singularity Spectrum

WLMA is described in detail in other sources (Ciuciu et al., 2008, 2012; Jaffard, 2004; Jaffard et al., 2007; Serrano and Figliola, 2009; Wendt et al., 2007), and information concerning multifractal analysis is detailed elsewhere (Kantelhardt, 2012). Therefore, we only briefly highlight the main features of WLMA for calculating the singularity spectrum.

Multifractal analysis may either be done from a global perspective by measuring how the variability of the signal changes across multiple scales or from a local perspective by measuring the variability of the signal at each point in time. The local variability in the signal $X(t)$ may be defined by the local Hölder exponent $h$, which is the largest $h$: $h(t_0) = \sup\{h : X \in C^h(t_0)\}$ that satisfies the equation

$$|X(t) - P_{t_0}(t)| \leq C|t - t_0|^h$$  \hspace{1cm} (1)

where $C$ is a positive constant and $P$ is an $n$th degree polynomial with $n$ less than $h$. This definition comes from the theoretical usage of the Hölder exponent and describes the pointwise regularity of the signal $X(t)$ (Struzik, 2000). The information concerning the variations in regularity of the Hölder exponents along time can be quantified with the multifractal singularity spectrum, which plots Hausdorff dimension, $D(h)$, of the set of points with the same Hölder exponent. The singularity spectrum may be calculated directly from the log-transformation of the normalized probability distribution function of the local Hölder exponents. However, for real finite data sets with noise calculating the singularity spectrum directly from the local Hölder exponents requires fitting a Taylor
polynomial $P_{t_0}(t)$ to every $t_0$; this procedure becomes computationally expensive and prone to high variability (Ihlen, 2012; Struzik, 2000; Wendt et al., 2007).

3.3.4.4. Estimation of Singularity Spectrum with Wavelet Leaders and Log-Cumulants

As an alternative, the singularity spectrum may also be calculated from a global perspective by estimating the scaling exponents $\zeta(q)$ of a process with self-similar scale invariant structure such that

\[ E|X(at)|^q = |a|^{\zeta(q)} E|X(t)|^q \]  

(2)

where $a$ is the scaling parameter and $q$ is the statistical moment. For monofractal processes, $\zeta(q)$ is constant. For multifractal processes, $\zeta(q)$ is a range of power-law exponents. The distribution of local Hölder exponents and scaling exponents $\zeta(q)$ are related through a Legendre transform (Wendt and Abry, 2007). WLMA computes the scaling exponents by measuring how the absolute value of the wavelet coefficients $d_X$ changes as a function of scale. The first step in WLMA is to transform the data to the wavelet domain with a Discrete Wavelet Transform (DWT) (Fig. 3.3A).

\[ d_X(j,k) = \int_R X(t) 2^{-j/2} \Psi_0 \left( 2^{-j} (t - 2^j k) \right) dt \]  

(3)

where $\Psi_0$ is an appropriately chosen mother wavelet. For this work, a Daubechies wavelet with 4 vanishing moments was used. The wavelet coefficients $d_X$ are extracted from the DWT on a dyadic grid at scales equal to $2^j$ and time shifts equal to $2^j k$. The wavelet leaders $d_L$ are calculated from the wavelet coefficients for every point on this
dyadic grid by finding the maximum wavelet coefficient among the adjacent wavelet coefficients for the current scale and all smaller scales (Fig. 3.3B & 3.3C).

\[ d_L(j, k) = \max \{ dx(j', k') \} \]  
\[ \text{such that} \]  
\[ (k - 1)2^j \leq 2^j j' < (k + 2)2^j \]  

If \( n_j \) is the number of wavelet leaders \( d_L(j, k) \) available at every scale \( 2^j \) for the time series \( X(t) \), then the structure function \( S(j, q) \) can be defined as:

\[ S(j, q) = \frac{1}{n_j} \sum_{k=1}^{n_j} d_L(j, k)^q \]  

where \( k = 1, 2, \ldots n_j \). This function behaves as a power-law over analysis scale \( 2^j \) for a set range of scales and for a set range of statistical orders \( q \). The scaling exponent \( \zeta(q) \) may be calculated from the structure function \( S(j, q) \) to quantify changes in variability as a function of scale \( j \) and statistical orders \( q \):

\[ S(j, q) = F_q(2^j)^\zeta(q) \]  

where \( F_q \) is a constant independent of \( j \). Then, singularity spectrum (Fig. 3.3E, gray line) can be estimated directly from the scaling exponent using a Legendre transform (Wendt and Abry, 2007).
The scaling exponents may also be rewritten as second characteristic functions, a standard function expansion of the natural log of the time averaged wavelet leaders $C_p^j$ (Fig. 3.3D). The power law expansion of the first three terms of $C_p^j$ are

$$C_1^j = \mathbb{E}[\ln(dL)] = c_1^0 + c_1 \ln 2^j$$

$$C_2^j = \mathbb{E}[\ln(dL)^2] - (C_1^j)^2 = c_2^0 + c_2 \ln 2^j$$

$$C_3^j = \mathbb{E}[\ln(dL)^3] - 3 C_2^j C_1^j - (C_1^j)^3 = c_3^0 + c_3 \ln 2^j$$

The log-cumulants $c_1$, $c_2$, and $c_3$, which are calculated from the slope of $C_p^j$ versus scale (Fig. 3.3D), correspond to specific attributes of the multifractal singularity spectrum (Wendt et al., 2007; Wendt and Abry, 2007). The singularity spectrum of a one-dimensional time series may be approximated as a polynomial expansion around its maximum (Ciuciu et al., 2012; Wendt et al., 2007) (Fig. 3.3E, red line).

$$D(h) = 1 + \left(\frac{c_1}{2^1}\right) h - \frac{c_1^2}{c_2} + \left(-\frac{c_2}{3^1}\right) h - \frac{c_1^3}{c_2} + \ldots$$

$c_1$ is a self-similarity parameter, which measures long-range temporal dependencies. It takes values very closely related to the global Hurst exponent and therefore shares properties as discussed in section 3.2.2 (Wendt et al., 2007). $c_2$ is a function of the width of the multifractal spectrum and acts as a test of mono- vs multifractal. Monofractal signals are self-affine with a narrow range of Hölder exponents uniformly distributed throughout the signal. Multifractal signals deviate from pure self-affinity by expressing a wide range of nonuniformly dispersed variability throughout distinct regions. $c_2$ is always negative due to the inverted parabolic shape of the singularity spectrum. As $c_2$ becomes
more negative, it is indicates that the signal is more multifractal. $c_3$ is related to asymmetry in the distribution of scaling exponents on either positive or negative end of the singularity spectrum, and can be used to describe more complex multifractal models, such as compound Poisson cascades (Wendt and Abry, 2007). For this paper, we mainly focus on $c_1$ and $c_2$ because they describe the main features of the singularity spectrum, location and width, and are the most robust to calculate.

The Wavelet Leaders code was obtained from Wendt's freely available, online MATLAB toolbox, the WLMA Toolbox (Wendt et al., 2007; http://www.irit.fr/~Herwig.Wendt/software.html). For all of our analyses, $j$ ranged from $2^3$ to $2^8$ in integer increments, and statistical orders $q$ ranged from 5 to -5 in 0.5 increments (excluding zero). Analyses were performed using this code with MATLAB version R2013a.
Figure 3.3. Illustration of Wavelet Leaders-based Multifractal Analysis.  A. (Bottom) The interspike interval time series of one neuron recorded during an DNMS session (section 3.3.4.5).  (Top) The continuous wavelet transform of this interspike interval sequence.  A continuous wavelet transform is shown here for illustrational purposes only; a discrete wavelet transform was used for all data analysis presented in this paper.  B. The wavelet coefficients are selected on dyadic grid.  The measurement scale (bin size) increases moving up the vertical axis, and time is increasing along the horizontal axis.  At each point in the dyadic grid, the wavelet leader (circled) is the maximum wavelet coefficient among the wavelet coefficients to the immediate right and left and for all lower scales (the two gray boxes).  C. The wavelet leaders \( d_L \) are calculated as a function of measurement scale \( j \).  Since wavelet leaders are averaged according to the scale (bin size), this results in the fewer wavelet leader data points at each scale.  In order for the scale invariant structure of the wavelet leaders to be seen, the average wavelet leader is plotted according to its bin size.  As measurement precision increases from top to bottom, the measurement scale (bin size) decreases.  This is why the length of the ISI index does not change and the wavelet leaders appear to have higher resolution (Methods section 3.3.4.5).  D. The log-cumulants are derived from the slopes second characteristic functions derived from the natural log of the time averaged wavelet leaders.  \( c_1 \) is obtained from the slope of the red line, \( c_2 \) from the blue line, and \( c_3 \) from the green line.  E. The singularity spectrum can be estimated from the log-cumulants (red) or the scaling function (gray).  Both methods yield very similar estimates.
3.3.4.5. Wavelet Leaders Analysis of Hippocampal Interspike Intervals

The Wavelet Leaders analysis was performed on a time series of interspike intervals (ISI) from every neuron recorded in either CA3 or CA1 during the DNMS task. In figures with interspike intervals over time (i.e., Fig. 3.1, 3.3A bottom, and 3.5A), the “ISI Index” on the horizontal axes represents the sequential order of ISIs to illustrate the variability in ISI amplitude as spiking occurs over time. In these graphs, the vertical axes was normalized based on the largest ISI, and the width of ISI data line segments along the horizontal axes were normalized to fit all ISIs obtained during a single recording into one graphic. All recordings included in the analysis were at least one hour in length and contained a minimum of 2048 ISI data points. Log-cumulants were calculated for each neuron recorded during each session. The log-cumulants for the same neurons were averaged within drug conditions to obtain separate values for control and THC sessions used to construct scatter plots (Fig. 3.8) in section 3.4.5. For the overall FCT analysis described in section 3.3.4.2 and in Figure 3.7, cumulant values obtained for all neurons during all recording session were compared using repeated measures ANOVA.

3.4. Results

3.4.1. DNMS Task and Behavioral Performance with Cannabinoid Manipulations

The delayed nonmatch-to-sample task (DNMS) tests short term memory by requiring rats to retain spatial information acquired during the “Sample phase” throughout a subsequent variable delay period of 1-30s ending with the “Last nosepoke,” after which they make a decision based on their memory of the sample phase in the “Nonmatch phase” (Deadwyler et al., 1996). Analysis from hippocampal pyramidal cell recordings (CA3 and CA1) revealed that FCTs encode task-specific information regarding combinations of lever position and task phase (Fig. 3.2B; Hampson et al., 1999). Results using this task have shown that behavior and hippocampal neural
processing are impaired by relatively low doses of the cannabinoid receptor type 1 (CB1) partial agonist, delta-9-Tetrahydrocannabinol (THC; Fig. 3.4). Cannabinoids reduced ensemble information content, increased the number of neurons required to encode the same information as in control sessions, and impaired memory performance by reducing the strength of sample encoding (Hampson and Deadwyler, 2000). The sample encoding strength is a function of the firing rate of specific FCTs during the sample response (Fig. 3.2A, 3.2B), which has been shown to be critically important for DNMS performance (Hampson et al., 1999, 2012). THC reduced the behavioral complexity by producing stereotyped responding, revealed in the increased behavioral bias, or “preference”, for the same lever in the nonmatch phase (Fig. 3.4). The important distinction between control and THC can be noted by examining the difference between preferred lever and non-preferred lever selection within the respective drug condition. When challenged with delays >20 s, subjects in control sessions showed preferred responding, while preferred responding during THC sessions occurred at all delay intervals. Behavior becomes more stereotyped (less complex) during the task after THC administration compared to behavior produced by an uncompromised working memory system in the control condition.
Figure 3.4. Average DNMS task performance during control and THC sessions. Mean (±SEM) % correct nonmatch responses (NRs) summed across animals (n = 6) shows significant decline in accuracy ($F_{(5,595)} = 61.79, p < 0.001$) as a function of delay duration in 5.0s blocks. A main effect of drug condition ($F_{(1,119)} = 32.09, p < 0.001$) and interaction between drug condition and delay interval ($F_{(5,595)} = 2.36, p < 0.05$) were detected. Solid lines show THC significantly impaired performance at all six delay intervals ($p < 0.05$). The dashed lines illustrate correct performance according to lever preferences which demonstrate that stereotyped responding underlies reduction in performance during THC administration. Under THC, there was a larger difference between preferred and non-preferred choices at all delay intervals. Preferred responding begins in the control condition only when delays are greater than 20 seconds. A within-subjects design with at least 2 non-drug days between THC administration was used. All animals were given THC (1.0 mg/kg) for no less than five sessions spaced over multiple weeks.

3.4.2. Multifractal Analysis of Hippocampal Interspike Intervals

In time series, multifractal analysis describes the distribution and structure of variability across multiple temporal scales (Makowiec, 2010). Multifractal dynamics are exhibited by complex systems, like the brain (Di leva et al., 2013a, 2013b) from macroscopic brain oscillations (Ciuciu et al., 2012; Zorick and Mandelkern, 2013) to an intermediate scale of neuronal spiking (Biella et al., 1999) and to the microscopic scale ion channel fluctuations (Brazhe and Maksimov, 2006). To investigate complex interactions on the intermediate scale, a time series was constructed from the action potential timestamps recorded from individual hippocampal principal cells (CA3 and CA1). The differences between each successive spike were taken to create an
interspike interval (ISI) time series to analyze using Wavelet Leaders-based multifractal analysis (Jaffard, 2004; Jaffard et al., 2007; Wendt et al., 2007). By examining the ISI of recorded neurons, it is not readily apparent which neurons are FCTs or not, nor is it possible to determine which neurons were subjected to THC (Fig. 3.5A & 3.5B). The differences between FCTs and non-FCTs are not detected by measuring the average firing rate or standard deviation over an entire behavioral session (population analyses in sections 3.4.3-3.4.4 and 3.4.7, respectively). Only after calculating the range of scaling exponents and the multifractal singularity spectrums do differences between these neurons become visually apparent (Fig. 3.5C). Multifractal analysis quantifies the heterogenously distributed variability related to active working memory correlates across many spatiotemporal scales (Ihlen and Vereijken, 2010).
Figure 3.5. Multifractal differences of interspike intervals quantified by singularity spectra. The first four graphs each show the sequence of interspike intervals recorded during one daily DNMS behavioral session. The ISI amplitude is normalized to one for each session based on the largest ISI and the ISI index represents the temporal ISI pattern (Methods section 3.3.4.5). Above each graph, the bracketed numbers signify (in order): the measurement scale, the average firing rate in hertz, and the standard deviation of ISIs. On the right, the singularity spectra are plotted with Hausdorff dimension $D(h)$ on the vertical axis and Hölder exponent $h$ on the horizontal axis. A. The interspike intervals (ISI) of one FCT (top) and one non-FCT (bottom) recorded over one control session. B. The ISI of one FCT (top) and one non-FCT (bottom) recorded over one THC session; the same neurons as in A are shown in B when recorded under THC administration. C. In the FCT control condition (top, blue), the central location of the spectrum exists at a larger Hölder exponent (signified by larger $c_1$) and the spectrum is wider (signified by larger $c_2$) than all other illustrated neurons. THC reduces both $c_1$ and $c_2$, designated by the leftward shift and decreased width, respectively. The fractal characteristics of the non-FCT are unchanged by THC.
The Wavelet Leaders method produces log cumulant values, $c_1$, $c_2$, and $c_3$, which correspond to specific attributes of the multifractal singularity spectrum (see Methods section 3.3.4.4; Wendt et al., 2007). Multifractal singularity spectrums were constructed by analyzing ISI sequences of each hippocampal neuron recorded during each DNMS behavioral session. Recordings over all sessions for two FCTs, left and right trial type neurons each recorded from a different rat, are presented in the left plots of Figure 3.6. Trial type cells are an FCT that corresponds to specific nonmatch-rule combinations; left trial type neurons discharge during left sample and right nonmatch responses, while the right trial type neurons fire for the opposite combination (Fig. 3.2B). Each singularity spectrum in the left graphs was computed from one DNMS session from the Hölder exponents $h$ and corresponding dimensionality $D(h)$ values. The average log-cumulant values for each neuron during each condition were used to create average singularity spectra plotted on the right using the equations 6-9 in section 3.3.4.4. Within neuron drug-induced changes in $c_1$ and $c_2$ are seen by differing maximum $h$ values and spectrum widths, respectively. Both neurons had a statistically significant decrease in $c_1$, but insignificant changes in $c_2$. 
Figure 3.6. Multifractal singularity spectra of Trial-Type Cells during THC administration. Right trial-type cells discharge for the right sample response and the left nonmatch response, while left trial-types respond during the left sample and right nonmatch. Together, these cell types provide the most essential information for DNMS performance. In the left graphics, each trace represents the results for that specific neuron obtained during one session. Control sessions are in blue and THC sessions are in green. We used a repeated measures design to collect data from the same neuron on a daily basis while interspersing control and THC days. Each of the two cells was recorded over multiple days from two different rats. The right figures represent the averaged spectrum by condition. The log-cumulant values in the table are averaged for each cumulant over all sessions the neuron was recorded. THC significantly reduces $c_1$ in these functional cell types ($p < 0.05$). However, THC non-significantly reduces $c_2$ ($p = 0.12$ for the left trial type and $p = 0.14$ for the right trial type).
3.4.3. Multifractal Analysis Facilitated Identification of New FCTs

Previous work identified four different FCT groups: position, phase, conjunctive and trial-type cells. The results of multifractal analysis revealed that some neurons unidentified solely based on combinations of sample and nonmatch lever responses exhibited multifractal dynamics. Further FCT analysis labeled the last nosepoke, which coincides with termination of the delay and extension of levers for the choice phase (methods section 3.3.4.1), as another event considered for FCT classification and increased the number of analyzed behavioral events from four to six. This allowed for a new type of phase cell, “nosepoke phase,” two conjunctive cell types, “left nosepoke” and “right nosepoke,” and two more trial-type cells, “left-trial sample-nosepoke” and “right-trial sample-nosepoke” to be identified (Table 3.1).

The addition of nosepoke events also allowed creation of two new FCT-groups: “Nosepoke-Nonmatch” and “3 Peaks.” These were not previously defined as a distinct FCT-group based on the subjectivity used in defining FCTs (Hampson et al., 1999), but multifractal analysis provided additional insight into the information processing of hippocampal neurons that allowed designation of new FCTs exhibiting significant peaks during the last nosepoke. Twenty-three neurons previously identified as nonmatch conjunctive cells were found to exhibit paired firing rate peaks during the last nosepoke and nonmatch response events; these are now classified as “Nosepoke-Nonmatch” neurons. These cells may be the same cells identified previously as a subset of nonmatch-conjunctive cells that fired in the nonmatch phase of the task as well as in the late (>15 s) portion of the delay (Deadwyler and Hampson, 2004). In addition, five neurons responded significantly to a combination of three out of six behavioral events, and these are now signified as “3 Peaks.” In total, including z-score analysis of the nosepoke event allowed us to classify 48 additional neurons as “new FCTs” that were
distinguished by containing at least one significant peak around the last nosepoke event (Table 3.1).

<table>
<thead>
<tr>
<th>New FCT Classification</th>
<th># Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nosepoke phase</td>
<td>10</td>
</tr>
<tr>
<td>Left nosepoke conjunctive</td>
<td>4</td>
</tr>
<tr>
<td>Right nosepoke conjunctive</td>
<td>2</td>
</tr>
<tr>
<td>Left sample-nosepoke trial-type</td>
<td>4</td>
</tr>
<tr>
<td>Right sample-nosepoke trial-type</td>
<td>0</td>
</tr>
<tr>
<td>Nosepoke-Nonmatch</td>
<td>23</td>
</tr>
<tr>
<td>3 Peaks</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 3.1. New FCT Classification. By including the last-nosepoke event, a total of 48 new neurons could be identified as FCTs. In section 3.4.4, these neurons were classified as “new FCTs.” In section 3.4.5, they were designated into their respective FCT-group.

3.4.4. Multifractal Correlates of FCT Designation Scheme

Long-range temporal dependences and multifractal properties were examined at a population level by grouping hippocampal principal cells based on FCT firing characteristics as described in section 3.3.4.1. 20 non-FCT neurons, 68 classic FCT neurons and 48 neurons exhibiting novel FCT classification were recorded in a total of six animals. Chronic hippocampal single-unit recordings allowed us to measure neuronal activity over many sessions to repeatedly measure activity over multiple control and THC sessions. The neurons within each FCT classification were subdivided by control and THC sessions and average $c_1$ and $c_2$ values were calculated (Fig. 3.7C & 3.7E). For $c_1$, a repeated measures ANOVA revealed a main effect of drug condition ($F_{(1,133)} = 29.58, p < 0.0001$), an interaction between drug condition and FCT group ($F_{(1,133)} = 3.41, p = 0.036$), but no significant main effect of FCT group ($F_{(1,133)} = 2.24, p = 0.11$). It should be noted that all averaged $c_1$ values are greater than 0.5, which implies long-range temporal correlations and persistent structure. For $c_2$, a repeated measures ANOVA showed main effects of drug condition ($F_{(1,133)} = 33.73, p < 0.0001$), FCT group ($F_{(1,133)} = 3.91, p = 0.022$) and an interaction between the two ($F_{(1,133)} = 11.72, p <$
0.0001). Large, negative values of $c_2$ in FCTs indicate inhomogenous diffusion of interspike interval variability. $c_2$ is always negative, but for illustrational purposes, we plotted the absolute value $|c_2|$ (Fig. 3.7E). 5 posthoc t-tests were performed for $c_1$ and $c_2$ using Tukey-Kramer correction for multiple comparisons; drug effects were examined within each FCT group, and both classic and new FCTs were compared to non-FCTS (control condition only). THC reduced $c_1$ and $|c_2|$ in both classic FCTs and new FCTs (Fig. 3.7C & 3.7E). In addition, both classic FCTs and new FCTs recorded in the control group had significantly larger $|c_2|$ compared to non-FCTs in the control condition.

As a control procedure, the mean firing rate over the entire session was computed and a repeated measures ANOVA was performed (Fig. 3.7A). No significant effect of FCT group ($F_{(1,133)} = 0.03, p = 0.97$), drug condition ($F_{(1,133)} = 0.05, p = 0.83$), or interaction between the two ($F_{(1,133)} = 0.97, p = 0.38$) was detected. FCTs could not be distinguished from non-FCTs using mean firing rate as the dependent variable (Fig 3.7A).
Figure 3.7. Effects of THC by FCT-group designation. A-B. THC did not significantly affect mean firing rate recorded throughout the entire behavior session using either FCT grouping scheme. C. THC significantly reduced long-range temporal correlations, as measured with $c_1$, in both classic and new FCTs. D. THC significantly reduced $c_1$ in Conjunctive, Trial-Type, Nosepoke-Nonmatch and 3 Peak FCT-groups. Recorded in the control condition, Trial-Type and 3 Peaks FCT-groups had significantly larger $c_1$ values than control non-FCTs. E. Both classic and new FCTs in the control condition have significantly larger $c_2$ values compared to non-FCTs in the control condition. THC significantly reduced multifractal complexity in both classic and new FCTs. F. All FCT groups recorded in the control condition, except position cells, exhibited greater multifractal complexity than non-FCTs from the control condition. THC reduced multifractal properties of phase, conjunctive, NP-NM and 3 Peaks FCT-groups. In all graphs, * $p < 0.05$, ** $p < 0.01$, # $p < 0.0001$. 

