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Foundations for In Vivo Nano-Scale Measurement of Memory Processes

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Abstract

An ongoing program of research and development is utilizing nanomaterials as a basis for observing and measuring neurophysiological processes. Work commencing in fiscal year 2007 will focus on expanding current capabilities to create nanoelectrode arrays that will allow nanoscale measurement of the activity of 10's to 100's of neurons. This development is a vital step in gaining scientific insights concerning network properties associated with neural representations and processes. Specifically, attention will be focused the representation of memory in the hippocampus, for which extensive research has been conducted using laboratory rats. This report summarizes background research providing a foundation for work planned for fiscal year 2007 and beyond. In particular, the neuroanatomy and neurophysiology of the hippocampus is described. Additionally, several programs of research are described that have addressed the relationship between neurophysiological processes and behavioral measures of memory performance. These studies provide insight into methodological and analytic approaches for studying the representation of memory processes in the hippocampus. The objective of this report is to document relevant literature in a reference document that will support future research in this area.

Contents

Introduction

There has been an enduring interest in the mechanisms by which the brain forms, maintains and retrieves records of experiences (i.e. memories). A common account of these mechanisms links the brain's record of experiences with spatial, and perhaps temporal, patterns of activation across a collection of neurons (Eichenbaum, 2002; O'Keefe & Nadel, 1978). These patterns of activation and the accompanying neural substrate have been described as "neural assemblies," "neural microcircuits," or "neural ensembles" (Klimesch, 1996; Lin et al 2005; Moser & Paulsen, 2001; Wittenberg & Tsien, 2002). It is important to note that these are theoretical conceptualizations. Current techniques for recording single-unit neural activation only allow crude sampling from a population of neurons. Nonetheless, reproducible patterns of activity corresponding to different experiences have been reported (e.g. Wittenberg & Tsien, 2002). While these findings support the general theoretical concept, techniques enabling higher fidelity measurement of neural activity are necessary to uncover the mechanisms for memory storage and retrieval.

Through earlier work (Fan et al, 2005), Sandia National Laboratories has demonstrated the use of biocompatible nanocrystals to signal subcellular neural events (i.e. presynaptic reuptake of neurotransmitter packets). A technical approach has been formulated that involves fabrication of nanoelectrode arrays that conceptually, would function similarly to current microelectrode arrays, but at a smaller scale. Once such arrays have been successfully fabricated and implanted in targeted brain regions, it should be possible to measure neural activation, including subcellular processes, at a level of fidelity sufficient to elucidate the mechanisms for neural encoding of memories.

Development and testing of the proposed nanoelectrode array technology is the subject of the Sandia Fiscal Year 2007 Neural Assembly Models Derived through Nano-scale Measurements Laboratory Directed Research and Development (LDRD) project. This report summarizes work conducted in preparation for the FY07 LDRD through an FY06 late-start LDRD. The objective of this project has been to establish a foundation for future work with this report providing a reference document summarizing relevant research concerning neurophysiological processes underlying memory processes.

Functional Neuroanatomy of the Hippocampus

Whereas a variety of brain structures have been implicated with various roles in memory processes, the greatest attention has been focused on the hippocampus. This emphasis is product of lesion studies illustrating the devastating effects of hippocampal damage on performance of tasks involving the formation and retrieval of memory (Olton et al, 1978), and electrophysiological, pharmaceutical and genetic studies elucidating the functional operation of the hippocampus in memory (Chen et al, 2001; Lin et al, 2005; Montag-Sallaz & Montag, 2006). Due to this rich understanding, the material summarized here is largely centered on the functions of the hippocampus.

Circuitry of the Hippocampus

The hippocampus is a deep brain structure that may be readily distinguished due to its shape and texture. Figure 1 provides four alternative depictions of the hippocampus and its component structure. The hippocampus consists of various fields and layers that most prominently include the dentate gyrus, CA1, CA2 and CA3 areas, subiculum and entorhinal cortex. The basic circuitry within the hippocampus involves a unidirectional flow of excitatory pathways. The entorhinal cortex provides the primary source of sensory input to the hippocampus, with a secondary source supplied through the retrosplenial cortex. Significant processing of sensory information occurs primarily through the entorhinal cortex, including the integration of sensory input from different sensory modalities (Burwell, 2000). Neurons from layer 2 of the entorhinal cortex project through the subiculum terminating in the dentate gyrus and CA3, while neurons from layer 3, as well as the lateral and medial entorhinal cortex project to CA1.

As noted previously, mossy fibers from the dentate gyrus synapse with proximal dendrites of CA3 pyramidal cells. These CA3 pyramidal cells subsequently project heavily to other areas of CA3, and to CA1 (i.e. *Schaffer collateral projections*). Then, CA1 neurons project back to the subiculum and entorhinal cortex, with neurons arising from the deep layers of the entorhinal cortex returning to form synapses in the sensory cortices from which input to the entorhinal cortex originated. This provides a semi-closed circuit in which excitatory input from sensory cortices travels to the hippocampus and then back again via the entorhinal cortex with the processing occurring along the way conjectured to be essential for long-term memory storage (Johnston & Amaral, 2004).

Dentate Gyrus

The dentate gyrus consists of three layers: (1) the granule cell layer, (2) the largely acellular molecular layer located above the granule cell layer and (3) the diffusely cellular polymorphic cell layer located below the granule cell layer. The principle neurons in the dentate gyrus are the granule cells. These cell have small, approximately 10 mm diameter, spherically-shaped cell bodies which are arranged 4-6 cells thick in the granule layer. The dendrites of granule cells extend perpendicular to the granule cell layer into the molecular layer of the dentate gyrus. It is in the polymorphic and molecular layers that the dentate gyrus receives inputs through cholinergic, dopaminergic serotonergic and noradrenergic synaptic connections with axons from several sources (i.e. entorhinal cortex, septal nuclei, posterior hypothalamus, locus coeruleus and raphe nuclei).

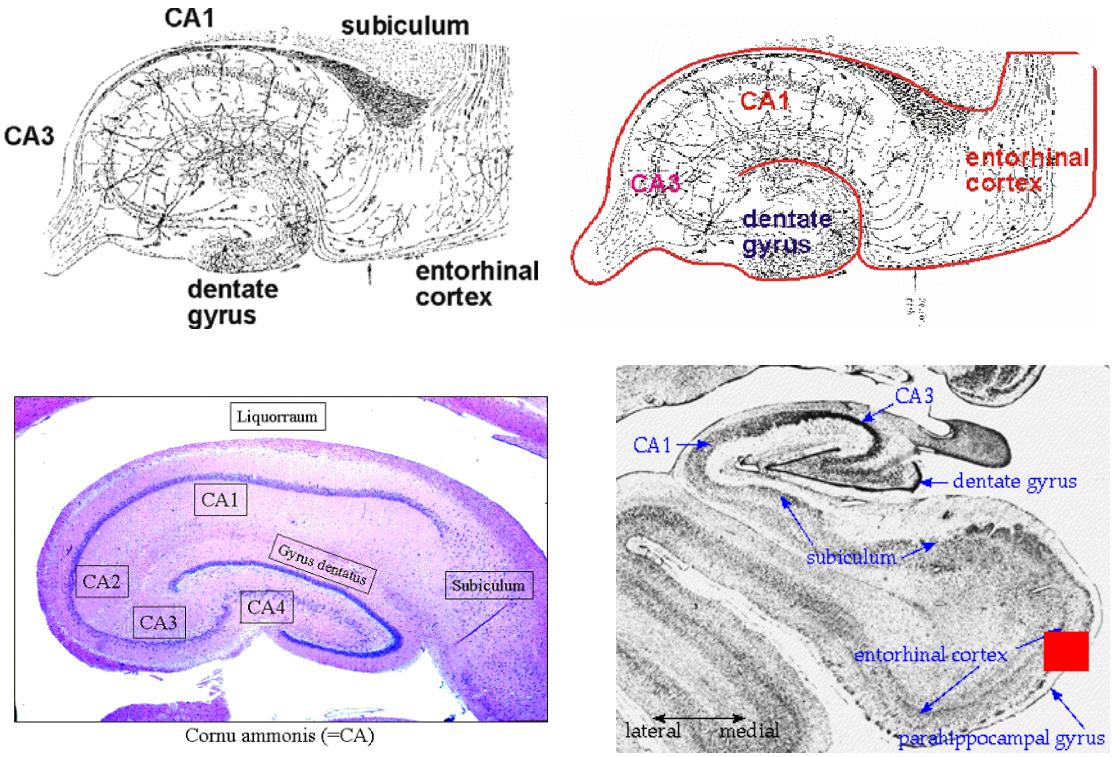

Figure 1. Four alternative depictions showing the basic structure and anatomical components that constitute the hippocampus.