*Figures and data are not provided in this text. They should be referred to in the original article for accurate representation.*
3.4.5. Multifractal Correlates of FCT Groups

Based on the positive results obtained from including nosepoke events, all FCTs were further subdivided into designated groups based on increased firing rate around a combination of task-related events using a standard z-score (Methods section 3.3.4.1; Hampson et al., 1999). The neurons exhibiting significant nosepoke peaks were sorted into their respective classifications (sections 3.3.4.1 and 3.4.3) from the classic FCT grouping system (i.e., the nosepoke phase cells were sorted as phase cells, etc.) or labeled as Nosepoke-Nonmatch (NP-NM) or 3 Peaks. This grouping yielded 10 position, 34 phase, 33 conjunctive, 11 trial-type, 23 nosepoke-nonmatch, and five “3 peak” cells that were used for all subsequent analyses in this section.

The first log-cumulant, \( c_1 \), measures long-range temporal correlations in the data and is very similar to the Hurst exponent (Wendt et al., 2007; Ciuciu et al., 2012). Repeated Measures ANOVA for \( c_1 \) revealed a significant main effect of drug condition \((F_{(6,129)} = 36.46, p < 0.0001)\), and an interaction between FCT group and drug condition \((F_{(6,129)} = 2.25, p = 0.0427)\). However, there was not a significant main effect of FCT group \((F_{(6,129)} = 2.1, p = 0.0576)\). Two sets of posthoc t-tests were conducted using Tukey-Kramer adjustment for multiple comparisons. Each FCT-group was compared to itself in control and THC conditions, and all control FCTs were compared to control non-FCTs (Fig. 3.7D). Trial-type and 3 Peaks in the control condition contained significantly greater \( c_1 \) values than control non-FCTs. All FCTs, except position and phase cells, showed a significant decrease in \( c_1 \) after THC administration (Fig. 3.7D).

The second log-cumulant, \( c_2 \), quantifies the multiplicative interactions across spatiotemporal scales as indicated by multifractal dynamics. By convention, \( c_2 \) is negative, but the absolute value \(|c_2|\) was used for graphing purposes (Zilber et al., 2013; Fig. 3.7E and 3.7F). Repeated Measures ANOVA for \( c_2 \) revealed a significant main effect of drug condition \((F_{(6,129)} = 34.68, p < 0.0001)\), a significant interaction between
FCT group and drug condition ($F_{(6,129)} = 4.37, p = 0.0005$), but no main effect of FCT group ($F_{(6,129)} = 1.73, p = 0.1181$). The same series of posthoc t-tests as performed for $c_1$ were done for $c_2$. All control FCT-groups, except position cells, were significantly more multifractal than control non-FCTs (Fig. 3.7F). All FCT-groups, except position and trial-type cells contained significantly more multifractal complexity in the control condition compared to the THC condition (Fig. 3.7F).

As a control, the same analysis was also performed using mean firing rate over the entire DNMS session (Fig. 3.7B). There was no main effects of drug condition ($F_{(6,129)} = 1.79, p = 0.18$) or FCT group ($F_{(6,129)} = 0.62, p = 0.72$). No interaction was detected ($F_{(6,129)} = 0.48, p = 0.82$). FCTs could not be distinguished from non-FCTs by using mean firing rate as the dependent variable.

The effects of THC on the dynamics of the entire recorded neuronal population is illustrated in figure 3.9 by comparing mean firing rate (Fig. 3.8A), $c_1$ (Fig. 3.8B), and $c_2$ (Fig. 3.8C & 3.8D) across drug conditions. In all of these plots, each neuron is represented by one dot labeled by its corresponding FCT-group. It is visually apparent that THC reduces $c_1$, as seen by a larger cluster on the right side of the midline. THC reduces multifractality as indicated by $c_2$ closer to zero and the clustering towards the left of the midline (Fig 3.8C & 3.8D). To appreciate the density of data points, neurons with $c_2$ less than -0.1 (designated by a black square in Fig. 3.8C) are also shown in a bigger plot (Fig. 3.8D). THC had no significant effect on mean firing rate since all data points reside near the midline (Fig. 3.8A).
Figure 3.8. Scatter plot comparision of control versus THC condition. These scatter plots show the entire recorded neuronal population coded by color-symbol patterns based on FCT-group designation. The midline is plotted on all graphs to visually show deviations from equal dependent variables between control and THC conditions. A. The mean firing rate of the neuronal population did not change due to THC administration, and data points cluster around the midline. B. THC reduced $c_1$ in many different neurons across FCT-groups, as noted by the rightward-shifted cluster below the midline. C. THC reduced multifractal complexity as measured by $c_2$ values closer to zero. This is visually seen by the clustering of neurons to the upper left of the midline. D. The “dense” section of C, designated by the black square, is enlarged with axes limits to -0.1 instead of -0.6.
3.4.6. Similar Multifractal Dynamics within Hippocampus

Repeated Measures ANOVA results looking for an effect of hippocampal location (CA3 or CA1) or hemispheric location (right or left) revealed no significant differences. When comparing $c_1$ values, there was no effect of hippocampal location ($F_{(1,135)} = 0.49$, $p = 0.48$) or lateralization ($F_{(1,135)} = 0.68$, $p = 0.41$). When comparing $c_2$ values, there was no significant effect lateralization ($F_{(1,135)} = 0$, $p = 0.97$), but a trend towards an effect of hippocampal location ($F_{(1,135)} = 3.67$, $p = 0.06$) with CA1 exhibiting slightly greater multifractal properties than CA3 (data not shown). This demonstrates that classifying neurons based on their multifractal properties is more useful than classification based on anatomical location.

3.4.7. THC Increases Standard Deviation of Hippocampal ISIs

Another series of control analyses examined if the standard deviation of ISI sequences could distinguish between FCT and non-FCTs in control or THC conditions. When using three FCT groups from section 3.4.4 (non-FCTs, classic FCTs, new FCTs), a repeated measures ANOVA revealed a significant main effect of drug condition ($F_{(1,133)} = 8.53$, $p = 0.0041$), but no main effect of FCT group ($F_{(1,133)} = 0.02$, $p = 0.98$) or interaction between the two ($F_{(1,133)} = 0.31$, $p = 0.28$). When using all 7 FCT groups (section 3.4.5), a significant main effect of drug condition ($F_{(6,129)} = 12.77$, $p = 0.0005$) was found, but FCT groups ($F_{(6,129)} = 0.78$, $p = 0.58$) could not be distinguished. An interaction could not be detected ($F_{(6,129)} = 0.77$, $p = 0.59$). In both cases, THC increased ISI standard deviation as measured throughout the entire behavioral session (data not shown). It was not possible to distinguish between FCT groups based on ISI standard deviation alone.
3.5. Discussion

3.5.1. Multifractal Features of Hippocampal FCT Neurons

In the present work, we showed that WLMA and corresponding log-cumulants (Wendt et al., 2007) differentiated groups of neurons based on their memory-related responses (FCT characteristics), confirming our first two hypotheses. The ISI trains of hippocampal principal neurons recorded during a working memory task exhibit long-range dependencies and multifractal complexity. The finding that FCTs exhibit very multifractal discharge properties is an interesting property previously unattributed to memory-correlated neurons. It is known that FCTs are essential for sensory processing relevant for task performance (Hampson et al., 1999). Sensory processing occurring over sequential trials and across days may generate correlations in the neuronal response patterns (i.e., bursting sequences) detected by $c_1$. FCTs could present increased multifractal dynamics due to the relatively large degree of effective connections necessary to promote successful memory performance. FCTs are known to represent memory processing during the DNMS task, and multifractal analysis provides an empirical method for quantifying their dynamical complexity that could be applied to many other forms of data. Importantly, the differences between FCT neurons and non-FCT neurons were not readily detected by mean firing rate and standard deviation calculations over the entire signal, and this emphasizes the importance of analyzing the structure of ISI variability with multifractal analysis.

Multifractal analysis revealed that some neurons unidentified using previous techniques exhibited a large degree of multifractal complexity, and this finding elicited investigation into their relationship to the DNMS task. Firing rate responses around the last nosepoke event were analyzed which allowed for creation of two new FCT-groups: Nosepoke-Nonmatch and 3 Peaks. Previous work could have identified these neurons as FCTs if nosepoke events were included in the analysis (Hampson et al., 1999), but
the subjectivity involved in this classification allowed these cell types to be undetected for 15 years. Grouping neurons with significant nosepoke peaks as new FCTs revealed that they are similar to classic FCTs, as both are more multifractal than non-FCTs and both show significant decreases of log-cumulants after THC administration (Fig. 3.7E). However, even with the inclusion of the last nosepoke event, FCT designation could not be assigned to all neurons with multifractal dynamics. For example, one non-FCT neuron with a $c_2$ near -0.3 could have a functional role in the task that was not detected by the standard FCT analysis using z-score (Fig. 3.8C), such as delay activity or rhythmic modulation, and future work is planned to thoroughly investigate these possibilities.

Z-score analysis allowed for qualitative descriptions of FCTs into specific groups, while multifractal analysis allowed us to quantify the information processing capacity of these FCT-groups. We showed that trial-type and 3 peak FCTs groups contained more long-range temporal correlations and exhibited more multifractal complexity than non-FCTs. Phase, conjunctive, and nosepoke-nonmatch cells did not have greater $c_1$ values than non-FCTs but were more multifractal than non-FCTs (Fig. 3.7F). Position cells were not distinguished from FCTs by either log-cumulant. The lack of significance may represent an important link to the proposed “functional hierarchy” (Hampson et al., 1999), where position cells are labeled as least complex. It is hypothesized that connections from position and phase cells converge onto conjunctive and trial-type cells as information becomes more specific (Hampson et al., 1999, 2012). Results from multifractal analysis lend support to this interpretation and suggest that multifractal properties of FCTs may arise from these hypothesized interactions. However, future work combining cross-correlation and multifractal analyses is needed to substantiate this claim.
Five FCT-groups, excluding position cells, were significantly more multifractal than non-FCTs in the control condition (Fig 3.7F), but the scatter plot reveals some very interesting information when comparing the magnitude of multifractal complexity to FCT-group designation. Surprisingly, “very multifractal” neurons (i.e., $c_2 < -0.1$) are drawn from all FCT-groups. Previous results from z-score analysis and FCT-group designation (Hampson et al., 1999) suggested that trial-type cells are hierarchically more complex than position and phase cells. Additionally, it has been demonstrated that the MIMO model preferentially extracts trial-type cells to optimally boost performance with electrical stimulation (Hampson et al., 2012). However, our present results show that neurons from all FCT-groups exhibit a large degree of multifractal complexity (Fig. 3.8C). The absence of clear clustering based on FCT groupings suggests that neurons within specific FCT-group may not contribute equally to the DNMS task and supports the hypothesis that designing models based on FCT group alone might miss important information uncovered with multifractal analysis. The resulting spectrum of neurons with differing degree of multifractal complexity suggests that it may be advantageous to give a “functionality score” based on log-cumulants for neuronal modeling and microstimulation experiments (Berger et al., 2011; Hampson et al., 2012, 2013; Opris, 2013). One of the requirements of an effective MIMO model is recording and stimulation of neurons with high information content (Hampson et al., 2012), and multifractal analysis has allowed us to explore task-relevant neural firing on a deeper level by identifying a spectrum of multifractal neurons with different correlates to the behavioral task. In general, our results showed that 3 Peaks are the most multifractal and nosepoke-nonmatch are second (Fig. 3.7F). However, this grouping still contains variability as it is visually apparent that three of the five FCTs with 3 peaks (left side of Fig. 3.8C) are more multifractal than the remaining two that can also be seen in Fig. 3.8D. Since multifractal analysis quantifies the structure of variability occurring
throughout the entire recorded DNMS session, it is hypothesized that the FCTs exhibiting greater multifractal complexity than others in the same FCT group may exhibit additional roles undetected with z-score-based FCT classification.

3.5.2. Multifractality represents interactions across multiple spatiotemporal scales

Hippocampal neurons specifically involved in task-related mnemonic processing (i.e., FCTs) are multifractal and exhibit more multifractal properties than uninvolved neurons, which supports interaction-dominant model (Dixon et al., 2012; Ihlen and Vereijken, 2010) and suggests that these neurons may be involved with more active memory-related interactions than neurons without task-specific activity. The interactions suggested by multifractality may arise from involvement of specific hippocampal neural ensembles with other memory-related brain structures, interactions between frequency components, and/or coordination between macroscopic and microscopic brain processing mechanisms. In future work, it will be very important to examine cross-correlations between recorded neurons and determine if multifractality arises directly from interactions within the recorded hippocampal population.

The multifractal nature of FCTs may arise from a greater amount of reciprocal connections with other anatomical regions. Interaction-dominant theory states that multifractal properties describe intermittency in signals due to exchange of information within and between cognitive structures across multiple temporal scales (Ihlen and Vereijken, 2010). The DNMS task is a working memory paradigm that necessitates interactions among all three classical memory modalities: memory encoding of the sample position, maintenance or short-term storage throughout a delay interval, and a recognition-based form of memory recall during the nonmatch phase. This “layered” form of memory processing may lead to multifractal dynamics. These three memory modalities are known to be distributed among various cortical and limbic structures, including the entorhinal cortex (Deadwyler et al., 1976; Newmark et al., 2013; Stepan et
al., 2012; Talnov et al., 2003), prefrontal cortex (Hyman et al., 2010, 2011), subiculum (Deadwyler and Hampson, 2004, 2006), medial septum (Hasselmo and Stern, 2014; Mitchell et al., 1982; Rawlins et al., 1979) and parahippocampal gyrus (Barredo et al., 2013). It has been shown that the DNMS task involves reciprocal connections from the hippocampus to the subiculum (Deadwyler and Hampson, 2004, 2006) and theta-entrainment of cells in the medial prefrontal cortex during correct responding (Hyman et al., 2010, 2011). Multifractal analysis of simultaneous recordings of neuronal ensembles and local field potentials in these and other memory-related brain structures will enrich our understanding of cognitive processing related to multiscale spatiotemporal interactions.

Multifractal properties could correlate with neuronal bursting events specific to memory processing or bursting induced by endogenously generated brain rhythms, like theta or gamma rhythms. The requirements of the DNMS task synchronize neuronal action potentials (Berger et al., 2011; Hampson et al., 2012) and may elicit neuronal avalanches (Beggs and Plenz, 2004; Palva et al., 2013; Ribeiro et al., 2010) during memory encoding or recall, and this bursting activity could produce the detected power-law distribution of interspike intervals. Specific, recurring spatiotemporal bursts of neuronal activity were shown to possess the diversity and long-term stability required as a memory substrate (Madhavan et al., 2007) activated during both encoding and retrieval (Ji and Wilson, 2007; Nádasdy et al., 1999). Similar spatiotemporal activity patterns may underlie memory transmission and storage mechanisms described as the memory engram (Liu et al., 2014; Ramirez et al., 2013), neuronal avalanches (Beggs and Plenz, 2003, 2004; Ribeiro et al., 2010), and spatial memory encoding during the sample lever press (Berger et al., 2011, 2012; Deadwyler et al., 2013).

It has also been postulated that brain rhythms, specifically theta and gamma rhythms, can coordinate neuronal assemblies by precise temporal modulation of network
excitability (Buzsáki and Draughn, 2004; Buzsáki and Moser, 2013; Chrobak et al., 2000). Theta-entrainment of specific medial prefrontal neuronal populations is known to occur during successful nonmatch responses in the DNMS task (Hyman et al., 2010, 2011). Cross-frequency theta-gamma coupling correlates with memory success in rats (Shirvalkar et al., 2010; Tort et al., 2009) and humans (Axmacher et al., 2010; Canolty et al., 2006). Theta-gamma comodulation produces “up-states” (Mölle and Born, 2011) during which memory-specific neuronal ensembles can become coordinated as nested representations (Jensen, 2006; Mathis et al., 2012) or bursts within bursts (Linkenkaer-Hansen et al., 2001) across many resolution scales. The notions of nested neuronal resolutions and cross-frequency comodulation can be viewed as comparable to self-affinity, intermittency, scale-invariance, and temporally-specific activity patterns characteristic of multifractal dynamics, especially when viewed as evidence for interaction-dominant theory (Ihlen and Vereijken, 2010). All of these phenomena could potentially generate multifractal dynamics in single neurons activated by the memory sequence. Interactions between frequencies have been postulated to represent an exact timing mechanism extractable from local field potentials, while multifractal interactions between temporal scales suggest a precise temporal structure of neuronal interspike intervals. These views from two measurement scales may reflect self-affine timing processes. Multifractal analysis provides a method to understand how macroscopic oscillations interact with neuronal spike trains and could facilitate development of a more coherent timing paradigm through future theory integration.

Fractal processes are described by $1/f^\alpha$ power-law distributions and self-affine patterns appearing over macroscopic to microscopic scales. This relationship can be conceptualized in the brain as the different measurement scales ranging from surface EEG to local field potentials to neuronal spiking and to more microscopic levels of channel currents and biochemical molecular interactions. Modeling of recurrent fractal
neural networks (Bieberich, 2002) and topological structure of brain modules and clusters in fMRI data (Gallos et al., 2012a, 2012b) reveal fractal dynamics at the most macroscopic level. Self-organized criticality (metastability) is suggested by the presence of $1/t^\alpha$ power-law distributions (Bak et al., 1987). Networks are “optimized” in the critical state: interpretation of sensory stimuli (Linkenkaer-Hansen et al., 2004), fluctuations between cognitive states (Linkenkaer-Hansen, 2001), adaptation to novel circumstances (Alstrom and Stassinopoulos, 1995), neuronal integration within avalanches (Begg and Plenz, 2003, 2004) and maintenance of large memory repositories (Ribeiro et al., 2010) occurs in an augmented fashion during metastability (Kinouchi and Copelli, 2006). Modeling work predicts coupled neural oscillators in a metastable (critical) state produce long-range temporal correlations and fractal properties of interspike intervals (Fronczak et al., 2006; Usher et al., 1995). We hypothesize that long-range dependencies and multifractal features provide empirical measures of persistent activity structure and increased network interactivity of FCTs compared to non-FCTs. Future experiments are planned to improve upon the current methodology by combining multifractal analysis with spectral, wavelet and unsupervised machine learning algorithms to understand what other aspects of neuronal activity may contribute to the multifractal complexity of FCTs.

3.5.3. Role of THC in Multifractal Behavior of Neural Firing

Tetrahydrocannabinol (THC), the main psychoactive component of cannabis (Gaoni and Mechoulam, 1964), administered prior to the DNMS task reduced memory performance, hippocampal memory encoding (Deadwyler et al., 2007), information content (Hampson and Deadwyler, 2000), long term dependencies and multifractal properties. THC administration disrupts the complexity of responding by evoking a more simplistic, homogenous behavioral pattern during performance of the DNMS task. The rats adopted a stereotyped performance strategy in an attempt to maximize rewards; they increasingly chose a “preferred” lever during the nonmatch phase during
challenging trials with long delays (Fig. 3.4). The ability of THC to impair behavioral performance seems correlated with its capacity to reduce long-range temporal correlations (Fig. 3.7C) and multifractal properties (Fig. 3.7E) in FCTs, which only partially confirms our third hypothesis since non-FCTs were unaffected by THC (section 3.2.3). These results highlight the usefulness of multifractal analysis in examining the effects of cannabinoids on brain and nervous system function and provide novel insight into THC’s mechanism of action. The use of THC also provides reciprocal insight into what multifractal complexity of hippocampal neurons could reveal about memory processing. According to interaction-dominant theory (Ihlen and Vereijken, 2010; Kelty-Stephen et al., 2013), it could be hypothesized that THC reduces multifractal complexity of FCTs by inhibiting interactions with other task-relevant neurons or brain areas.

Since non-FCT neurons are not processing much, if any, task-related information, the lack of a THC effect on fractal measures is consistent with the presence of fractal modification only in areas receiving information (Linkenkaer-Hansen et al., 2004). The absence of a THC effect on either log-cumulant of position cells initially seems surprising (Fig. 3.7D & 3.7F), but it could be attributed to a smaller number of neurons within this group (10 neurons). The scatter plot (Fig. 3.8C) shows at least 3 position cells residing to the left of the midline because THC reduced their multifractal complexity; two position cells clearly show reduced $c_1$ after THC administration (Fig. 3.8B). THC did not significantly reduce $c_1$ of phase cells, but $c_2$ was significantly reduced by THC in this FCT-group. Trial-type cells showed the opposite effects, where THC reduced $c_1$ but not $c_2$. Before adjusting for multiple comparisons, all 4 of the above differences were detected, and this suggests that with more neurons in these FCT-groups, significance would have been achieved. The scatter plot (Fig. 3.8C) suggests that THC may preferentially reduce multifractality of very multifractal neurons from all FCT-groups. The lack of an effect of THC on some FCT-groups suggests that a weighting system of
scores based on event-related (FCT correlates) activity and fractal properties (log-cumulants) could accurately and objectively capture more relevant interactions than previously used FCT detection methods (Hampson et al., 1999).

In general, THC could be reducing temporally precise activity in the working memory network which was detected as reduced multifractal dynamics of FCTs. The two most commonly reported effects of cannabis use are short term memory deficits and a distorted sense of time (Atakan et al., 2012; Tart et al., 1970). THC disrupts temporally specific information and serial dependence related to sensory processing in the hippocampus (Hampson et al., 1989) and increases the probability of nonmatch information proactively interfering with encoding of the next sample position during the DNMS task (Deadwyler and Hampson, 1997; Hampson and Deadwyler, 1998, 2000). Within the conceptual framework of multiscale temporal processing, THC-induced impairment may predispose retrieval from inappropriate time scales (previous trials), which would be in line with impaired short term memory processing and reduced sequential memory differentiation. THC effectively causes decoupling and reduced cellular synchrony of intrahippocampal activity (Goonawardena et al., 2011). Theta phase-locking correlates with successful DNMS performance (Hyman et al., 2010, 2011), and in similar spatial working memory tasks, cannabinoid agonists impaired theta phase-locking of medial prefrontal cortical neurons to hippocampal LFP (Kucewicz et al., 2011), decreased theta power, and reduced temporal coordination of hippocampal principal cell ensembles (Robbe et al., 2006). In humans, THC reduces EEG theta power and synchrony (Ilan et al., 2005), working memory speed (Böcker et al., 2010), accuracy, (Ilan et al., 2004), and activation of the working memory network (Bossong et al., 2012). Interesting, it was shown that decreased frontal theta power correlates with increased default mode network (DMN) activity (Scheeringa et al., 2008). DMN activity correlates with stimulus-independent thought (Mason et al., 2007), and activity in this
network is inappropriately induced by THC during an executive function task in humans (Bossong et al., 2013). It has been proposed that effects of cannabis, such as mind-wandering, disorganized thought, and memory impairments could be related to the increased default mode network (DMN) activation (Bossong et al., 2013). These studies suggest that THC decreases working memory network function by facilitating DMN network activity, and this phenomenon may reduce interactions and multifractal dynamics of FCTs measured during the DNMS task.