The axons of granule cells arise from the portion of the cell body opposite to the dendrites and have a distinctive appearance which has led to their being referred to as *mossy fibers*. The mossy fibers extend into the polymorphic cell layer where some form synapses with neurons of this layer, but the bulk coalesce into fiber bundles that extend into the CA3 area. These mossy fiber projections expand within CA3 to establish wide varicosities (3-6 m diameter) distributed at intervals of 140 m along the mossy fiber axon (Claiborne et al, 1986). However, while making numerous synapses with a single CA3 pyramidal cell, a given mossy fiber tends to contact a relatively small number of pyramidal cells (i.e. no more than 14 along the entire trajectory of the fiber). Each CA3 pyramidal cell is estimated to receive input from approximately 50 granule cells of the dentate gyrus. The primary neurotransmitter associated with mossy fibers is glutamate, although some have been linked to the opiate peptides (van Daal et al, 1989) and immunoreactivity with GABA has also been established (Sloviter et al, 1996). Within the polymorphic cell layer, mossy fibers synapse with neurons that will form the pyramidal basket cells, as well as with other cells localized to the polymorphic layer. The dentate gyrus does not project to other brain regions and within the hippocampal formation, it only projects to CA3.

It has been observed that the vast majority of mossy fibers synapse onto interneurons to form inhibitory connections (Acsady, 1998). Aspiney neurons appear to be GABA-ergic mediating feedfoward inhibition of pyramidal neurons. However, mossy fibers also synapse with glutamatergic spiney neurons that mediate feedforward excitation to pyramidal neurons.

Within the granule cell layer of the dentate gyrus, these basket cells can form a densely concentrated terminal plexus of GABA-ergic neurons that can influence large numbers of granule cells (Struble et al, 1978). Other inhibitory influences on granule cells derive from the axo-axonic *chandelier-type* cells of the molecular layer and immunoreactive neurons within the polymorphic layer. In general, it appears that there are functional circuits in which the mossy cells provide feedback between the different layers of granule cells (Johnston & Amaral, 2004). Furthermore, projections originating as collaterals of mossy cells within the molecular layer of the dentate gyrus form the *ipsilateral associational-commussural projection* that functions to provide both feedfoward excitatory and inhibitory influences upon the granule cells.

CA1 and CA3 Regions of Hippocampus

Within the hippocampus proper (i.e. CA1, CA2 and CA3 areas), the principle neurons are the pyramidal cells which form the pyramidal cell layer, with their being a number of strata above and below this layer. The cell bodies of the pyramidal cells are arranged within the pyramidal cell layer in an orderly manner 3-6 cells deep. Pyramidal cells have extensive dendritic trees that extend in both directions from the cell body. The apical dendrites are longer and extend toward the center of the hippocampus (i.e. dentate gyrus) making synaptic contacts within each of the three strata they traverse. The shorter basal dendrites extend from the cell body to form synapses in the stratum oriens.

The hippocampus proper may be subdivided into major regions. There is a large-celled region closer to the dentate gyrus that encompasses the CA2 and CA3 regions. The pyramidal cells of this region exhibit extensive dendritic trees that greatly vary in length with those closer to dentate gyrus being shorter and those closer to CA1 being longer. CA2 composes a thin strip (~250 m) that may be distinguished from CA3 due to the absence of the thorny excrescences typical of CA3 pyramidal cells. CA2 has also been distinguished on the basis of a denser representation of cholinergic neurons, as well as the calcium binding protein parvalbumin (Bainbridge & Miller, 1982). The latter observation has been cited as a potential basis for observations that CA2 neurons tend to be less susceptible to ischemic or excitotoxic cell death, and epileptic seizures (Corsellis & Bruton, 1983).

A smaller-celled region is more peripheral to CA2 and CA3 and is referred to as CA1. A typical CA1 pyramidal cell is estimated to have 30,000 excitatory and 1,700 inhibitory synapses with the proximal dendritic shaft being heavily innervated by inhibitory synapses (Megias et al, 2001).

Pyramidal cells are densely covered with dendritic spines with most having the typical cortical-shape. This includes the unusually large thorny excrescences of CA3 pyramidal cells which synapse with mossy fibers from the dentate gyrus. These spines form complex branching structures which are surrounded by a single mossy fiber bouton. Enclosed within the bouton, there are multilobulated branching spine-like processes that may form as many as 37 synaptic contacts with a single CA3 pyramidal cell (Chicurel & Harris, 1992).

In the hippocampus and dentate gyrus, interneurons are numerous. These cells target relatively limited areas, lack spines and are GABA-ergic (i.e. release the neurotransmitter GABA or gamma-aminobutyric acid). There are three types of interneurons found in the pyramidal layer. The axo-axonic cells synapse onto the initial segments of pyramidal cell axons and influence the initiation of axon potentials. Basket cells synapse with the cell bodies of pyramidal neurons forming a basket-like structure encompassing multiple pyramidal neurons. The bistratified cells synapse with the dendrites of pyramidal cells. All three types of interneurons project into each strata of the hippocampus and may receive feedback from pyramidal cells in the local region (Buhl et al, 1996), as well as inhibitory connections between interneurons which operate to synchronize interneuron activity producing oscillatory firing in the theta (5 Hz) and gamma (40 Hz) bands (Jefferys et al, 1996). Unlike excitatory neurons, inhibitory interneurons can fire repetitively at high rates (Schwartzkroin & Mathers, 1978). Thus, excitatory input to interneurons can evoke a high-frequency train of action potentials with prolonged inhibition of postsynaptic neurons.

Functional Structure of CA3 Region

The pyramidal cells of CA3 give rise to broadly distributed , highly-ordered connections extending throughout the hippocampus. The projections terminating within CA3 have been termed *associational connections*, whereas those to CA1 are known as *Schaffer collaterals*. Within CA3 recurrent excitatory connections are prominent producing positive feedback that can invoke synchronous activity within groups of CA3 neurons. It has been hypothesized that CA3 recurrent connections, and the positive feedback they provide, may represent the basis for autoassociative memories (Kohnomen, 1978). In fact, it has been demonstrated that autoassociative memory deficits may be induced through disabling the NMDA receptors of CA3 pyramidal neurons (Nakazawa et al, 2002).

CA3 pyramidal neurons have dendrites that typically receive approximately 25,000 excitatory synapses over an average length of 16 mm, with there being substantially fewer inhibitory synapses (Ishizuka et al, 1995). These dendrites exhibit both passive conductance of electrical potentials along their lengths (i.e. conductance in accordance with cable properties) and active conductance in which the spread of the electrical potential from point-to-point is facilitated by opening voltage-gated ion channels (Johnston & Amaral, 2004). With respect to the former, EPSPs exhibit a length constant of 2 mm (i.e. over a distance of 2 mm, the potential induced by an EPSP should dissipate) and time constant of 50 msec (i.e. at its origin, the potential induced by an EPSP should dissipate within 50 msec). Electrical potentials may also originate from the back-

propagation of action potentials with these potentials dissipating much more rapidly than those originating with EPSPs. Synaptic scaling or normalization has been reported with CA3 neurons whereby a higher density of AMPA receptors at distal dendrites, as compared to proximal dendrites, results in higher amplitude membrane potentials compensating for the decay that occurs as the signal is conducted along the length of the dendrite (Magee & Cook, 2000).