Evidence for fractal dynamics in spike-timing and receptor dynamics exists at a more microscopic level, where cannabinoids modulate channel currents, synaptic plasticity and neurotransmitter release. Ion channel kinetics (Liebovitch and Sullivan, 1987; Lowen et al., 1999), AMPA receptor state transitions (Szárics et al., 2000), and quantal release events (Lamanna et al., 2012) exhibit fractal behavior. Channels have been successfully modeled using chaotic (Bandeira et al., 2008) and multifractal properties (Brazhe & Maksimov, 2006). Endogenous cannabinoids, or “endocannabinoids,” alter synaptic currents by activating CB1-receptors (Mechoulam and Parker, 2013; Morena and Campolongo, 2013). Endocannabinoids can act preferentially on either glutamate or GABA neurons (Domenici et al., 2006), and thereby modulate the “spontaneous” levels of excitation and inhibition (for review, see Diana and Marty, 2004; Lutz, 2004). Endocannabinoids were shown to enhance spike-time precision through selective inhibition of GABAergic neurons (Dubruc et al., 2013). Cannabinoid receptor activation inhibits calcium currents (Twitchell et al., 1997), increases potassium conductance (Mu et al., 1999), and inhibits long-term potentiation (Terranova et al., 1995). These mechanisms demonstrate numerous ways whereby cannabinoids can modulate neurotransmitter release and network excitation. Critical state dynamics are characterized by a balance between network stability and information transfer, and another way to interpret this would be a balance between excitation and inhibition.
Our data suggests that THC might reduce the criticality of the network as measured by decreased fractal properties, and this may be, in part, due to the modulation of excitation and inhibition at the cellular level caused by exogenous cannabinoid administration (Xu et al., 2010).

3.5.4. Multifractal Analysis as a Biomarker

Multifractal analysis could be used as a biomarker for identification, detection and classification of recorded biological signals to improve computational models and predictions based on experimental data. Much evidence exists suggesting that multifractal analysis could be used to improve our understanding of clinically defined disorders. Multifractal can enhance placement precision of deep brain stimulators needed for Parkinson’s disease by distinguishing between brain areas (Zheng et al., 2005). Some indication exists demonstrating the efficacy of patterned spatiotemporal electrical stimulation in suppressing seizures (Osorio et al., 2005; Sandler et al., 2013; Sun et al., 2008). Seizure events measured in mouse and human hippocampus show decreased nonlinearity and reduced multifractal character (Serletis et al., 2012), and together, these results suggest the multifractal analysis might provide critical information relevant for seizure detection, prediction and prevention.

Our data supports the idea that multifractal analysis could be incorporated into brain-computer interface detection algorithms as a biomarker for neurons or signals that might be functioning in the task (Hu et al., 2013). Multifractal analysis describes the temporal evolution of scale-invariant movement patterns over time and across environments (Ihlen and Vereijken, 2013). Understanding how movement patterns are encoded in the motor cortical regions is critically important for advancing the learning rate for users and improving decoder algorithms (Ortiz-Rosario and Adeli, 2013). Multiplicative interactions between temporal scales reflects healthy performance and adaption to external environmental influences (Ihlen and Vereijken, 2013), and integrating naturally occurring
modes of movement and adaptation into BCI algorithms should be ideal for mimicking biologically produced motions. By combining multifractal analysis with established BCI algorithms, like band-power features, we believe that improved classification and decoding performance of the technology can be achieved (Brodu et al., 2010).

3.6. Conclusions

We have shown that the heterogenous distribution of task-relevant hippocampal interspike intervals possesses long-range temporal correlations and multifractal properties, and these results support the interaction-dominant theory. We suggested mechanisms for these interactions: different memory-related brain regions may preferentially connect with FCTs, multifractal ISI distributions may be a cellular correlate of cross-frequency coupling, and/or multifractal ionic currents may alter firing properties.

Future work could bridge the gap over spatial scales by relating the multifractal activity of ion channels to the membrane potential of neurons, to action potential intervals, and to interactions between neurons. In addition, combinations of acquisition modalities, including fMRI, EEG, MEG, and multi-unit recording, could improve our understanding of the multifractal interactions occurring within the brain across multiple spatiotemporal scales. Additional sophisticated techniques, like unsupervised machine learning and wavelet coefficient analyses, could also be combined with results from multifractal analysis to generate deeper insight into these mechanisms. One major benefit of multifractal analysis is that if can be applied to previously collected neurophysiology data to further understand its complexity and provide evidence either for or against interaction-oriented dynamics within a given system. Multifractal analysis is a very translational approach, and by capitalizing on this advantage, better clinical theories and treatments may be established. By incorporating multifractal analysis with other proven neuroscience techniques, we will be able to improve data analysis and develop richer interpretations that would advance progress in many clinically relevant areas.
The central theme of the proposed mechanisms generating multifractal complexity in FCTs relies on dynamical interactions across spatial and temporal scales. Previous experiments have shown that the MIMO model relies heavily on information from trial-type neurons (Hampson et al., 2012), but the current results suggest that neurons with the largest degree of information content, as measured by multifractal complexity, may be represented from all different FCT-groups. Information processing relevant for performance of the task can be detected using multifractal analysis, as demonstrated by the reduction in log-cumulants after memory-impairing doses of THC. Multifractal analysis promoted the identification of two new classes of FCTs: Nosepoke-Nonmatch and 3 Peaks. Together, our results show that multifractal analysis can detect neuronal processing relevant for memory performance. We hypothesize that multifractal log-cumulants can provide an objective measure for pre-screening neurons to include in neuroprosthetic models and devices, including MIMO and BCI, which overcomes the subjectivity associated with z-score-based FCT classification.

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3.7. References


CHAPTER 4: DISTINGUISHING COGNITIVE STATE WITH MULTIFRACTAL COMPLEXITY OF HIPPOCAMPAL INTERSPIKE INTERVAL SEQUENCES

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The following manuscript was published in *Frontiers in Systems Neuroscience*, September 2015, volume 9:130, and is reprinted under the terms of the Creative Commons Attribution License. Stylistic variations are due to the requirements of the journal. D. Fetterhoff collected behavioral and electrophysiological data, performed data analysis and wrote the manuscript. R.A. Sandler and V.Z. Marmarelis provided algorithms for Fourier analyses. D. Fetterhoff and R.A. Kraft conceptualized the study. R.A. Kraft, C.A. Sexton, R.E. Hampson, and S.A. Deadwyler edited the manuscript.
4.1. Abstract

Fractality, represented as self-similar repeating patterns, is ubiquitous in nature and the brain. Dynamic patterns of hippocampal spike trains are known to exhibit multifractal properties during working memory processing; however, it is unclear whether the multifractal properties inherent to hippocampal spike trains reflect active cognitive processing. To examine this possibility, hippocampal neuronal ensembles were recorded from rats before, during and after a spatial working memory task following administration of tetrahydrocannabinol (THC), a memory-impairing component of cannabis. Multifractal detrended fluctuation analysis was performed on hippocampal interspike interval sequences to determine characteristics of monofractal long-range temporal correlations (LRTCs), quantified by the Hurst exponent, and the degree/magnitude of multifractal complexity, quantified by the width of the singularity spectrum. Our results demonstrate that multifractal firing patterns of hippocampal spike trains are a marker of functional memory processing, as they are more complex during the working memory task and significantly reduced following administration of memory impairing THC doses. Conversely, LRTCs are largest during resting state recordings, therefore reflecting different information compared to multifractality. In order to deepen conceptual understanding of multifractal complexity and LRTCs, these measures were compared to classical methods using hippocampal frequency content and firing variability measures. These results showed that LRTCs, multifractality, and theta rhythm represent independent processes, while delta rhythm correlated with multifractality. Taken together, these results provide a novel perspective on memory function by demonstrating that the multifractal nature of spike trains reflects hippocampal microcircuit activity that can be used to detect and quantify cognitive, physiological and pathological states.
4.2. Introduction

By analyzing the mono- and multifractal properties of neural temporal dynamics, we may generate new insights concerning how the brain functions with implications for detection of cognitive, physiological and pathological states. Such analyses have been used successfully to detect pathological conditions such as heart disease (Ivanov et al., 1999), Alzheimer’s disease (Lahmiri and Boukadoum, 2013), Parkinson’s disease (Zheng et al., 2005) and epilepsy (Serletis et al., 2012; Dutta et al., 2014). Additionally, multifractal analysis detects clear differences in neural activity between wakefulness and sleep stages using EEG signals (Weiss et al., 2009; Zorick and Mandelkern, 2013). Multifractal complexity of time series is believed to indicate functional connectivity because such complexity would provide the essential temporal dynamics to support information transfer via variable patterns of neural activity. Interactions across brain regions, detected as multifractal complexity, regularly fluctuate between task and rest conditions specifically in regions associated with the task (Ciuciu et al., 2012). In order to assess cognitive state detection abilities, a paradigm was implemented to examine how multifractal complexity is reflected by active (i.e., task-related) hippocampal microcircuit processing.

Temporal coding analyses attempt to derive information about brain function from the timing of action potentials generated by neuronal ensembles or from rhythmic neuronal oscillations, such as theta rhythm (Jones and Wilson, 2005). Some temporal coding analyses assign physiological function to activity within frequency bands (i.e., theta-phase precession or cross frequency coupling), but we propose that multifractal analysis can provide new insights into mechanisms of neurological temporal coding because it quantifies the structure of variability and the self-similar (fractal) nature of physiological systems. Scale-free dynamics are often associated with long-range temporal correlations (Linkenkaer-Hansen et al., 2001; Ciuciu et al., 2012) and
quantified by the monofractal Hurst exponent. However, a single exponent does not capture the complexity of many physiological signals, supporting the use of a spectrum of scale invariant exponents that describe the multiple, co-existing fractal patterns (Dixon et al., 2012). Memory is commonly believed to occur through repetitive neuronal sequences (Hampson et al., 2012), and therefore multifractal analysis applied to spike train patterns may quantify a possible basis of memory detected as multifractal complexity.

To assess if active memory processing is reflected by multifractal measures, analyses of monofractal long-range temporal correlations (LRTCs) and multifractal complexity in hippocampal interspike interval (ISI) neural sequences were conducted during a working memory task. In vivo electrophysiological recordings of rat hippocampal CA3 and CA1 subregions were conducted during a resting state condition in a neutral (task-independent) context for 25-30 minutes both before and after performance of the delayed nonmatch-to-sample (DNMS) task. Between the pre-task recording and the DNMS task, rats were injected with vehicle or tetrahydrocannabinol (THC), a psychoactive component of cannabis known to impair memory function (Hampson and Deadwyler, 2000). Prior results demonstrated that hippocampal neurons with memory-correlated firing rate alterations (functional cell types, FCTs) recorded during the DNMS task were more multifractal than non-memory neurons (non-FCTs) and THC administration impaired memory while reducing multifractality (Fetterhoff et al., 2015). By examining the same neurons before, during and after the DNMS task, alterations in multifractality were assessed in a different context. These experiments and analyses were designed to extend previous findings by testing three new hypotheses and facilitating a stronger intuition concerning multifractal properties of hippocampal microcircuits. First, we hypothesized that LRTCs, as indicated by the Hurst exponent, would decrease during the DNMS task compared to resting (pre/post)
recording conditions. Since LRTCs arise when distant activity has a greater influence on future activity patterns, we hypothesized a decrease would occur due to the constantly changing requirements of the DNMS task. Second, we hypothesized that an increase in multifractal complexity reflects active memory processing in populations of hippocampal neurons and therefore, spike trains should be more multifractal during the DNMS task compared to both pre- and post-task recording phases. Third, we hypothesized that THC would decrease both multifractal complexity and Hurst exponents during the task and post-recording phases compared to vehicle control recordings during the same phase. Finally, to enrich conceptual interpretation of multifractal complexity and LRTCs and establish the difference between structure (multifractality) and amount of variability, classical spike train variability measures (coefficient of variation, ISI STD and mean ISI) were compared with the mono- and multifractal variables.

The primary goal of this study was to assess the capacity of multifractal analysis to distinguish between recording phases and drug conditions. Fourier analysis of neuronal signals is one commonly employed method to distinguish between physiological and cognitive states (De Carli et al., 2004; Jones and Wilson, 2005; Nguyen et al., 2008; Palva et al., 2010; Van Someren et al., 2011; Garn et al., 2014), and therefore, the performance of multifractal analysis was compared to the frequency content computed from the same spike trains. The results showed that the monofractal Hurst exponent and magnitude of multifractality could differentiate between more recording/drug conditions compared to frequency content (theta and delta) and further support utility of multifractal analysis for this objective (Weiss et al., 2009; Zorick and Mandelkern, 2013). Multifractal analysis has the potential to generate novel insights into the role of neuronal ensembles by quantifying different temporal features compared to other analyses.
4.3. Materials and Methods

4.3.1. Rats

Male Long-Evans rats (Harlan) aged 6-10 months (n=10) were tested under protocols approved by the Wake Forest University Institutional Animal Care and Use Committee, and in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care and the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023). All animals were individually housed and allowed free access to food with water regulation to maintain 85% of *ad libitum* body weight during testing. Upon termination of the study, all rats were anesthetized with ketamine (100 mg/kg) and brains were perfused with formaldehyde for preservation and subsequent histology to confirm electrode placement.

4.3.2.1. Apparatus

The behavioral testing apparatus for the delayed nonmatch to sample (DNMS) task as used in other studies (Hampson and Deadwyler, 2000; Hampson et al., 2012) consisted of a 43 × 43 × 50 cm Plexiglas chamber with two retractable levers (left and right) positioned on either side of a water trough on the front panel. A nosepoke device (photocell) was mounted in the center of the wall opposite the levers with a cue light positioned immediately above the nosepoke device. A video camera was mounted on the ceiling and the entire chamber was housed inside a commercially built sound-attenuated cubicle.

4.3.2.2. Delayed nonmatch-to-sample (DNMS) task

The DNMS task consisted of three main phases: Sample, Delay and Nonmatch. The Sample phase initiated the trial via presentation of either the left or right lever (50% probability), which required the animal to press and make the Sample Response (SR). The lever was then retracted and the Delay phase of the task initiated, as signaled by
the illumination of a cue light over a nosepoke photocell device on the wall opposite to where the lever was presented. At least one nosepoke (NP) was required following the interposed delay interval which varied randomly in duration (1-30 s) on each trial during the session. The Nonmatch phase began when the delay timed out, the photocell cue light turned off, and both the left and right levers on the front panel were extended. Correct responses consisted of pressing the lever in the Nonmatch phase located in the spatial position opposite to the position of the SR; in other words, a Nonmatch response (NR). This produced delivery of a 0.4 ml water reward in a reservoir between the two levers. After the NR the levers were retracted for a 10.0 second intertrial interval (ITI) before the Sample lever for the next trial was presented. A lever press at the same position as the SR (match response) constituted an “error” with no water delivery and turned off chamber house lights for 5.0s with the next trial presented 5.0 s later. Individual performance was assessed as % NRs (correct responses) with respect to the total number of trials (80-100) per daily (1 hr) session.

4.3.2.3. Task and Rest (non-task) recording paradigm

All rats were recorded for 25-30 minutes in a bare, white 38 x 29 x 30 cm plastic container that was inserted within the DNMS testing chamber both before (pre) and after (post) the DNMS task (Fig. 4.1A). This constituted a neutral environment used to record task-independent neuronal activity that was opaque to prevent rats from seeing task components, such as levers or the nosepoke device.
Figure 4.1. Rest (pre/post) and Delayed Nonmatch-to-Sample (DNMS) task recording paradigm. (A) Prior to each testing session, all rats were recorded in a white, rectangular plastic box for 25-30 minutes (pre-task recording). Upon completion of pre-task recording phase, the same rats were injected with either vehicle or delta-9-tetrahydrocannabinol (THC) 5-10 minutes before the start of delayed nonmatch-to-sample (DNMS) task. Immediately after completing the DNMS task, rats were put back into the same plastic chamber for another 25-30 minute recording (post-task recording). (B) Progression of the DNMS task is illustrated. A 10 second Intertrial Interval (ITI) precedes the Sample Presentation (SP). Rats must make the Sample Response (SR) and remember the lever position throughout the variable 1-30s delay that terminates after the Last Nosepoke (LNP). The LNP signals extension of both levers and rats receive a water reward (reinforcement) for appropriately making a Nonmatch Response (NR).
4.3.3. Drug preparation and administration

$\Delta^9$-THC was obtained from the National Institute on Drug Abuse as a 50 mg/ml solution in ethanol. Detergent vehicle was prepared from Pluronic F68 (Sigma, St. Louis, MO), 20 mg/ml in ethanol. $\Delta^9$-THC was added to the detergent-ethanol solution (0.04-0.12 ml of THC), and then 2.0 ml of saline (0.9%) was slowly added to the ethanol-drug solution. The solution was stirred rapidly and placed under a steady stream of nitrogen gas to evaporate the ethanol (\sim 10 min). This resulted in a detergent-drug suspension (12.5 mg/ml THC), which was sonicated and then diluted with saline to final injection concentrations (1.0–3.0 mg/ml THC). On testing days, animals were injected intraperitoneally with the detergent vehicle solution or the THC drug-detergent solution (1 ml/kg) immediately after the pre-task recording phase and approximately 5-10 minutes before the start of the behavioral session. At least one vehicle day was imposed between each drug-testing day. All rats received THC on 5-8 days. All drug solutions were mixed fresh each day.

4.3.4.1. Hippocampal Electrode array surgery.

All surgical procedures conformed to National Institutes of Health and Association for Assessment and Accreditation of Laboratory Animal Care guidelines, and were performed in a rodent surgical facility approved by the Wake Forest University Institutional Animal Care and Use Committee. Electrode arrays and recordings were the same as described in several prior publications from this laboratory (Hampson et al., 1999, 2012; Hampson and Deadwyler, 2000). After being trained to criterion performance level in the DNMS task animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic frame. Craniotomies (5mm-diameter) were performed bilaterally over the dorsal hippocampus to provide for
implantation of 2 identical array electrodes (Neurolinc, New York, NY), each consisting of two rows of 8 stainless steel wires (diameter: 20 μm) positioned such that the geometric center of each electrode array was centered at co-ordinates 3.4 mm posterior to Bregma and 3.0 mm lateral (right or left) to midline (Paxinos and Watson, 1997). The array was designed such that the distance between two adjacent electrodes within a row was 200 μm and between rows was 400 μm to conform to the locations of the respective CA3 and CA1 cell layers. The longitudinal axis of the array of electrodes was angled 30° to the midline during implantation to conform to the orientation of the longitudinal axis of the hippocampus, with posterior electrode sites more lateral than anterior sites. The electrode array was lowered in 25-100 μm steps to a depth of 3.0 - 4.0 mm from the cortical surface for the longer electrodes positioned in the CA3 cell layer, leaving the shorter CA1 electrodes 1.2 mm higher with tips in the CA1 layer. After placement of the array the cranium was sealed with bone wax and dental cement and the animals treated with Ketoprofen (3.0-5.0 mg/kg) for pain relief over the next 4-6 hrs. The scalp wound was treated periodically with Neosporin antibiotic and systemic injection of penicillin G (300,000 U, intramuscular) were given to prevent infection. Animals were allowed to recover from surgery for at least 1 week before continuing behavioral testing (Berger et al., 2011; Hampson et al., 2012).

4.3.4.2. Electrophysiological monitoring, acquisition and waveform sorting of neuronal data

Animals were connected by cable to the recording apparatus via a 32-channel headstage and harness attached to a 40-channel slip-ring commutator (Crist Instruments, Hagerstown, MD) to allow free movement in the behavioral testing chamber. Single neuron action potentials (spikes) were isolated by time-amplitude window discrimination and computer-identified individual waveform characteristics using
a multi-neuron acquisition (MAP) processor (Plexon Inc., Dallas, TX, USA). Single neuron spikes were recorded daily and identified using waveform and firing characteristics within the task (perievent histograms) for each of the DNMS events (SR, LNP & NR). To maintain waveform shape across days, all recorded data was concatenated into one file (separately for each rat) and offline sorting was performed using principal component analysis, peak-valley, and nonlinear energy algorithms in Offline Sorter (Fig. 4.S1; Plexon Inc., Dallas, TX, USA). Hippocampal neuron ensembles used to distinguish recording phases and drug treatment conditions consisted of 10-30 single neurons, each recorded from a separate identified electrode location on either of the bilateral arrays. Only isolated neural spike waveforms exhibiting firing rates of CA1 and CA3 principal cells (i.e. 0.5-8.0 Hz average firing rate) and consistent multifractal properties across sessions were included in analyses. Previous work has shown that hippocampal neurons recorded identified in this manner exhibit consistent mean, baseline and DNMS task modulated firing rate over multiple task sessions (Deadwyler et al., 1996; Hampson et al., 1999, 2003).

4.3.5. Multifractal Detrended Fluctuation Analysis (MF DFA)

Detailed descriptions of multifractal detrended fluctuation analysis (Kantelhardt et al., 2002; Kantelhardt, 2012) and associated Matlab code (Ihlen, 2012) are available elsewhere. We briefly summarize and illustrate the main components of the MF DFA method. A demonstration of this method (Fig. 4.2) was constructed using recordings of a single neuron (Fig. 4.S1) recorded during the DNMS task over two sessions/days: one vehicle (blue) and one THC (green). First, a neuronal spike train is converted to a sequence of interspike intervals (ISIs; Fig. 4.2A), represented as x, and commonly referred to as a noise-like time series (Ihlen, 2012). The ISIs are converted into a
“random walk-like” time series \( Y(i) \) by subtracting the mean and integrating the ISI signal \( x \):

\[
Y(i) = \sum_{k=1}^{i} [x_k - \langle x \rangle], \ i = 1, \ldots, N
\]  

(1)

The random walk \( Y(i) \) (Fig. 4.2B inset, blue line) is divided into \( N_s \) non-overlapping segments of equal length \( s \) (scale). The local quadratic trend \( y_v \) (Fig. 4.2B inset, black line) is calculated for each of the \( N_s \) segments \( v \) by a least-square fit of the series to determine the local root mean square variation \( F \) for each segment \( v \) (Fig. 4.2B):

\[
F^2(v, s) = \frac{1}{s} \sum_{i=1}^{s} \{ Y[(v-1)s + i] - y_v(i) \}^2
\]  

(2)

In this way, \( F^2(v, s) \) is essentially the mean-square error difference between the quadratic fit \( y_v \) and the walk-like ISI sequence \( Y \). Visualization of the walk like ISI sequence at multiple q-order statistical moments permits observation of the structure of variability quantified by multifractal analysis (Fig. 4.2B). Periods of low activity (i.e., clusters of short ISIs, faster firing rate) are amplified with negative moments (Fig. 4.2B). Conversely, periods of high activity (i.e., clusters of longer ISIs, slower firing rate) are amplified with positive moments (Fig. 4.2B, bottom). Next, the qth order fluctuation function is determined by averaging over all segments \( v \) for each qth power (Fig. 4.2C):

\[
F_q(s) = \left\{ \frac{1}{N_s} \sum_{v=1}^{N_s} [F^2(v, s)]^{q/2} \right\}^{1/q}
\]  

(3)

The scaling behavior of the fluctuation function is seen by analyzing log-log plots of \( F_q(s) \) versus \( s \) for each qth power (Fig. 4.2C). Standard (monofractal) Detrended Fluctuation Analysis calculates the Hurst exponent from the slope of the power-law
regression line between the overall root mean square variation $F$ across multiple scales, $s$, for a single statistical moment, $q = 2$. Multifractal analysis performs the same linear regression for a broad range of statistical moments $q$. $F_q(s)$ increases as a power-law for large values of $s$ if the signal $x$ contains long-range temporal correlations (LRTCs):

$$F_q(s) \sim s^{H(q)}$$

The Hurst exponent (Hurst, 1951) is the slope of $F_q(s)$ on this log-log plot for $H(q=2)$. A greater slope yields a larger Hurst exponent and indicates increased LRTCs which defines how fast the overall root mean square variation grows with increasing segment size (scale = $s$). Hurst exponents greater than 0.5 indicate the time series contains positive correlations (i.e., persistent structure), Hurst exponents ranging from 0 to 0.5 indicate negative correlations and uncorrelated Gaussian noise has a Hurst exponent equal to 0.5. In multifractal signals, changes in LRTCs occur at different $q$-order statistical moments. These variations are visualized by comparing the $q$-order Hurst exponents (slopes of regression lines) on log-log plots of $F_q(s)$ versus scale $s$, where each line is computed from a different (integer) $q$th power ranging from -3 to 3 (Fig. 4.2C). Our example shows more variable slopes in the vehicle condition (Fig. 4.2C, blue) compared to the similar slopes in THC condition (Fig. 4.2C, green), indicating greater multifractality during the vehicle condition. The $q$th order fluctuation function $F_q(s)$ is one way to visualize multifractal properties of variability, but generally the multifractal singularity spectrum (Fig. 4.2D) is constructed to illustrate the distinction between (mono)-fractal and multifractal analyses. The $q$-order Hurst exponent $H(q)$ is converted to the $q$-order mass exponent $\tau(q)$:

$$\tau(q) = qH(q) - 1$$

Finally, a Legendre transform relates \( \tau(q) \) to the fractal dimension \( D(h) \) and Hölder exponent \( h \):

\[
h = t'(q) \quad \text{and} \quad D(h) = qh - \tau(q)
\]

The singularity spectrum is like a histogram of the q-order Hurst exponents (slopes of regression lines in Fig. 4.2C). The magnitude of multifractality is determined by the width of the singularity spectrum and consequently the range of Hölder exponents \( h \) covered by the ISI signal (Fig. 4.2D). The Hurst exponent is closely approximated by the Hölder exponent at the apex of the singularity spectrum (where \( D(h) = 1 \)). In our example, the singularity spectrum for the THC condition is narrower, and thus less multifractal than the singularity spectrum computed for the control condition (Fig. 4.2D). The parameters used for all analyses were determined by viewing log-log plots of \( F_q(s) \) versus \( s \) for each qth power and multifractal singularity spectra that resembled those found in the literature (Kantelhardt et al., 2002; Ihlen, 2012). MFDFA was performed by fitting a second-order polynomial, scales used ranged from 16 to 256, and singularity spectrum width was determined by computing \( h(q = 3) - h(q = -3) \). All analyses were performed in Matlab using publicly available MFDFA code (Ihlen, 2012).
Figure 4.2. Multifractal Detrended Fluctuation Analysis. An illustration of the MFDFA method was created using one CA1 neuron recorded on two different days: one vehicle condition (blue) and one tetrahydrocannabinol (THC) condition (green). (A) The interspike interval sequence (ISI) of each neuron is shown for the first 2000 ISIs. Five seconds were subtracted from the entire THC ISI sequence for illustration purposes only. (B) The fluctuation function $F$ is shown at scale 16 ($s = 16$) for four different q-order statistical moments. Negative q-order statistical moments amplify small fluctuations, while positive moments amplify large fluctuations. Inset: $F^2(v, s)$ is the root mean-squared residual between the fit $y_v$ (black) of one segment $s$ from the walk-like time series $Y$ (blue). (C) The changes in variability across scales are indicated by variable slopes at different qth powers (integer q-values from -3 to 3). The q-order Hurst exponent $H(q)$ is the slope of each regression line. Blue lines are from the vehicle recording and green lines are from the THC recording. Dots indicate individual values from each scale (19 scales ranging from 16 to 256). (D) Multifractal complexity is visualized with the multifractal singularity spectrum. The Hurst exponent is closely related to the $h$ value at the apex of the singularity spectrum (black data points). The width is obtained by subtracting $h$ values at each end of the spectrum (independent of $D(h)$ values) indicated by the black arrow. The singularity spectrum for the vehicle condition is wider than the THC condition, indicating THC decreases multifractality.
4.3.6. Fourier transform

A fast Fourier transform of the spike train data in 1 ms binary bins where 1 = spike and 0 = no spike was performed for every neuron recorded during every recording phase. Delta and theta power were measured by taking the ratio of signal power in the delta (0.5 – 4.0 Hz) or theta range (4-8 Hz) over the total power of the normalized signal, bandpass filtered from 0.5 to 12 Hz. All analyses were performed using Matlab.

4.3.7. Statistical analyses

All calculations were performed in Matlab, results were recorded spreadsheets and imported into Statistical Analysis Systems (SAS) software (SAS Institute, Cary, NC) to perform repeated measures ANOVAs and correlations. A total of seven repeated measures ANOVAs were performed using these dependent variables: the Hurst exponent, singularity spectrum width, coefficient of variation, ISI standard deviation, mean ISI, delta power and theta power. Neurons were the subject identifier, each session as a within-subjects effect, and no group identifier was used. The covariance structure used was compound symmetry. Main effects of drug condition or recording phase were only discussed when the interaction between drug condition and recording phase was non-significant. When significant ANOVA effects were found, posthoc tests were performed with 10,000 Monte Carlo permutations to determine two-tailed p-values. Significance levels were set to p < 0.0083 to control for multiple comparisons (Bonferroni correction). Correlations (Spearman's rho) were used to examine the associations between mono- and multifractal variables (Hurst exponent and singularity spectrum width) and coefficient of variation, ISI standard deviation, mean ISI, delta and theta power. 55 total correlations were performed to assess the relationships for monofractal and multifractal variables during all three recording phases (pre-task, task, post-task).
4.4. Results

To investigate dynamical interspike interval (ISI) patterns associated with different hippocampal microcircuit processing states, a comparison was made between those generated during the DNMS task (Deadwyler et al., 1996; Hampson et al., 1999) and those occurring during a resting state after either vehicle or THC administration (Hampson and Deadwyler, 2000). Hippocampal principal (pyramidal) cells were identified based on mean firing rate (0.5 – 8.0 Hz), and neurons exhibiting rates outside this range were excluded. Identified neurons from selected wires were “tracked” from day to day by waveform and multifractal properties (i.e., Hurst exponent and width). A total of 197 hippocampal principal (pyramidal) cells were recorded from 10 different rats and each neuron was recorded from the same electrode over multiple days (10-16 recording days per rat). 117 CA1 and 80 CA3 neurons were analyzed for this study. Every neuron included in the analyses was recorded during at least 4 days (2 vehicle and 2 THC).

4.4.1. Delayed Nonmatch-to-Sample Task

Hippocampal spike trains were recorded during a resting state condition in a neutral (i.e., task-independent) environment both before (pre) and after (post) the DNMS task (Fig. 4.1A) to assess the influence that active memory processing (during the task) exerts on the structure of spike train variability, as indicated by multifractal analysis. This approach was designed to assess electrophysiological distinctions between three different recording phases (pre-task, task, post-task) and in two drug conditions. Since drug injections (pluronic vehicle or THC) were given immediately after the pre-task/pre-drug resting state recording phase, all computed measures for the pre-task phase are equal across the two drug conditions. THC, the main active ingredient in cannabis (Gaoni and Mechoulam, 1964), was chosen because it impairs memory encoding during the DNMS task (Hampson and Deadwyler, 1999, 2000), reduces LRTCs and multifractal
complexity of task-related neuronal spike trains (Fetterhoff et al., 2015) and impairs theta frequency-related working memory performance in both rats (Robbe et al., 2006) and humans (Ilan et al., 2004; Böcker et al., 2010). THC doses were chosen to maximally impair DNMS performance in order to examine effects on associated multifractal spike train characteristics using previously established dose-response relationships (Hampson and Deadwyler, 2000). Working memory was assessed in ten rats after vehicle or THC administration using the DNMS task (Fig. 4.1B). A within subjects design was used to assess behavioral performance and hippocampal electrophysiology for 5 to 8 days per drug condition (vehicle or THC) per rat. The DNMS performance was inversely correlated with delay length, as all animals performed worse at longer delays (Fig. 4.3). THC (green line) impaired performance compared to vehicle (Fig. 4.3, blue line).

![Figure 4.3. Delayed nonmatch-to-sample behavioral performance during vehicle and tetrahydrocannabinol (THC) sessions.](image)

Mean correct nonmatch responses summed across all rats (n=10) shows the delay-dependent decline in performance under both conditions. A within subjects design with at least one non-drug day between THC administration was used. All animals were given THC (1.0-3.0 mg/kg) for at least five sessions spaced over consecutive weeks. Error bars indicate S.E.M.
4.4.2. Frequency and singularity spectra of hippocampal spike trains

Interactions between frequency bands are believed to represent coordination and exact timing relationships within neuronal ensembles (Buzsáki and Moser, 2013). Multifractal dynamics are believed to arise from interactions across spatiotemporal scales (Ihlen and Vereijken, 2010; Kelty-Stephen et al., 2013) and therefore may represent phenomena related to frequency-specific activity. Additionally, both analyses are commonly used to assess differences in sleep/arousal state, and increasing interest is focused on multifractal analysis as an automatic sleep stage detection method (Weiss et al., 2009; Zorick and Mandelkern, 2013). The performance of multifractal and Fourier analyses was evaluated here in terms of their ability to distinguish between recording phases and drug treatment conditions (Fig. 4.1) based on analyses of hippocampal spike trains. Fourier transforms were used to measure both delta (0.5 – 4 Hz) and theta (4 – 8 Hz) power in binary spike train representations. These frequency bands were chosen due to their prominence during the DNMS task (Hyman et al., 2010, 2011) and pervasive presence in working memory literature (Sato and Yamaguchi, 2003; Mormann et al., 2008; Clemens et al., 2013; Assenza et al., 2014; Hasselmo and Stern, 2014). MFDFA quantifies the structure of variability of an ISI sequence and permits estimation of the multifractal singularity spectrum (Ihlen, 2012) that describes a given spike train. The singularity spectrum presents two important pieces of information: the Hurst exponent and magnitude of multifractality. The Hurst exponent $H$ is a self-similarity parameter that quantifies long-range temporal correlations (LRTCs) and takes values nearly equivalent to the Hölder exponent $h$ value at the center apex of the singularity spectrum (Fig. 4.2D) where $D(h) = 1$ (Ciuciu et al., 2012; Ihlen, 2012). The Hurst exponent quantifies the (mono)-fractal structure of the ISI time series, however, many physiological signals exhibit a wider range of dynamical activity that is better described as multifractal. The magnitude of multifractality quantifies the structure of variability in
an interspike interval (ISI) sequence and is directly proportional to the width of the singularity spectrum. The width is defined by the range of local Hölder exponents $h$ (Fig. 4.2D) which quantify the dynamical profile of a time signal.

The singularity and frequency spectra for two different example neurons are shown in Figure 4.4 while the population singularity spectra are shown in Figure 4.5. Repeated measures ANOVA results from population analyses are briefly mentioned here and presented fully in the subsequent sections. The first example permits comparison of all three recording phases taken from vehicle treatment conditions (Figs. 4.4A & B, 4.5B). One example neuron exhibits increased multifractal complexity during the DNMS compared to either resting state recordings (Fig. 4.4A). The frequency spectra for this same neuron exhibits both delta and theta power in all recording phases (Fig. 4.4B). This neuron illustrates the same effect found in the population (Fig. 4.5B): multifractal complexity (width) increases from post-task to pre-task to task (Fig. 4.7F) and LRTCs (Hurst exponent) are larger during the resting states (pre-task and post-task) compared to the task (Fig. 4.6F). Although the singularity spectra are discernable across task phases for this neuron, the frequency spectra were not (Fig. 4.4B). However, the population analyses revealed increased theta power during vehicle resting state recordings (pre-task and post-task) compared to vehicle task recordings (Fig. 4.8E).

Another example neuron was chosen to illustrate the finding that THC reduced multifractal complexity (width) during the DNMS task compared to vehicle (Fig. 4.4C). This neuron was recorded over 16 total days, 8 vehicle days and 8 THC days. Individual and average singularity spectra are shown (Fig. 4.4C). The frequency spectrum for this neuron shows delta rhythm but not theta (Fig. 4.4D). This neuron is representative of the entire population: during the DNMS task, THC reduces multifractality (width; Figs. 4.5C, 4.7D & 4.7F), but has no significant effect on the Hurst exponent (Fig. 4.6D &
4.6F). Additionally, THC did not affect frequency content during the DNMS task compared to vehicle (Fig. 4.8D-E).
Figure 4.4. Singularity and frequency spectra of example neurons. Singularity and frequency spectra pairs are shown for two different CA1 neurons. Single neurons were measured across multiple days in various recording phases (pre-task, task, post-task) and drug conditions (vehicle or THC) and each spectrum trace represents the multifractal complexity (A & C) and frequency content (B & D) computed from one recording phase on one day. (A) Singularity spectra are shown for each recording phase over three vehicle experiment days (diamonds for day 1, squares for day 2, triangles for day 3). Singularity spectra are wider, thus multifractal complexity is greater, during the task compared to either resting state recording phases. (B) Frequency spectra are shown for the same neuron recorded during the same three days as in A and color coded to match the legend in A. This neuron exhibits both delta (0.5-4 Hz) and theta (4-8 Hz) frequency activity during all recording phases on all days. (C) Singularity spectra computed from the DNMS task are compared between vehicle and THC conditions for one example neuron recorded over 16 total days. Individual session singularity spectra are plotted in thin blue lines for vehicle and green lines for THC sessions. The average singularity spectra for this neuron is plotted as a dashed orange line for vehicle and as a dashed red line for THC. (D) Frequency spectra are shown for the same neuron recorded during the same 16 days as in C and color coded to match the legend in C. Only spectra from individual neurons were plotted. This neuron exhibits delta rhythm only.
Figure 4.5. **Average singularity spectra across recording phases and drug conditions.** Average spectra were computed by averaging all neurons within the respective recording phase and drug condition. A total of 197 hippocampal neurons were recorded from 10 different rats. Each neuron was recorded from the same electrode over multiple days and multifractal analysis was performed on a total of 5,143 individual ISI sequences. 771-1004 individual ISI sequences were averaged for each condition. The legend in the upper right corner of panel A holds true for all figures. (A) Average singularity spectra were obtained from all neurons recorded during their respective recording phase and drug condition. (B) Average singularity spectra from all recording phases during vehicle treatment are plotted for comparison. Neurons exhibit greater multifractal complexity (i.e., wider singularity spectra; wider range of Hölder exponents $h$) during the task compared to either resting state. Long-range temporal correlations, indicated by the Hurst exponent, which is closely related to the Hölder exponent at the apex of the singularity spectrum (where $D(h) = 1$), are stronger during the resting states compared to the task. (C) Average singularity spectra from both drug conditions during DNMS task recordings show that THC reduces multifractal complexity, as indicated by decreased singularity spectra width. (D) Average singularity spectra taken from post-task recording phases show that THC reduces LRTCs (i.e., decreased Hurst exponent) compared to vehicle recordings; this effect is seen as the leftward shift in the THC spectrum compared to the vehicle one. Multifractal complexity was unchanged by THC during post-task recordings.
4.4.3. Temporal correlations and multifractal complexity of hippocampal population

Two repeated measures ANOVAs were performed to assess the capacity of monofractal and multifractal variables, the Hurst exponent and singularity spectrum width, to distinguish between recording phases and drug treatment conditions. Hurst and width were used as dependent variables to examine main effects of drug condition (vehicle versus THC) and recording phase (pre-task/pre-drug, DNMS task, post-task) and an interaction between the two. Only interactions (not main effects) are discussed when significant. We primarily wanted to evaluate the ability of computed variables to establish 6 ad-hoc chosen differences: vehicle pre-task vs. task, vehicle task vs. post-task, vehicle pre-task vs. post-task, vehicle task vs. THC task, vehicle post-task vs. THC post-task, and THC task vs. THC post-task. Since the distributions were long-tailed, posthoc Monte Carlo permutation tests were performed with significance levels set at p < 0.0083 (Bonferoni correction). All analyses were performed on a total of 5,143 individual interspike interval (ISI) sequences: 829 (495 CA1, 334 CA3) individual ISI sequences from vehicle pre-task recordings, 1004 (592 CA1, 412 CA3) from vehicle task recordings, 848 (510 CA1, 338 CA3) from vehicle post-task recordings, 771 (443 CA1, 328 CA3) from THC pre-task/pre-drug recordings, 923 (550 CA1, 373 CA3) from THC task recordings, and 768 (453 CA1, 315 CA3) from THC post-task recordings.

The Hurst exponent is a monofractal self-similarity parameter that quantifies LRTCs in an ISI sequence. A significant main effect of drug condition ($F_{(1,196)} = 97.61, p < 0.0001$), a significant main effect of recording phase ($F_{(2,379)} = 182.7, p < 0.0001$), and a significant interaction $F_{(2,369)} = 80.77, p < 0.0001$) were found when using the Hurst exponent as the dependent variable. The significant drug treatment by recording phase interaction revealed three out of six important group differences (Fig. 4.6F): both pre-task and post-task vehicle recordings contain greater LRTCs than task recordings (Fig.
4.6A & 4.6B) and THC reduces LRTC\(\text{s}\) compared to control during the post-task recording (Fig. 4.6E). No significant difference was found between the Hurst exponent from vehicle pre-task recordings compared to vehicle post-task recordings (Fig. 4.6C), nor was a difference established between the Hurst exponent computed from vehicle or THC task recordings (Fig. 4.6D). After THC treatment, the Hurst exponent of the task condition was similar to those from the post-task (Fig. 4.6F).
Figure 4.6. Fractality (Hurst exponent) across recording phases and drug conditions. The scatter plots show each data point obtained by averaging the Hurst exponent for individual neurons over all recordings. All neurons included in the analysis were recorded for 2-8 days of vehicle and THC administration. Thick gray lines are y = x. Data points from vehicle only sessions are displayed in A-C. (A) Neurons exhibited greater Hurst exponents during the pre-task recordings compared to DNMS task recordings. (B) The Hurst exponent of ISIs was also greater during the post-task compared to task recordings. (C) Hurst exponents were similar during pre-task and post-task recordings. (D) THC had no effect on the Hurst exponent of neurons recorded during the DNMS task. (E) THC significantly reduced the Hurst exponent during post-task recordings. (F) Each bar was obtained by averaging Hurst exponent values from individual spike trains within specified recording phase and drug treatment combinations (n = 771-1004 neurons per group). Errors bars represent S.E.M. Statistical significance is designated by * indicating p < 0.0083.
Multifractal complexity reflects energy flow through all scales of a dynamical system (Dixon et al., 2012; Kelty-Stephen et al., 2013) and is greater in memory processing neurons compared to randomly spiking ones (Fetterhoff et al., 2015). Therefore, multifractality may selectively arise in hippocampal microcircuit processing when task-specific input-output transformations occur (Berger et al., 2012; Hampson et al., 2012). A repeated measures ANOVA using multifractal width as the dependent variable yielded a significant main effect of drug condition ($F_{(1,196)} = 11.09, p = 0.001$), a significant main effect of recording phase ($F_{(2,379)} = 30.8, p < 0.0001$), and a significant interaction $F_{(2,369)} = 13.87, p < 0.0001$). The significant drug condition by recording phase interaction established four out of six significant differences (Fig. 4.7F): in the vehicle condition, all three recording phases are different from each other (Fig 4.7A-C; task > pre > post). Multifractal complexity was greatest when hippocampal ensembles were processing task-relevant information. THC reduced multifractal complexity (width) during the task (Fig. 4.7D) but had no effect on multifractal complexity during post-task recordings (Fig. 4.7E). Therefore, the effect of THC to reduce multifractality of hippocampal neurons only occurred when memory was impaired during the task. Additionally, multifractal complexity (width) of neurons after THC administration was similar during DNMS task and post-task phases (Fig. 4.7F). The lack of multifractality adjustments between task and post-task recordings after THC administration suggests that THC impairs functional transitions in hippocampal microcircuit activity detected as multifractal complexity.
Figure 4.7. Multifractality (singularity spectrum width) across task and rest conditions after vehicle or THC administration. The scatter plots show each data point obtained by averaging singularity spectrum width for individual neurons over all recordings. All neurons included in the analysis were recorded for 2-8 days of vehicle and THC administration. Thick gray lines are $y = x$ and thin black lines are regression lines. Data points from vehicle only sessions are displayed in A-C. (A) Neurons exhibit greater multifractal complexity (defined by the width or the singularity spectrum) during the DNMS task compared to pre-task recordings. (B) Multifractal complexity of ISIs is greater during the task compared to post-task recordings. (C) On average, multifractal complexity is greater during the pre-task resting state recording compared to the post-task recordings. (D) THC significantly reduces multifractal complexity during the DNMS task. (E) THC has no effect on multifractal complexity during the post-task recordings. (F) Each bar was obtained by averaging multifractality (width $h$) from individual spike trains within specified recording phase and drug treatment combinations ($n = 771-1004$ neurons per group). Errors bars represent S.E.M. Statistical significance is designated by * indicating $p < 0.0083$. 
4.4.4. Standard variability measures are marginally task-specific

Multifractal analysis quantifies the structure of variability (Ihlen, 2012); therefore, to establish the differences between structure and amount of variability, we compared multifractal indices to standard variability measures of the same hippocampal multi-neuron data. To determine if the amount of variability of hippocampal spike trains can account for distinctions between recording phases and drug conditions, the coefficient of variation (CV) was computed for all hippocampal ISI sequences by dividing ISI standard deviation by mean ISI. A CV greater than 1 is an indicator of neuronal bursting and a CV equal to 1 indicates a poisson process. Repeated measures ANOVAs were performed for all three variables: CV, ISI STD and mean ISI. For CV, there was no significant main effect of THC administration ($F_{(1,196)} = 0.68, p = 0.4096$), nor was there a significant interaction between drug condition and recording phase ($F_{(2,369)} = 1.61, p = 0.2008$). A significant main effect of recording phase was found ($F_{(2,379)} = 3.76, p = 0.0241$) and posthoc assessment showed that CVs were higher during the pre-task recording phase compared to the task phase (Fig. 4.8A). For ISI standard deviation, neither main effect was significant (drug condition: $F_{(1,196)} = 2.72, p = 0.1004$; recording phase: $F_{(2,379)} = 0.1, p = 0.9017$), but the interaction between drug condition and recording phase was significant ($F_{(2,369)} = 3.72, p = 0.0252$). Posthoc assessment of ISI STD revealed only two significant differences: amount of variability is greater during the task compared to either before or after in the vehicle condition (Fig. 4.8B). For mean ISI, neither main effect was significant (drug condition: $F_{(1,196)} = 3.84, p = 0.0514$; recording phase: $F_{(2,379)} = 1.82, p = 0.1633$), but there was a significant interaction ($F_{(2,369)} = 7.2, p = 0.0009$). Posthoc assessment for mean ISI revealed three significant differences: neurons fire slower after THC compared to vehicle during post-task recordings, and neurons fire slower during the task compared to pre- and post-task after vehicle administration (Fig. 4.8C). Both the Hurst exponent and multifractal complexity (width)
appear to be better indicators of hippocampal microcircuit processing than these standard variability measures since they were able to distinguish task phase and drug condition combinations.