A somewhat topographic layout exists in which cells originating in specific regions of CA3 project to specific regions of CA1, although each CA3 cell makes contact with numerous CA1 cells. It has been estimated that each CA1 cell may synapse with as many as 5,000 CA3 pyramidal cells (Amaral et al, 1990). The size and shape of the CA1 dendritic spines onto which CA3 pyramidal axons synapse varies greatly with this being the basis for differential efficacy of CA1 synapses (Jonhston & Amaral, 2004). Along axonal fibers of a Schaffer collateral, synapses occur at intervals of approximately 2.7 m (Shepherd & Harris, 1998). The total length of a CA3 axonal fiber can be as long as 150-300 mm and may contact 30,000-60,000 neurons (Li et al, 1994). However, while a single Schaffer collateral may synapse with numerous CA1 neurons, there are typically only one or two synaptic contacts with any given neuron (Sorra & Harris, 1993).

Whereas the primary projections from the hippocampus to other brain regions pass through the subiculum, a sizable projection exists from CA3 to the lateral septal nucleus. It has been asserted that nearly all the CA3 pyramidal cells project to both CA1 and the lateral septal nucleus (Swanson et al, 1980). Likewise, the lateral septal nucleus provides the primary source of input to CA3 originating outside of the hippocampal formation. These projections generally terminate on GABA-ergic synapses of CA3 interneurons.

Functional Structure of CA1 Region

Unlike CA3 which has extensive associative collateral projections, the neurons of CA1 exhibit very few such projections (Amaral et al , 1991). In contrast, CA1 cells generally project toward the subiculum. While some axons of CA1 pyramidal cells form recurrent excitatory synapses with other CA1 neurons, the incidence is substantially less than with CA3, and the positive feedback effects are much weaker (Radpour and Thomson, 1991). In general, CA1 does not possess the mass associative network that characterizes CA3.

Whereas CA1 receives a substantially smaller projection from the lateral septal nucleus than CA3, there is a significant projection that enters CA1 from the amygdaloid complex. Two major projections within the hippocampus formation originate within CA1. The first innervates the deep layers of the entorhinal cortex. Second, there is a topographically organized projection into the adjacent subiculum that unlike other similar projections within the hippocampus that form spatial gradients, terminates in relatively well-defined columns. It has been observed that within the subiculum, neurons projecting to different subcortical regions may be differentiated on the basis of their cell body diameters: those projecting to the thalamus are the smallest, nucleus accimbens are intermediate and hypothalamus are the largest.

Cellular Electrophysiology of the Hippocampus

A series of studies have characterized the basic response of hippocampus neural networks to electrical stimulation of either input pathways originating in the entorhinal cortex, mossy fibers arising from the dentate gyrus or Schaffer collaterals linking CA3 to CA1 (Langmoen & Andersen, 1981). Specifically, there is a momentary excitation of target neurons followed immediately by inhibition. The excitation leads the inhibitory response by a few milliseconds. The inhibition results as a product of feedfoward and recurrent feedback circuits with there being a fast and slow phase.

Due to the orderly arrangement of pyramidal cells within the hippocampus, extracellular electrical field potentials exhibit fluctuations that are consistent with the directional flow of neuronal currents. This is evidenced by symmetrical fields in the longitudinal axis and asymmetrical fields in the dendritic-somatic axis. Furthermore, the time course of fluctuations in the electrical field potential is approximately equivalent to the time course of synaptic currents (Johnston & Wu, 1995).

Recorded *in vitro* from a slice of hippocampal tissue applied electrical stimulation, a sequence of events may be observed that begins with a brief negative-going transient attributable to a volley of action potentials in the presynaptic fibers. The amplitude of this transient will vary in proportion to the number of activated presynaptic neurons. Next, there is a slower negative-going potential that reflects the summed postsynaptic response to a population of activated presynaptic neurons. The slope of this response corresponds to the intensity of synaptic activity. The current flowing into neurons will exit near their cell bodies causing a positive-going current at this level. Finally, with action potentials, the inward current flow will produce a negative-going extracellular potential with the amplitude proportionate to the number of neural units exhibiting action potentials.