![Graphs showing frequency spectra and spike train variability measures.](image)

**Figure 4.8. Distinction between recording phases and drug conditions using frequency spectra and spike train variability measures.** Each bar was obtained by averaging values from individual spike trains within specified recording phase and drug treatment combinations (n = 771-1004 neurons per group). Vehicle (blue) or THC (green) was given after pre-task recording, so measures obtained during the pre-task recording should be the same for drug conditions. Statistical significance is designated by * indicating p < 0.0083 (Bonferroni correction). Errors bars represent S.E.M. (A) Repeated measures ANOVA using coefficient of variation (CV) as the dependent variable yielded a significant main effect of recording phase but no significant interaction between drug condition and recording phase. CV was greater during pre-task recordings compared to recordings during the DNMS task. (B) A significant interaction between recording phase and drug condition revealed that ISI standard deviation is greater during the task compared to pre- and post-task recordings. (C) A significant interaction between recording phase and drug condition revealed that ISIs recorded under vehicle administration were larger during the task vs. pre- and post-task conditions. THC increased mean ISI only during the post-task recording but had no effect during the task. (D) A significant main effect of recording phase was found when assessing delta power: Delta power was larger during task and post-task vs. pre-task sessions. (E) Theta power of hippocampal neurons was higher during the task-independent (pre- and post-task) resting phases compared to during DNMS task performance.
4.4.5. Frequency content varies in a task-specific manner

Independent delta and theta rhythms were found in the human medial temporal lobe and are hypothesized to perform separate cognitive functions (Mormann et al., 2008). Delta power is a classical marker used to assess physiological/sleep state (Harmony et al., 1996; Pereda et al., 1999; Clemens et al., 2013) and is negatively correlated with human working memory performance (Axmacher et al., 2010). Additionally, theta frequency activity is known to coordinate neuronal interactions in working memory networks (Jones and Wilson, 2005), has been correlated with performance during the DNMS task (Hyman et al., 2010, 2011) and is reduced by cannabinoid administration (Ilan et al., 2004; Robbe et al., 2006; Böcker et al., 2010; Kucewicz et al., 2011). Therefore we assessed the performance of these frequency components in distinguishing between recording phases and drug conditions. Delta and theta power in all hippocampal neurons were assessed by calculating the total power in the delta (0.5-4 Hz) and theta bands (4-8 Hz) using a fast Fourier transform (Fig. 4.4B & 4.4D). These two activity bands were chosen because of their prominence in hippocampal spike train recordings. Two repeated measures ANOVAs were performed using delta and theta power as the dependent variable to determine if alterations in frequency content can account for the same differences detected with multifractal analysis.

When using delta power as the dependent variable, a significant main effect of different recording phases was found ($F_{(2,379)} = 5.23, p = 0.0057$). Posthoc analysis of the recording phase effect showed that delta power is greater during task and post-task compared to pre-task (Fig. 4.8D). There was no significant main effect of drug condition ($F_{(1,196)} = 2.19, p = 0.141$) or interaction between drug condition and recording phase ($F_{(2,369)} = 2.52, p = 0.082$).
Theta power was assessed in the same manner. The overall effect of drug condition was not significant ($F_{(1,196)} = 3.1, p = 0.0796$). However, there was a significant main effect of recording phase ($F_{(2,379)} = 41.91, p < 0.0001$) and a significant drug condition by recording phase interaction ($F_{(2,369)} = 13.28, p < 0.0001$). Posthoc analysis of the interaction revealed that theta power was significantly higher during both the pre-task and post-task recording sessions compared to the task (Fig. 4.8E).

4.4.6. Correlation Analysis

The aforementioned results suggest a relationship between spike train temporal coding properties quantified by fractality (Hurst exponent/LRTCs/self-similarity), multifractality (singularity spectrum width), variability measures (mean ISI, ISI STD, CV), and frequency content variables (delta and theta). Correlation analyses were performed in order to improve theoretical understandings of multifractal variables and provide insight for developing computational models of the nervous system that reproduce LRTCs and multifractal complexity of neuronal spike trains. Spearman’s rho values were computed for all recording phases and drug condition combinations separately. Correlations between the Hurst exponent and singularity spectrum width yielded small positive rho values ranging from 0.10-0.28 (data not shown). This indicates that the Hurst exponent and multifractal width quantify different spike train properties with respect to hippocampal microcircuit processing. Fifty additional correlation analyses were performed to determine relationships of LRTCs (the Hurst exponent) and magnitude of multifractality (width) with CV, ISI STD, mean ISI, delta and theta power for each recording phase and drug condition. Spearman’s rho values greater than |0.5| were considered to indicate “strong” correlations. Pre-task THC values were not reported because they were similar to pre-task vehicle values in all cases.

We tested whether LRTCs were associated with changes in variability or frequency content using correlation. A strong relationship was found between the Hurst
exponent and mean ISI during task recordings (Fig. 4.9A), indicating that LRTCs are more prominent with shorter average ISIs (i.e., when neurons fire more frequently). The Hurst exponent was negatively correlated with ISI STD during the task (Fig. 4.9A), indicating that LRTCs and self-similarity occur more frequently when neurons fire with less variable ISIs. Both of these correlations were weaker during the resting phase recordings (pre-task and post-task), suggesting a task-specific relationship of fractal vs. firing rate and variability patterns. None of the other three relationships were strong, which indicate that LRTCs and self-similarity quantified by the Hurst exponent are independent from CV, theta and delta power.

To develop a deeper conceptual understanding of multifractal complexity, the singularity spectrum width was correlated with standard variability measures and frequency content. Multifractal complexity (width) was strongly correlated with relative variability, expressed as the coefficient of variation (CV; Fig. 4.9B), during all recording phases and drug conditions. Despite this strong correlation, multifractal complexity was able to distinguish between recording phases and drug conditions better than CV (Fig. 4.7F vs. Fig.4.8B). This indicates that multifractal complexity is a more sensitive measure for detecting memory-specific alterations in hippocampal microcircuit processing. A strong positive correlation was found between multifractal complexity and delta power during the task that decreased during the resting state recordings (Fig. 4.9B), suggesting that low frequency activity may support multifractal complexity during working memory. Multifractal complexity strongly correlated with “Poison-ness” (CV) and delta power but not with mean ISI, ISI STD or theta power. Taken together, these results suggest that fractality, multifractality and theta frequency activity quantify different properties of spike train temporal coding structure.
Figure 4.9. Correlations of fractality (Hurst exponent) and multifractality (singularity spectrum width) with spike train variability and frequency spectrum measures. Correlations (Spearman’s rho) are plotted with 95% confidence intervals as error bars. (A) The Hurst exponent is negatively correlated with mean ISI and ISI STD during task recordings independent of drug condition – indicating that faster spiking and less variable firing patterns are correlated with greater LRTCs during the task only. (B) The coefficient of variation (CV) was positively correlated with multifractal complexity (width) - indicating “burstiness” correlates with multifractality. Delta power was positively correlated with multifractality (width) during the task phase, regardless of drug condition.
4.5. Discussion

Multifractal analysis revealed something never shown before: the nature of the temporal structure in hippocampal spike trains is altered by performing a working memory task. Monofractality (Hurst exponent) decreased but multifractality (width) increased from pre-task to task and these trends reversed from task to post-task. Prior indications that the neuronal encoding during the task is essential for successful performance (Hampson et al., 2012) support the hypothesis that spike trains represent information with multifractal temporal coding properties (Fetterhoff et al., 2015). Previous results showed that microcircuit activity patterns occurring when encoding information during the sample phase are correlated with correct performance (Berger et al., 2011; Opris et al., 2012) and support the notion that memory functions through repetitive neuronal ensemble codes (Deadwyler and Hampson, 1997; Berger et al., 2012). Therefore, multifractal, self-similar spiking patterns detected during this study might constitute a substrate for memory information transmission.

The main goal of this study was to elucidate the multifractal properties of active hippocampal microcircuit processing by assessing differences between resting state and working memory conditions. The presented results show that multifractal complexity (indicated by singularity spectrum width) permits distinction between all recording phases and support the use of multifractal analysis in extracting variables related to cognitive state better than other commonly applied methods. Our first hypothesis was verified by the finding that long range temporal correlations (LRTCs), indicated by the Hurst exponent, were decreased during task performance compared to the resting state (Fig. 4.6A-C & F). We verified our second hypothesis that multifractal complexity (width) of hippocampal ISI sequences is greater during active memory processing (task recording phase) compared to the resting state (pre-task and post-task; Fig. 4.7A-C & F). Unexpectedly, we found that pre-task was more multifractal than post-task (Fig. 4.7A-C & F).
4.7C). We believe this could be due to a “priming” effect because the rats are more motivated for water and their hippocampal ensembles are preparing to perform the DNMS task by some form of mental rehearsal. Due to the rats’ experience with the habitual daily testing procedure, they may learn to associate pre-task recording conditions with subsequent task recordings when reward is available. The rats are also more satiated during the post-task recording after receiving water during the task and this may reduce the hippocampal inputs responsible for generating multifractality during the task. This finding highlights the advantage of using multifractal analysis for detecting even subtle differences in cognitive states. These results are consistent with other findings showing that task performance elicits multifractal fluctuations restricted to task-related regions while LRTCs decrease in all analyzed brain regions, independent of task involvement (Ciuciu et al., 2012). The structure of variability, detected as multifractal complexity, becomes more intricate when hippocampal microcircuits exhibit a wider range of dynamics required for memory processing (during the task) and this alteration may elicit breakdowns of LRTCs (Fig. 4.6F & 4.7F). These results suggest that enhancement of the theoretical and conceptual foundations of neuroscience is possible by applying multifractal analysis in order to achieve the larger goal of producing a dynamic portrait of the functioning brain.

THC impairs hippocampal information transmission by disrupting the multifractal spike train patterns that may constitute a basis of memory processing. THC administration reduced multifractal complexity during the DNMS task and decreased LRTCs during post-task recording phase (Figs. 4.6F & 4.7F). Under control conditions, multifractal complexity (width) and LRTCs became lower and higher, respectively, when rats transitioned from the task to the post-task, but THC administration inhibited these changes. Therefore, it is possible that THC effectively prevented endogenous microcircuit dynamics that facilitate neurophysiological adjustments from memory.
processing to a resting state. These findings are consistent with others demonstrating that THC promotes default mode network activity at inappropriate times (Bossong et al., 2013), and they support the application of multifractal complexity as a marker of network involvement if the reduction of multifractal complexity is due to decreased memory-related interactions that occur when DNMS performance is impaired by THC.

Computational models attempting to reproduce the temporal coding properties of neuronal spike trains (Goris et al., 2014) can likely be improved by replicating endogenously occurring LRTCs (Fürstenau, 2010) and multifractal complexity. Interpretation of the structure of variability assessed using multifractal analysis was enhanced by comparing performance and relationships between monofractal and multifractal variables (Hurst and width) and standard measures of variability. The correlation analysis showed that the Hurst exponent is negatively correlated with mean ISI and ISI STD during the DNMS task under both vehicle and THC conditions (Fig. 4.9A). This task-specific correlation demonstrates that the increase of LRTCs during resting phase (pre/post) recordings (Fig. 4.6F) is not due to independent changes in mean ISI and ISI STD but may be related to alterations in their ratio when expressed as CV (Fig. 4.9A). The strongest relationship was found between multifractal complexity (width) and the coefficient of variation (Fig. 4.9B), and this finding would suggest that multifractal complexity reflects information directly related to how ISI variability scales with ISI mean. However, the superiority of singularity spectrum width in distinguishing between examined cognitive states is clear in the population ANOVA results: multifractality (width) confirmed four out of six ad hoc distinctions from the interaction between drug condition and recording phase while CV failed to yield a statistically significant interaction and could only distinguish between the pre-task and task recording phases (Fig. 4.7F vs. 4.8A).
Examination of brain oscillations via frequency content is a commonly used method to study temporal information processing in the nervous system (Lisman, 2005; Axmacher et al., 2006; Dragoi and Buzsaki, 2006). Therefore, it is important to understand how these methods compare to new ways of quantifying and describing temporal coding properties, such as multifractal analysis. It was found that the combination of delta and theta power quantification distinguished between recording phases to a lesser extent than multifractal complexity (width) and LRTCs (Hurst). We found that theta power determination permitted distinction between two out of six ad hoc differences (Fig. 4.8E), and interestingly, both were identical to changes detected by the Hurst exponent (Fig. 4.6F). Further inspection using correlation analyses promoted discovery that the Hurst exponent was not correlated with oscillatory activity in the tested frequency bands and therefore suggests that they are independent dynamical processes. Theta and delta power were negatively and positively, respectively, correlated with multifractal complexity (width). This supports the finding that both delta and theta rhythms exist independently in the human hippocampus (Mormann et al., 2008) and suggests that temporal coding properties detected as multifractality may preferentially arise from the delta frequency activity. It is possible that the precise action potential timing in the delta frequency range, from 1 second to 250 ms, conveys essential information utilized to support cognitive function and reflected as multifractality. It is shown here that multifractal analysis can be used to detect changes in hippocampal processing better than standard Fourier spectrum analyses. However, it will be necessary to compare both methods in order to integrate multifractal concepts with prior findings describing frequency content and to ultimately synthesize new hypotheses about how the brain functions.

Learning and memory require information to be carried from the past into the future, and multifractal complexity of hippocampal neurons may fluctuate depending on
how strongly information is received, processed and sent by these neurons. To put our results into a more general perspective, hypothetical singularity spectra were constructed to match our qualitative findings with respect to memory processing (Fig. 4.10). We hypothesize that physiological states are characterized by specific monofractal and multifractal features. All different states must fall within a range of possible dynamics (Fig. 4.10, gray spectrum) and thus a range of possible multifractality. Active memory processing (Fig. 4.10, blue) recruits this system to a stronger degree than resting (Fig. 4.10, orange) and therefore neurons recorded during the task exhibit stronger multifractality. This multifractality may facilitate memory processing by offering a larger range of spike train variability and greater processing capacity. When THC or other memory impairing agents are administered during the task (Fig. 4.10, green), the normal level of multifractal complexity exhibited is reduced and memory performance suffers. Alterations in multifractal complexity may reflect the degree of presently embedded information and therefore would provide information relevant for detection of physiological state.
Figure 4.10. Summary of how multifractal complexity relates to memory processing. Hypothetical singularity spectra were constructed based on the qualitative results of the study. The gray spectrum represents the maximal amount of multifractal complexity possible in a system (i.e., hippocampal spike trains in this example). Active memory processing recruits a large portion of this potential, but additional resources are still available to support more cognitively demanding instances. THC impaired working memory and reduced multifractal complexity. During resting conditions, the singularity spectrum shrinks compared to the task (blue) condition. Multifractality may be highest when embedded information relevant for working memory is being processed. Consequently, resting and pharmacological impairment could reduce multifractal complexity by decreasing the fraction of utilized resources.
The application of multifractal analysis revealed that repeating spatiotemporal activity patterns detected in hippocampal spike trains may form a previously undiscovered contribution to the temporal coding of memory information. This demonstrated how analysis of the multifractal structure of temporal dynamics can enable new insights about how the brain functions and can facilitate methodological improvements for detection of alterations in cognitive, physiological and pathological states (Suckling et al., 2008; Wink et al., 2008). Multifractal analysis is being utilized in pathology diagnosis and has already been successfully applied to Parkinson’s Disease (Zheng et al., 2005), seizure detection (Dutta et al., 2014), Alzheimer’s Disease (Vysata et al., 2013), and many others (Slezin et al., 2007; Di Ieva et al., 2015). As for physiological state, multifractal analysis was proposed as a method for automatic detection of sleep stages (Weiss et al., 2009; Zorick and Mandelkern, 2013), and an fMRI study showed changes in multifractality selectively occur in task-related brain regions (Ciuciu et al., 2012). The presented assessment of cognitive state can be integrated with these results concerning physiological state: deep sleep contains more LRTCs and less multifractality compared to REM sleep (Weiss et al., 2009), REM sleep contains larger LRTCs than waking (Zorick and Mandelkern, 2013), and as shown here, hippocampal neurons during a task-independent (resting) state exhibit greater LRTCs but less multifractality compared to when recorded during the working memory DNMS task. It is therefore possible that consciousness, described in terms of cognitive/physiological state, occurs on a spectrum that can be quantified and understood via computation of the multifractal singularity spectrum. Multifractal analysis quantifies the scale invariant, self-similar structure that is pervasive throughout biological processes and application of this method to neurophysiological data will improve our understanding of how the nervous system processes cognitive information.
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4.6. References


4.7. Addendum: Supplementary Figures

Figure 4.S1. Waveform Identification across recording sessions. All waveform files (.plx) were concatenated into one large file, and waveform sorting was performed only on this merged file. After sorting, the file was separated into appropriate files based on digital timestamps acquired during recording. Waveforms, PCA space and ISI histograms are shown for unsorted noise (gray) and two identified neurons (yellow and green) from one wire located in CA1. The green neuron was used as the example in figure 2. Images were taken directly from Plexon Offline Sorter. (A) Waveforms (left), PCA space (upper right), and ISI histograms (bottom right) from the merged file are displayed. Sorting a merged file ensures that similar waveform shaped are isolated on every session. (B) Waveforms, PCA space and ISI histograms are shown for the vehicle session. (C) Waveforms, PCA space and ISI histograms are shown for the THC session.
Figure 4.S2. Monofractal and Multifractal changes in Hippocampal CA3 and CA1 regions. (A) and (C) are reproduced from figures 6F and 7F for comparison purposes. Errors bars represent S.E.M. Statistical significance is designated by * indicating p < 0.0083. (A) Each bar was obtained by averaging Hurst exponent values from individual spike trains within specified recording phase and drug treatment combinations (n = 771-1004 neurons per group). (B) Three differences were found between CA3 and CA1 for the Hurst exponent, signified by asterisks. CA3 neurons exhibited stronger monofractality during vehicle resting states (pre and post) compared to CA1. Conversely, the Hurst exponent of CA1 neurons was larger during the task. Besides that, the same 3 differences were found within each region as the population data shown in (A), but within region significance is not denoted to preserve clarity. Each bar was obtained by averaging Hurst exponent values from individual spike trains within specified recording phase and drug treatment combinations (n = 592-443 CA1 neurons and 412-315 CA3 neurons per group). (C) Each bar was obtained by averaging multifractality (width $h$) from individual spike trains within specified recording phase and drug treatment combinations (n = 771-1004 neurons per group). (D) Two between-region differences were detected and are marked by asterisks: CA3 neurons recorded during the task are less multifractal than CA1 neurons recorded during the task under both drug conditions. The within-region differences are signified on the graph using lines between groups. CA3 neurons had greater multifractality during task control recordings compared to task THC but did not present the other three population differences shown in (C). CA1 neurons had 3 of 4 detected population differences (pre-vehicle vs. task-vehicle, task-vehicle vs. post-vehicle, and task-vehicle vs. post-vehicle). Pre-control vs. post-control was not significant within CA1. Each bar was obtained by averaging multifractality values from individual spike trains within specified recording phase and drug treatment combinations (n = 592-443 CA1 neurons and 412-315 CA3 neurons per group).
CHAPTER 5: ALTERATIONS OF LONG RANGE TEMPORAL CORRELATIONS AND MULTIFRACTAL COMPLEXITY INDUCED BY REVERSAL LEARNING

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5.1. Abstract

Cognitive flexibility was assessed using a reversal learning paradigm that required rats to initially learn a delayed match-to-sample task before the rule was switched to nonmatch. Reversal learning requires extinction of an originally learned rule before learning of a second (opposite) association occurs. Behavioral and neuronal activity occurring at specific task events and time intervals between events were found to correlate with changes in the fractal structure of spike trains. Multifractal analysis was used to assess long range temporal correlations and the structure of variability of hippocampal spike trains. Our results indicated that changes in both monofractal and multifractal complexity followed alterations in behavioral latency throughout reversal learning and suggest that task complexity is reflected as spike train complexity. Determination of local variability, by calculation of local Hölder exponents, allowed long-range temporal correlations to be linked to working memory maintenance during persistent delay activity and to microcircuit alterations associated with the extinction component of reversal learning. Extinction was found to initially elicit strong increases in both monofractal and multifractal complexity that subsided throughout second rule learning and successful post-reversal performance. Multifractal analysis also provided evidence that long-range temporal correlations are preferentially detected within CA1 spike trains while the recurrent connections within CA3 favorably exhibited multifractal complexity. Interactions between multiple timescales of memory, i.e. long-term vs. short-term, required for successful reversal learning are likely reflected as changes in the monofractal and multifractal structure of the hippocampal spike trains.
5.2. Introduction

Learning and memory research aims to understand how information is represented and transformed in neural firing patterns during encoding, retention, and retrieval stages of working memory. A reversal learning paradigm was implemented to inspect how cognitive flexibility and task complexity modulate interactions between long-term and short-term memory within the hippocampal CA3 and CA1 neuronal ensemble. Reversal learning examines cognitive flexibility because it requires an originally learned association to undergo extinction before second-rule learning can occur (Egerton et al. 2005). Rats were originally trained to perform a Delayed Match-to-Sample (DMS) task up to 1-30 second delays (Hampson et al. 1993; Heyser et al. 1993) prior to reversal to Delayed Nonmatch-to-Sample (DNMS) because extended delay training was previously shown to increase the number of task correlated neurons (Goonawardena et al. 2010) and alter the input-output dynamics of hippocampal subregions (Chan et al. 2010). By more strongly activating the hippocampal microcircuitry, we aimed to identify neural correlates of memory state adaptation from stable to perturbed before return to a newly learned, stable state.

Operant conditioning involves formation of a hierarchical associative structure (Rescorla, 1992) when stimuli presented during the sample phase elicit responses correlated to rewarded and non-rewarded outcomes. Reversal learning induces alterations of this structure (Colwill and Rescorla, 1990; Rescorla, 1992). Three phases of reversal learning were defined in order to dissect specific components of mental flexibility. The pre-reversal DMS task, phase A, was broken down into extended delay (i.e., 1-30s) sessions (phase A1) and two 1 sec. delay sessions immediately preceding rule reversal (phase A2). The transition stage, phase B, began when the nonmatch rule was implemented. Previous studies showed that extinction, the first component of
reversal learning (Chan et al. 2001), begins within one behavioral session (Ragozzino and Choi, 2004; Pamplona et al. 2006) and therefore the first session was defined as phase B1. Extinction is thought to occur when an inhibitory association overrides a previously learned response (Chan et al. 2001) after which second rule learning begins. Therefore we defined phase B2 as all sessions after the B1 (extinction) session, and continuing until rats achieved at least 75% correct responses. The post-reversal (phase C) phase occurred once the new rule was learned and performance stabilized, indicating that reversal learning was complete. Reversal learning induced alterations in the hierarchical associative structure (Colwill and Rescorla, 1990; Rescorla, 1992), neurophysiologically defined by selective responses of “functional cell types” during sample (encoding) and choice (retrieval) phases of the DNMS/DMS task (Hampson et al. 1999; Goonawardena et al. 2008; Fetterhoff et al. 2015) were quantified using multifractal analysis (Wendt and Abry, 2007; Ihlen, 2012; Kantelhardt, 2012).

Fractal analysis quantifies irregularities in objects and time series by computing an underlying correlative structure across multiple temporal and spatial scales. The spatial scales of the nervous system begin at the level of the brain and decrease in size from brain-wide networks (Linkenkaer-Hansen et al. 2001) to microcircuits to single neurons (Teich et al. 1990; Biella et al. 1999; Das et al. 2003; Gebber et al. 2006; Fetterhoff et al. 2015) and finally to ion channels (Lowen et al. 1997, 1999; Takeda et al. 1999). In the temporal scale, interspike intervals (ISI), i.e. the intervals between successive action potentials within single neuron spike trains, are commonly analyzed for patterns related to information content related to neural information processing (Hampson and Deadwyler, 1999; Hampson et al. 2001, 2005). When information from these spatiotemporal scales is coordinated for memory processing, the interactions can generate multiple, simultaneously occurring fractal patterns that are best described using
Multifractal analysis (Wendt et al. 2007; Ihlen and Vereijken, 2010). Wavelet Leaders-based Multifractal Analysis (WLMA) was used to estimate the monofractal and multifractal (henceforth referred to as mono/multifractal) structure of ISI sequences recorded from hippocampal CA3 and CA1 neurons throughout reversal learning. Important features of the singularity spectrum, the main output of multifractal analysis, can be summarized using log-cumulants, \( c_1 \) and \( c_2 \). The monofractal log-cumulant \( c_1 \) quantifies long-range temporal correlations (LRTCs) and describes past activity influences future dynamics (Wendt et al. 2007). This log-cumulant is qualitatively the same as the more commonly used global Hurst exponent \( H \) (Hurst, 1951; Ciuciu et al. 2012; Ihlen, 2012). The second log-cumulant \( c_2 \) quantifies how the correlative structure varies through time and provides a measure of multifractal complexity (Wendt et al. 2007). Results obtained using this global method (WLMA) can be visualized and directly linked to specific spike train components and task events by quantifying local variability with local Hölder exponents of the ISI time series (Ihlen, 2012; Trujillo et al. 2012). This method allows multifractality and the singularity spectrum to be understood in terms of the distribution of variability within a time series.

The primary goal of this approach was to understand how the cognitive flexibility required for reversal learning was reflected as mono/multifractal complexity of hippocampal spike trains. Previous work using the DNMS task (Hampson and Deadwyler, 1996; Deadwyler and Hampson, 2008) revealed that behavioral strategies appear during periods of inactivity between trials (from one choice response to the next sample) and within trials (during delay periods and from delay termination to the choice response). These strategies bias responding of animals to increase their potential for reward and suggest that behavioral adaption relevant for reversal learning may occur between these task events. Behavioral latencies compared across reversal phases
demonstrate that temporal aspects of the DMS/DNMS tasks may be reflected in mono/multifractal dynamics. LRTCs and multifractal complexity increase during rule induction (Anastas et al. 2011; Stephen et al. 2012) and the largest changes of hippocampal cell firing occurred when reward locations were switched (Eschenko and Mizumori, 2007). Therefore, we hypothesized that stronger LRTCs and greater multifractality would occur during the extinction session (phase B1) compared to before reversal (phase A2) and after successful reversal learning (phase C). We further predicted that stable DMS performance (phase A2) would be similar to stable, post-reversal DNMS performance (phase C). Finally, we hypothesized that pre-reversal sessions with extended delays (phase A1) would exhibited strong LRTCs and multifractal complexity compared to pre-reversal 1 second delay sessions (phase A2).

Our results indicate that the associative, recurrent networks of CA3 (Ishizuka et al. 1990; Le Duigou et al. 2014) may trigger population activity during learning via alterations in multifractal complexity while the primarily feedforward fibers of CA1 (Cenquizca and Swanson, 2007) may preferentially exhibit learning via monofractal LRTCs. Estimates of the local variability (local Hölder exponents) illustrate how cognitive flexibility and task complexity are reflected as LRTCs and multifractal complexity. Thus, by presenting results obtained from global and local variants of multifractal analysis, a novel feature of learning and memory processing is described.

5.3. Methods

5.3.1. Rats

Male Long-Evans rats (Harlan) aged 5-10 months (n=7) were tested under protocols approved by the Wake Forest University Institutional Animal Care and Use Committee, and in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care and the National Institute of Health Guide for the Care and Use
of Laboratory Animals (NIH Publication No. 8023). All animals were individually housed and allowed free access to food with water regulation to maintain 85% of ad libitum body weight during testing.

5.3.2.1. Apparatus.

The behavioral testing apparatus for the delayed match/nonmatch-to-sample (DMS/DNMS) task as used in other studies (Hampson and Deadwyler, 2000; Hampson et al 2012) consisted of a 43 × 43 × 50 cm Plexiglas chamber with two retractable levers (left and right) positioned on either side of a water trough on the front panel. A nosepoke device (photocell) was mounted in the center of the wall opposite the levers with a cue light positioned immediately above the nosepoke device. A video camera was mounted on the ceiling and the entire chamber was housed inside a commercially built sound-attenuated cubicle.

5.3.2.2. Delayed match-to-sample (DMS) and delayed nonmatch-to-sample (DNMS) tasks.

The DMS/DNMS task consisted of three main phases: Sample, Delay and Choice (Fig. 5.1). The Sample phase initiated the trial via presentation (Sample Presentation, SP) of either the left or right lever (50% probability), which required the animal to press and make the Sample Response (SR). The lever was then retracted and the Delay phase of the task initiated, as signaled by the illumination of a cue light over a nosepoke photocell device on the wall opposite to where the lever was presented. At least one nosepoke (NP) was required following the interposed delay interval which varied randomly in duration (1-30 s) on each trial during the session. The Choice phase began when the delay timed out and the rat performed the Last Nosepoke (LNP) which caused the photocell cue light to turn off and both the left and right levers on the front panel to be extended. For the DMS task, correct responses consisted of pressing the
lever in the Choice phase located in the same spatial position as was the SR. For the DNMS task, correct responses occurred when rats pressed the lever in the opposite position from the SR. Correct Choice Responses (CR) produced delivery of a 0.4 ml water reward in a reservoir between the two levers. After the CR the levers were retracted for a 10.0 second intertrial interval (ITI) before the Sample lever for the next trial was presented. A lever press at the same position as the SR (incorrect choice response) constituted an “error” with no water delivery and turned off chamber house lights for 5.0s with the next trial presented 5.0 s later. Individual performance was assessed as % CRs (choice responses) with respect to the total number of trials (100) per daily (30-60 min) session.

5.3.2.3. Reversal learning paradigm.

All rats (N = 7 rats) were initially trained to perform the DMS task at 1-30s delays for five consecutive days prior to hippocampal electrode implant surgery. After surgical recovery, rats were allowed to return to pre-surgery performance levels while electrophysiological recordings were conducted with 1-30s delays (reversal phase A1). After achieving > 75% overall correct responses for at least 5 days of DMS 1-30s, the delay was reduced to 1 s for two days (reversal phase A2). On the following day, the contingency changed to DNMS with the same 1 s delays (reversal phase B1). The rats performed poorly for the next five days, achieving less than 75% correct overall (reversal phase B2). When at least 3 consecutive sessions of DNMS occurred with above 75% correct it was considered the final phase of the reversal learning paradigm (reversal phase C).
Figure 5.1. Reversal learning paradigm. Rats were trained to perform the delayed match-to-sample (DMS) task at 1-30 second delays before electrode implantation. After recovery and reaching stable performance, the rule was reversed to nonmatch. The colored dots correspond to specific task events: Sample Response (SR) blue, Last Nosepoke (LNP) green, Choice Response (CR) red, and to designate critical task time intervals: Sample Presentation (SP)-SR: pink, SR-LNP (delay): orange, and LNP-CR: cyan. The same markers are used to signify task-related activity in figures 5, 7 and 8.

5.3.4.1. Hippocampal Recording Array Surgery.

All surgical procedures conformed to National Institutes of Health and Association for Assessment and Accreditation of Laboratory Animal Care guidelines, and were performed in a rodent surgical facility approved by the Wake Forest University Institutional Animal Care and Use Committee. Electrode arrays and recordings were the same as described in several prior publications from this laboratory (Hampson et al 1999, 2012; Hampson and Deadwyler, 2000). After being trained to criterion performance level in the DNMS task animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic frame. Craniotomies (5mm-
diameter) were performed bilaterally over the dorsal hippocampus to provide for implantation of 2 identical array electrodes (Neurolinc, New York, NY), each consisting of two rows of 8 stainless steel wires (diameter: 20 μm) positioned such that the geometric center of each electrode array was centered at co-ordinates 3.4 mm posterior to Bregma and 3.0 mm lateral (right or left) to midline (Paxinos and Watson, 1997). The array was designed such that the distance between two adjacent electrodes within a row was 200 μm and between rows was 400 μm to conform to the locations of the respective CA3 and CA1 cell layers. The longitudinal axis of the array of electrodes was angled 30° to the midline during implantation to conform to the orientation of the longitudinal axis of the hippocampus, with posterior electrode sites more lateral than anterior sites. The electrode array was lowered in 25-100 μm steps to a depth of 3.0 - 4.0 mm from the cortical surface for the longer electrodes positioned in the CA3 cell layer, leaving the shorter CA1 electrodes 1.2 mm higher with tips in the CA1 layer. After placement of the array the cranium was sealed with bone wax and dental cement and the animals treated with Ketoprofen (3.0-5.0 mg/kg) for pain relief over the next 4-6 hrs. The scalp wound was treated periodically with Neosporin antibiotic and systemic injection of penicillin G (300,000 U, intramuscular) were given to prevent infection. Animals were allowed to recover from surgery for at least 1 week before continuing behavioral testing (Berger et al 2011; Hampson et al 2012).

5.3.4.2. Processing of hippocampal neuronal firing.

Animals were connected by cable to the recording apparatus via a 32-channel headstage and harness attached to a 40-channel slip-ring commutator (Crist Instruments, Hagerstown, MD) to allow free movement in the behavioral testing chamber. Single neuron action potentials (spikes) were isolated by time-amplitude window discrimination and computer-identified individual waveform characteristics using
a multi-neuron acquisition (MAP) processor (Plexon Inc., Dallas, TX, USA). Single neuron spikes were recorded daily and identified using waveform and firing characteristics within the task (perievent histograms) for each of the DMS/DNMS events (SR, LNP & CR). To maintain waveform shape across days, all recorded data was concatenated into one file (separately for each rat) and offline sorting was performed using principal component analysis, peak-valley, and nonlinear energy algorithms in Offline Sorter (Plexon Inc., Dallas, TX, USA). Only single neural spike waveforms exhibiting firing rates of CA1 and CA3 principal cells (i.e. 0.5-8.0 Hz average firing rate) and consistent multifractal properties across sessions were included in analyses described here. Previous work has shown that hippocampal neurons identified in this manner exhibit consistent DNMS task modulated firing rate over multiple sessions (Deadwyler et al 1996; Hampson et al 1999, 2003).

5.3.5.1. The Local Hölder Exponent

Multifractal analysis may either be performed from a global perspective by measuring how the variability of the signal changes across multiple scales (i.e., WLMA) or from a local perspective by measuring the variability of the signal at each point in time (Struzik, 2000). When calculated from a global perspective, multifractal analysis can be performed in the time domain, as in Multifractal Detrended Fluctuation Analysis (Kantelhardt et al 2002; Ihlen, 2012), or in the wavelet domain, as in Wavelet Transform Modulus Maximus (Arneodo et al 1993; Oświecimka et al 2006) and Wavelet Leaders-based Multifractal Analysis (WLMA) (Jaffard et al 2007; Wendt and Abry, 2007). WLMA was used here because it exhibits enhanced statistical performance, especially when signals contain regions of very low variability (Wendt et al 2007). Additionally, multifractal analysis was performed from the local perspective (Ihlen, 2012) to illustrate relationships between mono/multifractality and firing rate alterations around behavioral events.
throughout reversal learning. WLMA and multifractal analysis are described in detail in several prior reports (Jaffard, 2004; Jaffard et al. 2007; Wendt and Abry, 2007; Wendt et al. 2007; Serrano and Figliola, 2009; Ciuciu et al. 2012; Ihlen, 2012; Kantelhardt, 2012). The main properties of local Hölder exponents and WLMA used for calculating the singularity spectrum and log-cumulants are summarized here.

The local variability in the signal $X(t)$ is defined by the local Hölder exponent $h$, which is the largest $h$: 

$$h(t_0) = \sup \{ h : X \in C^h(t_0) \}$$

that satisfies the equation

$$|X(t) - P_{t_0}(t)| \leq C |t - t_0|^h$$  \hspace{1cm} (1)

where $C$ is a positive constant and $P$ is an $n$th degree polynomial with $n$ less than $h$ (Struzik, 2000). To illustrate this definition, fractional Brownian motion (fBm) was generated (Abry and Sellan, 1996) and analyzed using this method of local Hölder exponent $h$ determination. fBm is a monofractal signal whose variability depends solely on the global Hurst exponent $H$. The global Hurst exponent $H$ is the mode of a local Hölder exponent $h$ series (Ihlen, 2012) and represents the most frequently occurring form of variability in the analyzed time series. fBm with $H = 0.5$ is displayed (Fig. 5.2d, black line) with Hölder envelopes (Trujillo et al. 2012) for $H = 0.5$, 0.75 and 1 (Fig. 5.2d, red, blue, green lines) surrounding it. The local $h$ is calculated for every data point $t_0$ by computing this function (equation 1) across scales. The scales involved signify how many adjacent data points $t$ are used (Fig. 5.2b & d). For scale 7, the Hölder envelope (right side of equation 1) is fit to three adjacent data points in each direction (Fig. 5.2d, solid lines) while 8 data points are used for scale = 17 (Fig. 5.2d, dashed lines).
By choosing the Hölder exponent that satisfies equation 1 for every data point, the dynamical variations in Hölder exponent $h$ regularity through time can be quantified with the multifractal singularity spectrum, which plots the fractal dimension, $D(h)$, of the set of points with the same Hölder exponent $h$ (Fig. 5.2e, red). The singularity spectrum may be calculated directly from the log-transformation of the normalized probability distribution function of the local Hölder exponents (Fig. 5.2e, red spectrum). Note that the apex of this singularity spectrum is at $h = 0.5$ and its width is very small compared to the singularity spectra derived from the neuronal spike train (Fig. 5.3e, blue and black spectra). In this way, the singularity spectrum can be conceptualized as a frequency histogram of local $h$ values that summarize the pointwise regularity of an ISI sequence. By quantifying local variability of a signal using the local Hölder exponent (Fig. 5.2b), alterations in neuronal response dynamics (Fig. 5.2c) can be visualized and readily linked to behavioral activity. Matlab code for this analysis is available online (Ihlen, 2012).

5.3.5.2. Wavelet Leaders Multifractal Analysis (WLMA) and Log-Cumulants

As an alternative, the singularity spectrum may also be calculated from a global perspective by estimating the scaling exponents $\zeta(q)$ of a process with self-similar scale invariant structure such that

$$E|X(at)|^q = |a|^{\zeta(q)} E|X(t)|^q$$

where $a$ is the scaling parameter and $q$ is the statistical moment. For monofractal processes, $\zeta(q)$ is constant. For multifractal processes, $\zeta(q)$ is a range of power-law exponents. WLMA computes the scaling exponents by measuring how the absolute
value of the wavelet coefficients $d_X$ changes as a function of scale. A neuronal ISI sequence (Fig. 5.2c) was used to illustrate this method and compare it to the local Hölder exponent determination (Fig. 5.2b, d). The first step in WLMA is to transform the data to the wavelet domain with a Discrete Wavelet Transform (DWT) (Fig. 5.2a).

$$d_X(j,k) = \int_{\mathbb{R}} X(t) 2^{-j/2} \Psi_0(2^{-j} (t - 2^j k)) \, dt$$

where $\Psi_0$ is an appropriately chosen mother wavelet. For this analysis, a Daubechies wavelet with 4 vanishing moments was used. The wavelet coefficients $d_X$ are extracted from the DWT on a dyadic grid at scales equal to $2^j$ and time shifts equal to $2^j k$. The DWT enables extraction of ISI variability in a qualitatively similar way compared to the local $h$ method (Fig. 5.2b). The wavelet leaders $d_L$ are calculated from the wavelet coefficients for every point on this dyadic grid by finding the maximum wavelet coefficient among the adjacent wavelet coefficients for the current scale and all smaller scales.

$$d_L(j,k) = \max\{ d_X(j',k') \}$$

such that

$$(k - 1)2^j \leq 2^j k' < (k + 2)2^j$$

If $n_j$ is the number of wavelet leaders $d_L(j,k)$ available at every scale $2^j$ for the time series $X(t)$, then the structure function $S(j,q)$ can be defined as:
\[ S(j, q) = \frac{1}{n_j} \sum_{k=1}^{n_j} d_L(j, k)^q \]  

(5.1)

where \( k = 1, 2, \ldots, n_j \). This function behaves as a power-law over analysis scale 2 for a set range of scales and for a set range of statistical orders \( q \). Statistical orders \( q \) typically contain both positive and negative numbers in order to estimate multifractality. Positive \( q \)-values amplify periods of large variability while negative \( q \)-values amplify areas of low variability (i.e., short ISIs). Therefore, positive \( q \)-values estimate areas with small (near random) local Hölder exponents \( h \) and negative \( q \)-values estimate areas with higher local \( h \) values (i.e., LRTCs). The scaling exponent \( \zeta(q) \) may be calculated from the structure function \( S(j, q) \) to quantify changes in variability as a function of scale \( j \) and statistical orders \( q \):

\[ S(j, q) = F_q(2^j)^\zeta(q) \]  

(5.2)

where \( F_q \) is a constant independent of \( j \). The singularity spectrum (Fig. 5.2e, blue spectrum) can then be estimated directly from the scaling exponent using a Legendre transform (Wendt and Abry, 2007). The singularity spectrum calculated using WLMA closely resembles its counterpart calculated from the local \( h \) method (Fig. 5.2e, black spectrum) for \( h \) values less than 1.2. When the singularity spectrum is estimated using WLMA, positive \( q \)-values are located on the left side and negative \( q \)-values are located on the right. Accurate estimates with negative \( q \)-values are difficult to obtain because the signal can be influenced by noise. Therefore, WLMA sometimes underestimates very smooth areas containing LRTCs and this could be a reason why the WLMA spectrum ends before its counterpart estimated with the local \( h \) method.
The scaling exponents may also be rewritten as second characteristic functions, a standard function expansion of the natural log of the time averaged wavelet leaders $C_p^j$.

The power law expansion of the first two terms of $C_p^j$ are

$$C_1^j = \mathbb{E} \left[ \ln \left( \hat{d}_j \right) \right] = c_1^0 + c_1 \ln 2^j$$

$$C_2^j = \mathbb{E} \left[ \ln \left( \hat{d}_j \right)^2 \right] - \left( C_1^j \right)^2 = c_2^0 + c_2 \ln 2^j$$

The log-cumulants $c_1$ and $c_2$ are calculated from the slope of $C_p^j$ versus scale and correspond to specific attributes of the multifractal singularity spectrum (Wendt and Abry, 2007; Wendt et al 2007). The singularity spectrum of a one-dimensional time series may be approximated as a polynomial expansion around its maximum using the log-cumulants (Wendt et al 2007; Ciuciu et al 2012).

$$D(h) = 1 + \left( \frac{c_2}{2^1} \right) \frac{h-c_1^2}{c_2} + \left( \frac{-c_3}{3!} \right) \frac{h-c_1^3}{c_2} + \ldots$$

$c_1$ is a self-similarity parameter, which measures long-range temporal correlations (LRTCs) and it takes values very closely related to the global Hurst exponent $H$ (Wendt et al 2007). $c_2$ is a function of the width of the multifractal spectrum and acts as a test of mono- vs. multifractal. Monofractal signals are self-affine with a narrow range of Hölder exponents uniformly distributed throughout the signal. Multifractal signals deviate from pure self-affinity by expressing a wide range of nonuniformly dispersed variability throughout distinct regions. $c_2$ is always negative due to the inverted parabolic shape of the singularity spectrum. As $c_2$ becomes more negative, it is indicates that the signal is more multifractal. We mainly focus on $c_1$ and $c_2$ because they describe the main features of the singularity spectrum, location and width, and are the most robust to calculate. Note that the singularity spectrum for the ISI sequence (Fig. 5.2e, blue and
black) contains stronger LRTCs, indicated by a rightward shift in its apex and thus larger $c_1$ value, and greater multifractal complexity, indicated by a wider spectrum and thus larger $|c_2|$ value, compared to the monofractal fBm (Fig. 5.2e, red).

The Wavelet Leaders code was obtained from Wendt’s freely available, online MATLAB toolbox, the WLMA Toolbox (Wendt et al 2007; http://www.irit.fr/~Herwig.Wendt/software.html). For all of our analyses, $j$ ranged from $2^4$ to $2^8$ in integer increments, and statistical orders $q$ ranged from 5 to -5 in 0.5 increments (excluding zero). Analyses were performed using MATLAB version R2013a.
Figure 5.2. The singularity spectrum summarizes long-range temporal correlations and multifractal complexity. (a) Absolute coefficients obtained from a discrete wavelet transform of an interspike interval sequence. (b) Local Hölder exponents determined directly from the ISI sequence. At scale 7, 3 data points in both positive and negative directions are used to determine the local h value. Scale 17 requires 8 bidirectional data points. (c) Interspike interval sequence of an example hippocampal (CA3) neuron. (d) Fractional Brownian motion (fBm; black line) with Hurst exponent $c_1 = 0.5$ is used to illustrate the calculation of the local Hölder exponents. The Hölder envelope (colored lines) is fit to each data point to as in equation 1. This calculation performed with scale = 7 requires data points within the solid envelopes whereas with scale = 17 requires data points within the dashed lines. The chosen data point $t_0$ has a local Hölder exponent equal to 0.5 (red). (e) Singularity spectra calculated for the fBm signal from (d) in red, and from the interspike intervals series in (c) using WLMA (blue) and local Hölder exponent determination (black). The first log-cumulant ($c_1$) takes Hölder exponent values where $D(h) = 1$ at the apex of the singularity spectrum and signify long-range temporal correlations. The second log-cumulant ($c_2$) is a function of the width of the singularity spectrum and corresponds to multifractal complexity. Differences in singularity spectra obtained from global and local methods are discussed in section 3.3.5.2.
5.3.6. Statistical analyses.

All significance tests were performed in Matlab with 10,000 Monte Carlo permutations to determine two-tailed p-values. In order to maximize statistical power and minimize false positive rates, only 4 comparisons between reversal phases were made (A1 vs. A2, A2 vs. C, B1 vs. A2, and B1 vs. C). Significance levels were set at either \( p < 0.0125 \) when making between reversal phase comparisons or at \( p < 0.01 \) when making 5 comparisons (within reversal phases) using the Bonferroni correction. Since reversal phase B was defined based on performance criteria, statistical tests of behavioral performance of phase B1 vs. A2 or B1 vs. C were not made (Fig. 5.4a).

5.4. Results

5.4.1. Reversal Learning

The reversal learning paradigm was analyzed in three phases: pre-reversal DMS task (pre-reversal phase A), post-reversal DNMS task with poor performance (post-reversal phase B), post-reversal DNMS task with successful performance (reversal phase C). Previous work demonstrated that successful performance at extended delays (i.e., 30 sec.) involved hippocampal neuronal circuitry more than performance at short delays (<10 sec.) (Chan et al 2010; Goonawardena et al 2010). Therefore, 7 rats were trained to perform the delayed match-to-sample (DMS) task at extended delays of 1-30 seconds prior to hippocampal electrode implantation. After surgery and recovery, multiunit recordings of hippocampal CA3 and CA1 were obtained while average performance >75% was maintained with 1-30 second delays for at least 5 days (Fig. 5.3, phase A1). Following the 1-30 second delay sessions, two DMS sessions (days) with delays of only 1 second were presented (reversal phase A2) immediately prior to implementation of the reversal paradigm in which the opposite lever was rewarded in the choice phase of the task. The task was reversed to delayed nonmatch-to-sample
(DNMS) contingency, in which trials of 1 second delay constituted reversal phase B1. The first day following task transition when performance was below 75% correct was assessed separately from subsequent days (reversal phase B2). Animals achieved mean DNMS performance > 75% correct after an overall average of 5 transition days (range: 3-9 days) and continued to perform the DNMS task with 1 second delays for at least three days after achieving that performance level (reversal phase C).

During the transition phase (B1 and B2), all rats developed a behavioral lever bias during which they chose the same lever during the choice (nonmatch) phase (Fig. 5.3; dashed lines). Three of seven animals switched their lever bias near the later stage of the reversal, which can be seen at the start of phase C when the orange and green dashed lines switch positions.

**Figure 5.3. Rats acquired reversal learning over multiple days.** Rats were initially trained to perform the Delayed Match-to-Sample (DMS) task at 1-30s delays (phase A1). After achieving stable performance above 80% correct, the delay was lowered to 1 second for two days (phase A2) before the task was changed to nonmatch (phase B1). It took 5 consecutive days of DNMS exposure to achieve a mean performance level of greater than 75% correct nonmatch responses (phase B2). Delays were maintained at 1 second for 3-5 more days until stable performance was achieved (phase C).
Behavioral performance was assessed between reversal phases via average daily percent correct using Monte Carlo permutation tests as shown in Fig. 5.4a. Results showed that animals performed better during pre-reversal DMS 1 second delays (phase A2) compared to pre-reversal DMS 1-30s delays (phase A1) and post-reversal DNMS 1 second delays (phase C; Fig. 5.4a). Prior results from this laboratory have shown that performance is higher on short delays compared to long delays (Heyser et al. 1989; Deadwyler et al. 1996; Hampson et al. 1999), thus higher performance during A2 vs. A1 was expected. However, higher performance on A2 vs. C was likely due to prior experience when only one rule had been learned compared to exposure to an additional (nonmatch) rule which required actively suppressing of the first rule.

5.4.2. Behavioral latencies reflect reversal learning

Behavioral latencies between three task events were assessed to examine the behavioral substrate of reversal learning. The intervals assessed were: Sample Presentation (SP) to the Sample Response (SR), SR to Last Nosepoke (LNP), and LNP to Choice Response (CR). One comparison was made to determine if differences in successful performance (>75% correct) existed between the pre-reversal DMS task and post-reversal DNMS task: A2 vs. C. Two comparisons were made to determine how extinction was represented during the first day of rule reversal: A2 vs. B1 and B1 vs. C. A final comparison was made between A1 and A2 to determine if elongated delayed influenced other intervals outside the delay period. Comparisons were also made of correct vs. error trials within all 5 reversal phases.

Analysis of the latency from SP to SR showed that animals took longer to press the sample lever during pre-reversal phase A1 compared to phase A2 (Fig. 5.4c). This interval was also longer during phase B1 compared to both phases A2 and C. Rats
pressed the sample lever more rapidly during phase A2 compared to phase C. Correct or error trials were sorted by between-trial behavioral either by outcome of a) the prior trial (Fig. 5.4b) or b) the same trial (Fig. 5.4d). When based on the prior trial, the correct vs. error comparisons revealed 4 out of 5 significant within-phase differences (Fig. 5.4b).

During reversal phase A2, rats took longer to press the sample lever when they performed the immediately preceding trial incorrectly. This behavioral latency effect reversed during the DNMS task when longer SR lever press latencies occurred after correct trials (Fig. 5.4b).

The detected latency differences changed for correct and error trials based on the future (same trial) choice responding (Fig. 5.4d). Rats took longer to make SRs that eventually incurred incorrect choices during the CR only during reversal phases A2 and B2 (Fig. 5.4d).

The latency from SR to LNP coincided with the delay period and therefore was only assessed for sessions containing 1 second delays (excluding A1). It took longer to complete the LNP during the extinction phase (B1) compared to either A2 or C (Fig. 5.4e). Animals took significantly longer to terminate the delay during C vs. A2, and this may be an indication of increased difficulty in C vs. A2 (Fig. 5.4a). Animals had shorter latencies to LNP from SR during correct vs. error trials in reversal phases A2, B2 and C (Fig. 5.4f) but not during reversal phase B1. This agrees with prior literature showing that when animals complete the “delay” component of the task more rapidly, their performance improves (Deadwyler et al 1996).

A critical behavioral adjustment during this reversal learning paradigm should occur between the LNP and CR since the altered response contingency (i.e. press the opposite (nonmatch) lever instead of the same (match) lever) specifically affects physical responding during CR. Animals took significantly longer to perform the CR during the B1
phase compared to either pre-reversal A2 or post-reversal C phases (Fig. 5.4g). The interval between LNP and CR was longer during phase C compared to phase A2. Additionally, animals took longer to make the CR during the extended delay pre-reversal phase A1 trials compared to short delay trials in A2. Taken together, the results showed that extinction produced during phase B1 elicited longer CR latencies compared to when performance was improved in phases A2 and C. Rats performed correct and error CRs at the same rate during extinction in the B1 phase, but took longer to complete error trials when performing above 75% in phases A2 and C (Fig. 5.4h). These differences in behavioral latency were used to establish windows for assessing neural substrates of reversal learning using multifractal analysis.
Figure 5.4. Changes in behavioral performance and latencies revealed task complexity and cognitive demand. Animals performance is shown for all phases, in which successful responding was maximal during pre-reversal (phase A2) 1 second delay sessions (a). Behavioral latency was measured for three critical intervals between task events and differences due to correct vs. error were assessed. The intervals examined were from Sample Presentation (SP) to Sample Response (SR) (b-d), from SR to Last Nosepoke (LNP) (e-f) and from LNP to Choice Response (CR) (g-h). Average latencies are shown for all trials (n = 700 – 3500 intervals per reversal phase) and animals (n = 7). Error bars indicate SEM; asterisks (*) represent statistical significance after Bonferoni correction.
5.4.3. Visualizing multifractality of spike trains

Multifractal analysis quantifies the irregularity of a signal by detecting correlations across spatial and temporal scales (Kantelhardt, 2012). The global Hurst exponent (Hurst, 1951; Mandelbrot, 1982) is typically used to classify the average regularity of a signal, but local irregularity or smoothness can be quantified by the local Hölder exponent $h$ (Fig. 5.2d; section 3.3.5.1). The global Hurst exponent could be represented as the mode of local Hölder exponents in any signal (Ihlen, 2012). Local $h$ values can be determined for every point in an interspike interval (ISI) time series and describe how similar adjacent ISI times are to each other (Fig. 5.2b-e). Importantly, we note that the largest local $h$ values (approaching and exceeding 1) occurred when adjacent ISIs are very small during periods of neuronal bursting (Fig. 5.2b-c). Large local $h$ values ($\geq 0.5$) indicate long-range temporal correlations (LRTCs) and represent time intervals where similar values are likely to occur repeatedly in sequence. Local $h$ values of 0.5 indicate periods of randomness, while local $h$ values $\leq 0.5$ indicate anti-correlation (Brazhe and Maksimov, 2006). Multifractal signals contain a wide range of local $h$ values to describe their dynamic distribution of variability.

By visualizing a time sequence of local $h$ values, the manifestation of LRTCs and multifractality in an ISI series can be understood (Fig. 5.5a-b). Each local $h$ value corresponds to a specific ISI (Fig. 5.2b-c). When neurons fire around a task event or during an important task interval, the local $h$ values provide insight into the underlying information processing mechanisms. In this example local $h$ sequence (Fig. 5.5a-b), strong representations from SR (blue dots), delay (SR to LNP; orange dots), and LNP (green dots) were detected. Raster plots (Fig. 5.5c) and peri-event histograms (Fig. 5.5d) showed increased firing rate immediately after the SR that persisted throughout
the delay and terminated immediately after LNP. The increased firing rate after SR caused the local $h$ values to increase from near random (0.5) towards values close to and sometimes exceeding 1, indicating that LRTCs coincided with the increased firing rate and short ISIs occurring during SR. The local $h$ values hovered near 1 throughout the delay when the firing rate was maintained at an increased level relative to baseline (last 3 seconds of delay are from -3 to 0 in the LNP rasters and histograms; Fig. 5.5c right & 5d green). Sustained firing rate increases throughout the delay period coincided with neuronal spike trains exhibiting strong LRTCs. The firing rate drastically fell leading up to CR and stayed very low throughout the intertrial interval (Fig. 5.5d, red). ISIs bracketing the CR produced local $h$ values close to 0.5 (Fig. 5.5a-b, red dots), which were more random compared to other components of the task. These abrupt changes in firing rate occurring time-locked to behavioral events generated oscillating local $h$ values reflected as multifractal complexity.

Since local $h$ values depend on adjacent ISIs, the relationship between local $h$ values and ISI length can only reveal certain firing tendencies (Fig. 5.5e). This visualization helps to reiterate the fact that LRTCs ($h$ near 1) occurred when ISIs were relatively short. Local $h$ values close to 0.5 coincided with ISIs of any length due to the dependence on adjacent ISIs and long ISIs (>10 sec.) commonly resulted in local $h$ values near 0.5.
Figure 5.5. Ongoing memory processing was reflected as long-range temporal correlations and multifractal complexity. (a) An example sequence of local Hölder exponents $h$ is shown for one interspike interval (ISI) sequence from a CA3 neuron that exhibited LRTCs during the delay period (SR to LNP interval; orange dots). Multifractal dynamics were detected as the wide range of local $h$ values occurring during behavioral events: blue dots for Sample Response (SR), green dots for Last Nosepoke (LNP), red dots for Choice Response (CR). (b) A zoomed in version of the local $h$ sequence from (a) illustrates more precisely how local $h$ oscillations coincided with behavioral responses. (c) Raster plots around SR and LNP DMS task events. This neuron increased firing rate immediately after the SR that persisted throughout the delay and into the LNP. (d) Peri-event histograms for three critical task events: SR, LNP and CR. This neuron increased firing rate for the SR (blue line) that continued throughout the delay and terminated immediately after the LNP (green line). Firing rate was increased for CR (red line) as well. Z-scores were calculated based on the maximum firing rate observed in a one second window around events (light blue background) using a baseline from 2.5 to 2 seconds before SR (red background). Z-scores for each event are displayed in the legend. (e) Large local $h$ values occurred only when ISIs were very short, while smaller local $h$ values were detected with a wider range of ISI lengths. Responses during the SR (blue) and delay (orange) were generally very short and exhibited strong LRTCs, while those during the CR (red) were longer and closer to random ($h = 0.5$).
5.4.4. LRTCs and multifractal complexity of single neurons vary with reversal learning

While local Hölder exponent determination is useful for visualizing and comprehending LRTCs and multifractal complexity of neuronal ISI sequences, it is very computationally expensive and not a robust method for large datasets (Struzik, 2000; Ihlen, 2012). Alternatively, Wavelet Leaders-based Multifractal Analysis (WLMA) can rapidly yield singularity spectra and log-cumulants because it analyzes data from a global perspective (section 5.3.5.2). Therefore, WLMA was performed on all spike trains recorded during reversal learning to estimate a singularity spectrum (Fig. 5.2e, blue) for each neuron recorded during each daily session. A total of 247 neurons were recorded from 7 rats: 194-930 recordings per reversal phase with 88-426 from CA3 neurons and 106-504 from CA1 neurons per reversal phase. Log-cumulant values were obtained from the singularity spectra in order to summarize and compare changes in both monofractality (LRTCs), as indicated by log-cumulant $c_1$, and multifractality, indicated by $c_2$. The same four comparisons made between behavioral latency values in section 5.4.2 were performed here: phases A1 vs. A2, A2 vs. C, B1 vs. A2 and B1 vs. C.

Monofractality (LRTCs), detected as $c_1$, and multifractal complexity, quantified by $c_2$, closely tracked alterations in behavioral performance in such a way that supported their utility as a marker of task complexity and cognitive demand. One CA1 neuron from Rat 5 was chosen to illustrate this example (Fig. 5.6) that closely matched the detected population differences (Fig. 5.9). This animal nearly completed the reversal learning but instead adopted an opposite lever preference about midway through completion of phase B (Fig. 5.6a), possibly as a manifestation of spontaneous recovery (Rescorla, 2007). During the extinction session (phase B1; session 1 in Fig. 5.6), multifractal complexity reached its (tied) peak level (Fig. 5.6b, orange spectrum; Fig. 5.6c, session 1) before being drastically reduced during the next day's session (Fig. 5.6b, red
The behavioral bias disappeared when performance nearly reached the behavioral criterion of 75% correct on the 5th phase B session (Fig. 5.2a; session 3), and on the same day, multifractal complexity reached its third highest level (Fig. 5.6b, blue; 5.6c, session 3). In the next session, the opposite strategy of a strong preference for lever B persisted until reaching behavioral criterion on post-reversal day 10. Performance was with the opposite lever bias on sessions 4 and 5 (Fig. 5.6a) and reached the best post-reversal performance on session 6. Correspondingly, multifractal complexity was lower on sessions 4 and 5 (Fig. 5.6b, cyan and green spectra; 5.6c, sessions 4 and 5) when performed with an alternative strategy (i.e., lever preference) compared to when reaching maximal post-reversal behavioral performance (session 6) and exhibiting the (tied) greatest level of multifractal complexity (Fig. 5.6b, purple spectrum). It appeared that multifractal complexity of this CA1 neuron tracked ongoing cognitive demand and behavioral performance.
Figure 5.6. Reversal learning was neurophysiologically reflected as alterations in multifractal complexity. Behavioral performance from rat 5 and results obtained with multifractal analysis of a single CA3 neuron are illustrated for 6 sessions signified by numbers in all panels. (a) Immediately after the rule switch to DNMS, rat 5 showed bias on lever A for 4 days (including sessions 1 and 2) before nearly reaching behavioral criterion on post-reversal day 5 (session 3). On the sixth day, lever preference was reversed to lever B. The preference on lever B persisted for 4 days (including session 4 and 5) before reaching behavioral criterion and maximum post-reversal performance on session 6. (b) Singularity spectra from all 6 signified sessions in (a) show how multifractal complexity correlates with behavioral performance, which is explained in detail in section 5.4.4. (c) Log-cumulants for monfractality/LRTCs ($c_1$, black crosses) and multifractality ($c_2$, blue circles) of this CA3 neuron tracked behavioral performance from (a) and task complexity.
Mono/multifractal properties of a CA3 neuron from rat 1 tracked task complexity throughout the reversal learning paradigm (Fig. 5.7-5.8). This example illustrates how neuronal timing processes, indicated as LRTCs and multifractal complexity, correlated with increased behavioral latency during the extinction B1 session (Fig. 5.4). The log-cumulants obtained for all sessions during the reversal (Fig. 5.7a) resembled the group averages obtained for the entire neuronal population (Fig. 5.9). LRTCs were largest during the B1 session (Fig. 5.7a, black crosses). This neuron exhibited strong multifractal complexity during the second A2 session and B1 (extinction) session that heavily decreased during the first B2 (reversal learning) session (numbers 1, 2 and 3 in Fig. 5.7a, blue circles). The singularity spectra computed for this neuron over these three sessions are displayed in Figure 5.7b: Session 1 (blue) was anti-correlated and shifted towards the left but was just as wide (multifractal) as session 2 (green). Session 3 (orange) had intermediate LRTCs but was much less multifractal (h width) compared to both sessions 1 and 2. The local Hölder exponent sequences and peri-event histograms (PEHs) were computed for these three sessions (Fig. 5.7c-e, right panels). In the A2 session, this neuron began discharging faster during the SR (Fig. 5.7c, right, blue), peaked during the LNP (green) and fell during the CR (red). The large oscillations of the local Hölder exponent signify multifractal complexity generated by this dynamical discharge pattern according to task-specific behavioral fluctuations (Fig. 5.7c, left).

Large LRTCs and strong multifractal complexity coincided with extinction during the B1 session (Fig. 5.7d, left). This same CA3 neuron continued to exhibit qualitatively similar discharge properties (i.e., increasing firing rate near the end of the SR and peaking during the LNP), but quantitatively, these firing rate peaks (z-scores) were lower than during the prior session (Fig. 5.7d, right). At the very start of the B1 session, the
local Hölder exponent oscillated in the same manner as in the prior A2 session (Fig. 5.7d, left). Shortly into the session, a long pause (i.e., 60 sec.) was taken between a Sample Presentation (SP) and subsequent SR. During this behavioral break, this neuron exhibited strong LRTC s (Fig. 5.7d., left panel, pink dots under line z). This same behavior occurred again shortly afterwards (line y). Subsequent behavioral pauses between the LNP and CR were marked by strong LRTC s and oscillating local $h$ (Fig. 5.7d, left panel, cyan dots, lines x, w and v). Both LRTC s and multifractal complexity declined by the end of the extinction session (Fig. 5.7d, line u). The transition from strong LRTC s and multifractal complexity to random (local $h$ near 0.5) and simple dynamics coincided with the behavioral manifestation of extinction (Fig. 5.8).

During the first B2 session (session 3 in Fig. 5.7), the rat performed poorly while executing the old (match) behavioral rule. This CA3 neuron continued to fire after SR and during LNP (Fig. 5.7e, right panel). There were brief “bursts” of multifractal complexity during this session (Fig. 5.7e, left panel), but multifractality was very reduced compared to both previous sessions (Fig. 5.7b, orange; $c_2$, local $h$ oscillations).
Figure 5.7. Extinction elicited strong LRTCs and multifractal complexity in a CA3 neuron. One example CA3 neuron from rat 1 was used to illustrate the reversal learning phenomenon. Singularity spectra, local Hölder exponents, and peri-event histograms (PEHs) are shown for three sessions signified by numbers in (a). Z-scores were calculated from PEHs using the same method as figure 5.5. (a) Log-cumulant values ($c_1$ black crosses and $c_2$ blue circles) are shown for every session throughout behavioral reversal this this example neuron. Reversal phase A1 is denoted by a blue background and phases B1-B2 are denoted by a red background. The 3 sessions designated by numbers are displayed in the latter segments of this figure. (b) Singularity spectra are shown for the 3 sessions designated in (a). Session 2 (green) contained the strongest LRTCs (largest $c_1$) and therefore is located to the right of all other sessions. Session 3 (orange) contained intermediate LRTCs while session 1 (blue) contained weak LRTCs. Sessions 1 and 2 exhibited nearly equivalent degrees of multifractal complexity while session 3 was much less multifractal. (c) Prior to rule reversal, this neuron was very multifractal and showed large firing rate increases during the nosepoke and into the choice phase (right). The multifractal complexity can be seen as the large Hölder exponent oscillations ($h$ oscillations). (d) This neuron still fired for the LNP event, albeit with a weaker magnitude (right), and underwent “extinction” during the first day of reversal. Initially, two long pauses occurred between the sample presentations (SP) and sample response (SR; lines z and y) and then more occurred between the last nosepoke (LNP) and nonmatch/choice response (NR; lines x, w, and v). During these pauses, this neuron discharged quickly and exhibited strong long range
(continued) temporal correlations, as signified by local Hölder exponents near 1. LRTCs and multifractal complexity strongly decreased near the end of the session (line u). (e) During phase B2, this neuron continued to fire for the LNP (right) but exhibited much weaker LRTCs and multifractal dynamics, as evidenced by local $h$ values closer to 0.5 and decreased $h$ width, respectively (left).

Figure 5.8. Alterations of LRTCs and multifractal complexity coincided with reversal learning. The local $h$ sequences from figure 5.7c-e were reproduced and together to show consistent local $h$ patterns between sessions. Strong indications of LRTCs and multifractal complexity decreased near the end of the extinction session (reversal phase B1).
5.4.5. LRTCs and multifractal complexity of hippocampal population tracks reversal learning

Population results obtained by analyzing hippocampal neurons with multifractal analysis revealed a relationship between task complexity, cognitive demand and multifractal properties. When all hippocampal neurons were grouped together, both $c_1$ and $c_2$ yielded two common significant differences: B1 contained more LRTCs ($c_1$) and greater multifractal complexity ($c_2$) than either pre-reversal phase A2 or post-reversal phase C (Fig. 5.9a-b). Both mono- and multifractality were greater during the extinction (B1) sessions compared to successful performance sessions before or after rule reversal. These increases may related to synaptic plasticity mechanisms involved in breaking and/or forming associations (Stephen et al. 2012; Dong et al. 2013; Lamanna et al. 2015). Additionally, it was detected that reversal phase A1 presented stronger LRTCs (indicated by $c_1$) compared to phase A2, indicating that additional memory load enhanced LRTCs/monofractality of hippocampal spike trains. Extended delays could propagate LRTCs as shown in Figure 5.5.

When grouped by hippocampal subregion (CA3 or CA1), some distinct differences in LRTCs were found. Both CA3 and CA1 neurons recorded during reversal phase B1 contained stronger LRTCs than the same neurons recorded during either pre-reversal phase A2 or post-reversal phase C (Fig. 5.9c). Only the CA3 neurons had increased LRTCs during phase A1 compared to A2 (Fig. 5.9d), i.e., delays vs. no delays. The three differences detected in the CA3 neuronal population were identical to those found in the entire neural population (Fig. 5.9b).

The multifractal complexity of the CA3 population was significantly different between 3 reversal phases (Fig. 5.9d). Two were common to the entire population: B1 was more multifractal than either A2 or C. However, the increased multifractality
between pre-reversal phases A1 vs. A2 was only detected in the CA3 population (Fig. 5.9d). The CA1 population as a whole was more homogenous than the CA3 population and exhibited no significant changes in multifractality throughout the reversal learning paradigm. This suggests that recurrent connections of CA3 may be more adaptive for alterations in multifractal properties via synaptic plasticity mechanisms (Li et al 1994; Kali and Dayan, 2000) than CA1 neurons.

Within reversal phase comparisons were also made between hippocampal subregions to determine if monofractality or multifractality were more localized to specific areas (Fig. 5.9c-d). CA1 neurons exhibited greater LRTCs during phases A2, B2 and C compared to CA3 neurons. It is possible that LRTCs occur more readily in CA1 pyramidal cells because these neurons typically project to either subiculum or cortical regions (Commins et al 1998; Deadwyler and Hampson, 2004) instead in a recurrent manner as in CA3. No differences were detected between CA3 and CA1 neurons with respect to multifractal complexity (Fig. 5.9d).

Taken together, the results showed that task complexity correlated with alterations of mono/multifractal complexity of spike trains of hippocampal neurons. The differences in behavioral performance (Fig. 5.4a) and behavioral latency (Fig. 5.4b-h) indicated that reversal phase A2 was the simplest phase. Strong LRTCs during working memory maintenance (Fig. 5.5) may contribute to larger $c_1$ values detected in the extended delay sessions (Fig. 5.9a & c; phase A1). Increased cognitive load and task complexity coinciding with the rule change during the extinction session (phase B1) may be reflected as increased mono/multifractal complexity of hippocampal spike trains (Fig. 5.7-5.9).
Figure 5.9. LRTCs and Multifractality of CA3 and CA1 neurons fluctuate with reversal learning. Shown are average log-cumulants for all neuronal recordings (n = 3024 total recordings from 247 neurons; 930-194 [ 88-426 from CA3 and 106-504 from CA1 ] recordings per reversal phase). Error bars are SEM and asterisks (*) represents statistical significance after Bonferoni correction. (a) Average long range temporal correlations (LRTCs) from all ISI sequences were quantified by the first log-cumulant $c_1$. (b) Multifractal complexity ($c_2$) of ISI sequences was highest during the extinction session. (c) Average LRTCs ($c_1$) were different in hippocampal CA3 and CA1 subregions. CA3 ISI sequences exhibited the same differences as the entire population, while CA1 ISI Sequences showed 2 differences. CA1 spike trains had stronger LRTCs than CA3 spike trains in 3 reversal phases. (d) CA3 ISI sequences showed variable multifractal complexity ($c_2$) between reversal phases, but CA1 ISI sequences contained similar multifractal complexity.
5.5. Discussion

5.5.1. Reversal learning and memory were reflected as changes in LRTCs and multifractal complexity

A reversal learning paradigm was implemented to study how neural indicators of information processing and mental flexibility were reflected as changes in mono/multifractality of hippocampal microcircuits. Results presented indicate that extinction (phase B1) coincided with increased time spent disengaged from performing the task (i.e., longer behavioral latencies in Fig. 5.4). Changes of behavioral latency throughout the reversal could reflect variations in cognitive demand or memory load (D’Arcy et al. 2005; Jeneson et al. 2011; Sannino et al. 2012) detected as fluctuations in mono/multifractal complexity of concurrent hippocampal spike trains (Figs. 5.6-5.9).

When animals were trained to perform at 1-30s delays, task demands were diminished when delays were reduced to 1 second, and this may account for the decrease in LRTCs from reversal phase A1 to A2. The cognitive and memory workload demands of the task may have become more complex when the rule for reward was changed from match to nonmatch. As performance on the DMS task extinguished and performance on the second, DNMS task, improved (phases B1 to B2 to C), task novelty and associated cognitive challenge could have decreased along with mono/multifractality (Fig. 5.9). Fluctuations in mono/multifractal complexity may have coincided with reorganization of the hierarchical associative structure required for reversal learning (Rescorla, 1992).

Fractals are generated by repeating patterns. When such reiteration is understood to reflect feedback dynamics (Welch, 2010) within neural circuits, it could serve as a conceptual basis for the mono/multifractal properties of learning and memory. Learning occurs when associations are acquired, modified or reinforced by integrating information from multiple sources. In the reversal learning paradigm presented here,
animals altered encoding of positional information during the sample phase, followed by a change in the “rule” determining the response during the choice phase. These associational modifications served as the basis for correct behavioral performance utilizing short and long-term memory (Deadwyler et al 1996). Reversal of this response contingency to a nonmatch (reversed) rule requires flexibility and ultimately addition and/or modification of the stored memory reflecting the behavioral rule (Egerton et al 2005; Walsh et al 2011). Reorganization of reinforcement contingencies was reflected initially as increased mono/multifractal complexity, which resulted possibly from long-term depression (Dong et al 2013) or long-term potentiation (Lamanna et al 2015). LRTC.s imply “rich information content, high flexibility and purposeful variability” (Chen et al 1997; Wong and Shelhamer, 2013). Stronger LRTC.s detected during working memory maintenance (phase A1) and extinction (phase B1) support the notion of reflecting information content. Multifractal complexity can result from synergistic, interrelated activity utilized to extract common patterns during learning (Dixon et al 2012; Ihlen and Vereijken, 2013). Increased multifractality during extinction may indicate altered communication within the hippocampus (Dong et al 2013; Lamanna et al 2015), or increased communication between hippocampus and prefrontal cortex (Malá et al 2015) or hippocampus and striatum (Eschenko and Mizumori, 2007), all of which have been shown to support reversal learning.

The DMS/DNMS task requires integration of spatial (i.e., lever position) and nonspatial components (i.e., task phase -- sample or choice) for successful performance (Hampson et al 1999). Neuronal ensembles exhibit characteristic firing rate patterns for distinct lever positions that can be used to predict and enhance performance via electrical stimulation (Berger et al 2011; Hampson et al 2012). Both LRTC.s and multifractal complexity may be enhanced when these similar ensemble firing patterns
repeatedly occur throughout a behavioral session. The specific ensemble firing patterns identified for combinations of position and task phase (sample vs. choice) can alternatively be conceptualized as a manifestation of multistable states (Sakamoto et al 2013; Mazzucato et al 2015). According to this view, reversal learning would require rearrangement of the neuronal ensemble to combine previously unassociated states (i.e., left sample with right choice instead of left choice). An asynchronous firing regime may develop during extinction that destabilizes synapses (Kestler and Kinzel, 2006) and promotes increased LRTCs and multifractal complexity (Pavlov and Anishchenko, 2007).

5.5.2. Temporal associations were reflected as alterations of LRTCs and multifractality

Behavioral latency differences between correct and error trials during specific reversal phases may indicate fluctuations in cognitive demand reflected as mono/multifractal complexity. When performance of the pre-reversal DMS task was high (phase A2) and task difficulty (i.e., cognitive demand) was low, rats achieved the highest percent correct (Fig. 5.4a) and performed all task intervals faster than in post-reversal sessions (Fig. 5.4c, e, g). This evidence suggests that pre-reversal phase A2 required the least cognitive demand since ISI sequences recorded during this phase were shown to produce less LRTCs (Fig. 5.9a, c) and less multifractality (Fig. 5.9d) than when delays were longer and variable (phase A1). Decreased LRTCs during phase A2 may reflect decreased memory load due to previous, extended delay, DMS training (cf. Anastas et al 2011; Kelty-Stephen et al 2015). Stronger LRTCs detected during phase A1 may have resulted from hippocampal interactions with other brain structures under increased memory load conditions (Cave and Squire, 1991; Sannino et al 2012). Such enhanced LRTCs during the delay interval (Fig. 5.5) suggest involvement in the maintenance of working memory. LRTCs indicate that a signal contains memory of past activity in order
to increase information content (Wong and Shelhamer, 2013), and our results support
the interpretation that additional memory load required by extended delay intervals
elicited stronger LRTCs.

When animals are no longer rewarded for the originally learned match rule, they
could have adopted alternative strategies related to deciphering the new nonmatch rule
in phase B1. The pronounced lever bias (Fig. 5.3, dashed lines) and extended pauses
(Fig. 5.4c, e, g) of animals which occurred during phase B could indicate task extinction,
which likely occurred within a single session (Pamplona et al 2006). Prior to task
reversal, animals made correct responses faster than incorrect ones (phase A2; Fig.
4.4d, f, h), but this difference was lost during extinction when animals did not respond
accurately to the new associations (phase B1; Fig. 4.4d, f, h). DNMS task awareness
may have been manifested via quicker responses on correct trials during reversal
acquisition in phases B2 (Fig. 4.4d, f) and C (Fig. 4.4f, h).

Animals waited longer to initiate trials after completing correct trials in post-
reversal phases B-C which was opposite the trend in the pre-reversal phase A2 (Fig.
5.4b). If the increased post-error latency during A2 was an indication of post-error
behavioral adjustment (Narayanan et al 2013; Summerfield and Yeung, 2013), this
‘correct-error swap’ may have been a consequence of detection of the reversed rule
contingency (during B1) followed by continued positive reinforcement after correctly
executed nonmatch trials (Graham et al 2009). Increased mono/multifractality during the
B1 phase compared to both A2 and C (Fig. 5.9b, c) could therefore reflect how extinction
learning occurs via alterations in microcircuit/network structure (Egerton et al 2005;
Eschenko and Mizumori, 2007; Malá et al 2015) and synaptic plasticity (Dong et al 2013;
Lamanna et al 2015).
Contrary to our original hypothesis, behavioral performance and latencies for 1 second delay sessions after reversal (phase C) were significantly different from the same type sessions before reversal (phase A2). Average performance was better during phase A2 compared to C. Additionally, rats took significantly longer in all task intervals during phase C vs. A2 (Fig. 5.4c, e, g). A nonsignificant trend towards C having increased LRTCs vs. A2 could indicate increased activation of memory resources. Although behavioral measures indicate that A2 was easier than C, the results obtained with mono/multifractal log-cumulants did not show the same effect.

Processing of temporal information that is critical for DMS/DNMS task performance appeared in LRTCs and multifractality in hippocampal microcircuit processing. The long-term memory required to perform the DMS/DNMS task necessitates discrimination between lever responses based on task phase (i.e., SR or CR) and selection rule (i.e., match vs. nonmatch). Different temporal dynamics occur when the hippocampal circuit encodes information during the SR compared to retrieval during the CR (Deadwyler et al 1996; Hampson et al 1999), and such timing relationships may have been represented as oscillating local Hölder exponents (Figs. 5.5, 5.7, 5.8). Reversal learning required adjustments of these temporal associations, which likely occurred during the B1 (extinction) session. The neuron in Fig. 5.7c (left) exhibited oscillating local Hölder exponents that coincided with behavioral responses in the DMS task by sustaining an increased firing rate throughout single trials (from SR to LNP to CR; phase A2). These temporal associations could have been disrupted during long behavioral pauses in the extinction session (Fig. 5.4). The conveyed timing relationships might have disintegrated when strong LRTCs and multifractal complexity (Fig. 5.7d, left panel, lines z, y, x, w, and v) became more random and simple (Fig. 5.7d, left panel, line u). The extinction component of the reversal learning process (Fig. 5.8)
may be completed when strong LRTCs and multifractality decline in this manner (Fig. 5.7-5.8).

5.5.3. CA3 neurons preferentially exhibited multifractality while CA1 neurons showed stronger LRTCs

Interesting alterations of mono/multifractal complexity were detected in localized hippocampal CA3 and CA1 subregions that lend themselves to parsimonious neuroanatomically and physiologically based interpretations. Interneural pyramidal cell connections within the CA3 region are primarily recurrent (feedback) connections (Li et al 1994; Kali and Dayan, 2000) while the CA1 region consists primarily of parallel (feedforward) projections (Cenquizca and Swanson, 2007). Changes in multifractality (Fig. 5.9d) were only detected in CA3 and not CA1, which could reflect that fact that one form of processing necessary for reversal learning occurred primarily within recurrent connections. CA3 neurons are critical for encoding reversed reward contingencies (Nakazawa et al 2003) and their recurrent connections may flexibly modify goal-driven associations (Laje and Buonomano, 2013). The increased multifractal complexity during reversal phase B1 shown here may, therefore, be a marker for enhanced microcircuit processing within the CA3 connections.

The differences in monofractality between hippocampal CA3 and CA1 subregions could also be biologically interpreted as reciprocal information concerning microcircuit organization and long-range temporal correlations (LRTCs) detected by log-cumulant c₁ (Fig. 5.9c). Both hippocampal regions showed significant increases during the extinction phase compared to either pre- or post-reversal short-delay sessions. The long-term memory of the first (DMS) task had to be extinguished in order for reversal learning to occur and the LRTCs in both CA3 and CA1 were a viable marker for this process. CA1 spike trains exhibited stronger LRTCs compared to CA3 spike trains.
during reversal phases A2, B2 and C which could be linked to its mainly parallel (feedforward) connections. (cf. Gebber et al 2006). The recurrent connections of CA3 may be more selective to transmitting multifractal-based information while the parallel connections of CA1 more readily transmit LRTCs and "long-memory" processes to other structures (Wong and Shelhamer, 2013).

5.6. Conclusions

Mental flexibility allows animals to adjust behavioral output according to changing environmental demands and conditions (Egerton et al 2005). We report here that increased LRTCs and multifractal complexity occurred during extended delay and extinction sessions accompanying reversal of a learned behavioral task contingency. Behavioral performance decreased and response latencies increased during these sessions, indicating that mono/multifractal complexity in neural firing was associated with fluctuations in task difficulty and cognitive load during behavioral transitions. We further showed that working memory maintenance during delay intervals may be sustained with LRTCs, and that monofractal LRTCs may preferentially occur within CA1 spike trains, while multifractal complexity is more likely in CA3 spike trains. By providing local Hölder exponent sequences for consecutive sessions of reversal learning, we propose that extinction may occur when neural timing processes, indicated with multifractal analyses, are disrupted during extended behavioral pauses. The relevant timing mechanisms that support successful performance in DMS/DNMS task may be reflected as long-range temporal correlations and oscillating local Hölder exponents, hence multifractal complexity.

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5.7. References


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CHAPTER 6: DISCUSSION

New analytical tools can expand interpretations and promote a more comprehensive understanding of experimental data. A new methodological tool enlarges the perceptual window into the world. The previously unnoticed features of the world can be made visible by incorporating a fractal perspective to enable richer descriptions of psychological, neurophysiological and physical processes. The ubiquitous presence of fractal scaling in most physical and physiological systems, including multiple levels of nervous system temporal dynamics, hints at its existence following from a unifying mechanistic framework for life.

This dissertation provides several examples of ways in which fractals have been used to enhance knowledge and enhance descriptions of numerous forms of learning and memory (i.e., short-term vs. long-term, reversal learning), synaptic plasticity, and the effects of cannabinoids. I emphasize that the study of fractals, and especially multifractals, is still in its infancy and as new analysis methodologies develop, so will the experimental paradigms. This dissertation serves to integrate diverse fields of neuroscience, psychology, physiology and physics (fractals) and propel subsequent multidisciplinary research.

6.1. Synaptic Plasticity as Fractal and Multifractal Complexity

The central theme of this dissertation focuses on quantifying and describing novel, fractal and multifractal features of hippocampal memory processing. The idea that both memories and fractals form through reiteration is essential to their unification: long-term potentiation (LTP) occurs after repetitive activation of neuronal sequences and is manifested as stronger long-range temporal correlations (LRTCs) in the
interconnected neurons (Lamanna et al., 2015). Repeated activations of dynamically distributed hippocampal neuronal ensembles during specific instances of the DNMS task elicit formation of functional cell types (FCTs) studied in chapter 3 (Hampson et al., 1999; Goonawardena et al., 2010). FCT formation would require some form of synaptic plasticity, such as LTP, and would associate neurons into hierarchical assemblies in order to promote successful memory performance. I showed that FCTs exhibited stronger LRTCs and enhanced multifractal complexity compared to non-FCTs during the DNMS task (Chapter 3). The use of multifractal analysis also promoted discovery of a novel pair of FCTs (Chapter 3). The memory requirements of the DNMS task activate the auto-associative hippocampal network, and reverberating activity patterns may manifest as fractal and multifractal complexity detected in interspike interval sequences.

The subsequent study found specific memory processing signatures of LRTCs and multifractal complexity by comparing spike trains recorded during the DNMS task to resting periods (Chapter 4). Multifractal complexity was highest during the task when LRTCs were weakest indicating quantification of differential components of hippocampal processing. Strongest LRTCs during resting periods could arise from the lack of memory requirements and consequently lack of firing rate modulation with time, consistent with the decreased ISI variability and reduced firing rate during resting phases (Chapter 4). Correlation analysis confirmed this relationship between the Hurst exponent vs. mean ISI and ISI STD. Multifractal complexity may increase during the task due to mnemonic interactions between multiple neurons, neuronal ensembles and networks during the DNMS task, as suggested by interaction-dominant theory (Ihlen and Vereijken, 2010; Dixon et al., 2012). Dynamical variations in firing rate characteristic of FCTs during memory encoding and retrieval may be reflected as multifractal complexity specifically during the DNMS task. Multifractal complexity was strongly correlated with
CV, indicating that super-Poisson “burstiness” confers phenomenological features similar to multifractal complexity. The correlation between neuronal transmission in the delta range and multifractality suggests that low frequency interspike intervals could transmit memory information detected as multifractal variability within this range.

Tetrahydrocannabinol (THC) was used to impair memory performance in order to assess functional connectivity of the hippocampal microcircuit using multifractal analysis. I showed that THC impaired DNMS task performance and selectively reduced LRTCs and multifractal complexity of FCTs while sparing non-FCTs (Chapter 3). Since THC is known to inhibit LTP by preventing calcium entry into neurons (Misner and Sullivan, 1999; Sullivan, 1999), it is feasible that THC-induced decrements in the Hurst exponent and singularity spectrum width are another neurophysiological marker of LTP impairment. Additionally, modulation of short-term synaptic plasticity by THC, such as increased paired pulse facilitation and reduced EPSCs (Misner and Sullivan, 1999), may impair synaptic computation responsible for hippocampal-dependent working memory processing. It was shown that THC prevented hippocampal network transitions between resting and task neurophysiological states (Chapter 4). Under control conditions, hippocampal interspike interval sequences contained weaker LRTCs but stronger multifractality during the DNMS task compared to resting state recordings before or after it (Chapter 4), but THC prevented these alterations by inducing similar fractal and multifractal dynamics during and after the task. The hippocampal network becomes differentially engaged during memory processing vs. resting states and THC prevented the feature of this transition reflected as LRTCs and multifractal complexity.

Cognitive flexibility was assessed using a reversal learning paradigm that required animals to originally learn the delayed match-to-sample task before the rule was switched to nonmatch (Chapter 5). LRTCs and multifractal complexity of
hippocampal neurons tracked task complexity throughout reversal learning. Working memory maintenance during the delay period coincided with large local Holder exponents (i.e., strong LRTCs). Modulation of neuronal firing rate occurring during task events coincided with alterations of local Holder exponents generating multifractal complexity. This finding reiterates the original finding that FCTs are more multifractal than non-FCTs (Chapter 3): the dynamical firing patterns exhibited by FCTs during the task will produce multifractal sequences of local Holder exponents. Task-modulated firing rates reflected as increased multifractal complexity during the DNMS task vs. resting conditions (chapter 4) may reflect the dynamical modulation of task-specific firing rates exhibited by FCTs (chapter 5). Alterations in synaptic structure via both short-term and long-term plasticity would be required for behavioral expression of reversal learning, and my data indicates that increased LRTCs and multifractality may be a marker of such synaptic rearrangements.

6.2. Future Directions

The findings presented in this dissertation provide insight into the relationship between learning, memory, synaptic plasticity and fractals, but many unanswered questions remain. Although LRTCs are a demonstrated feature of LTP, no research has been done to implicate multifractal complexity. In order to firmly establish the relationship between multifractal complexity and synaptic plasticity, LTP could be induced by electrically stimulating the Shaffer collaterals while recording from CA1 neurons. Interaction-dominant theory could be tested by examining if levels of synaptic potentiation in groups of neurons summate after stimulation of multiple CA3 electrodes. I would hypothesize that multifractal complexity would increase as multiple associations are strengthened between groups of hippocampal neurons. The effects of different
stimulation patterns, such as theta burst frequency, high frequency, and MIMO, could be examined using multifractal analysis.

By knowing where and how to look, multifractal analysis permits quantification of complexity and information content in signals otherwise appearing to contain random fluctuations. However, to the best of my knowledge, further technical developments are required in order to measure multifractal complexity of multiple neurons simultaneously. Currently, multifractal analyses require input of a “noise-like” time series, such as an interspike interval sequence. If spike times of multiple neurons were combined before calculating interspike intervals, this would destroy the inherent LRTCs and produce artificial randomness (Das et al., 2003). Spike count analysis techniques, such as the Allan Factor, Fano Factor and Periodogram, (Lowen et al., 1997; Gebber et al., 2006; Lamanna et al., 2012) can estimate the scaling (Hurst) exponent using bin counts instead of interspike intervals, however, no such techniques currently exist to measure multifractal complexity of neuronal ensembles.

Multifractal analysis of population dynamics could be performed using local field potential data collected during the DNMS task and resting state conditions. Previous work has shown that by bandpass filtering EEG signals, fractal features of specific frequency bands indicated arousal state (Linkenkaer-Hansen et al., 2004; Nikulin and Brismar, 2004). Comparison of amplitude in specific frequency bands, such as delta, theta and gamma, to power-law exponents and multifractal complexity between and within frequency bands may reveal novel aspects of neurophysiological interaction.

Reversal learning could be performed during administration of multiple pharmacological compounds and/or during stimulation using a similar MIMO model (Hampson et al., 2012) adapted for reversal learning. Enhancers of endogenous
cannabinoid signalling are known to improve extinction and reversal learning in the Morris watermaze and would therefore be promising candidates for use in this reversal learning paradigm. By analyzing data generated in this dissertation (chapter 5), an extension of the MIMO could be constructed that facilitates flexible memory processing required for reversal learning.

Furthermore, neural network modeling represents a way to mechanistically describe neuronal ensemble formation and appearance of fractal and multifractal complexity. By mimicking learning in a self-organizing recurrent network (SORN) model (Lazar et al., 2009), the effects of synaptic plasticity could be investigated. Preliminary work with this model demonstrated the capacity for reversal learning correlated with alterations in synaptic structure, but binary spike trains did not exhibit multifractal complexity. Therefore, the model would have to be improved using integrate-and-fire or conductance based neuronal models to generate more realistic spike train dynamics in order to probe mechanistic realizations of fractality during learning.

6.3. Unanswered Questions and concluding remarks

This dissertation demonstrated that LRTCs and multifractal complexity are markers of mnemonic hippocampal processing. Previous studies showed that rule induction in a card sorting task elicited stronger LRTCs due to breaking and reforming of associations while providing evidence that increased multifractality augmented flexible processing (Anastas et al., 2011; Kelty-Stephen et al., 2015). THC-induced suppression LRTCs (chapter 3) and multifractal complexity (chapters 3 & 4) could be a neurophysiological marker of THC-induced inhibition of LTP (Misner and Sullivan, 1999). LRTCs and multifractality increased during extinction of the previously learning rule, possibly due to alterations in synaptic plasticity (chapter 5). Taken together with the
increased LRTCs after LTP induction (Lamanna et al., 2015), strong support exists for using LRTCs as a marker of synaptic plasticity and thus neuronal ensemble formation. Multifractal analysis provides a novel approach to measure dynamical transitions in physical and physiological systems. By integrating discoveries across diverse fields of science, such as neuroscience and physics, novel relationships will be revealed to move all fields forward.
6.4. References


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- Perform in vivo electrophysiology in behaving rats during operant working memory tasks
  - Operant conditioning experience using delayed nonmatch-to-sample and touchscreen tasks
- Perform perfusions and histology to confirm electrode array placement
- Proficiency using Microsoft Excel, SAS, Mathematica and Matlab for data analysis
- Analyze neuronal spike trains using multifractal analysis with Matlab
- Simulate reversal learning using a Self-Organizing Recurrent Model (SORN) in Matlab
  - Developed model for the Advanced Course in Computational Neuroscience final project
- Record and stimulate local field potentials in hippocampal slices
- Conduct electrophysiology experiments in anesthetized rats to induce LTP or LTD
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