It has been observed that the neurons in different regions of the hippocampus exhibit distinctive firing patterns (reviewed in Johnston and Amaral, 2004). The neurons of CA1 and the granule cell layer can fire repetitively at frequencies of several hundred hertz. In contrast, CA3 neurons fire in bursts of 5-10 action potentials with the interval between action potentials increasing and the amplitude decreasing as the pulse train progresses. Following the pulse train, there is a hyperpolarization of CA3 neurons that has been attributed to the activation of potassium channels due to an influx of calcium ions during the burst. Where mossy fibers synapse with CA3 neurons, due to the size of the fiber boutons with as many as 37 neurotransmitter release sites, larger EPSPs are produced than with other hippocampal neurons (Chicurel & Harris, 1992). This is the product of boutons each releasing around 5-10 quanta with a <1ms rise and about 5 ms decay constant (Jonas et al, 1993). Membrane events prompting glutamate release by mossy fiber boutons appear to involve a Ca^{2+} influx through voltage-gated Ca^{2+} channels and the Ca^{2+} induced release of Ca^{2+} from internal stores (Liang et al, 2002).

Intracellular recordings of CA1 neurons following activation of an afferent pathway indicate a sequence in which an excitatory postsynaptic potential (EPSP) is immediately followed by an inhibitory postsynaptic potential (IPSP). The amplitude of the EPSP can

range from less than 1 mV to several 10s of mV, with a rise (i.e. measured from 10% to 90% of peak) in the range of 5 ms when not accompanied by an IPSP. The EPSP decays consistent with the time constant of the cell membrane, generally on the order of 50 ms.

An EPSP of sufficient magnitude may evoke one or more action potentials. Typically, CA1 action potentials are initiated in the axon, and subsequently propagate to the axon terminals, as well as to the soma and dendrites. The threshold for initiating an action potential is variable such that in the absence of hyperpolarization, a depolarization of 20-25 mV from the resting membrane potential of -65 mV is adequate. However, the depolarization necessary to evoke an action potential will depend upon the prior history of the neuron (e.g. a lower threshold is observed following a prolonged hyperpolarization.

In contrast to CA1 EPSPs, the amplitude of IPSPs fall in a range from 1-10 mV with a fast component that peaks in 20-50 ms and decays in 100-500 ms and a slow component that peaks in about 100 ms and decays during a time period that could be greater than a second. Due to NMDA receptor responses, dendritic IPSPs typically exhibit a slower time course than somatic IPSPs. With CA1 neurons, spontaneous synaptic potentials are common. These potentials are the product of the postsynaptic response to the seemingly random firing and quantal release of neurotransmitter by presynaptic neurons.

A distinguishing feature of CA3 is the extensive presence of excitatory recurrent connections between pyramidal neurons. These connections are glutamatergic and promote the occurrence of large bursts of activity. Consequently, small shifts in the balance of excitatory to inhibitory activity in CA3 can produce rhythmic firing of large numbers of neurons that is the basis for epileptic seizures that begin in CA3 and readily spread to CA1.

The recurrent connections in CA3 also produce sharp waves involving synchronous bursts of a small group of neurons. Buzsaki (1989) has proposed that these sharp wave which are prominent during quiet wakefulness and slow-wave sleep may provide a basis for memory formation.

Neurochemistry of Hippocampal Neurons

Glutamate is the primary excitatory neurotransmitter in the hippocampus (Roberts et al, 1981) which is released by the entorhinal input fibers, mossy fibers, commissural-associational fibers, Schaffer collaterals and excitatory interneurons. Glutamate acts upon ionatropic receptors (Hicks et al, 1987). These receptors directly gate ion channels that are nonselective for monovalent cations Na^+ and K^+. Glutamate also acts upon metabotropic receptors which operate through an intermediary G-protein to either gate ion channels or activate second messenger molecules (Hicks et al, 1987).

The molecules constituting ionatropic and metabotropic receptors primarily consist of AMPA and kainite for fast EPSPs, and NMDA for slow-rising and slow-decaying EPSPs (Hollman & Heineman, 1994). Some AMPA and kainite and all the NMDA receptors are

permeable to Ca^{2+}. Additionally, NMDA receptors exhibit a voltage dependence such that when near the membrane resting potential, the channel is blocked by Mg^{2+} from extracellular influences (Mayer et al, 1984). Various combinations of AMPA, kainite and NMDA receptors have been found throughout the excitatory synapses of the hippocampus with metabotropic receptors appearing on both the postsynaptic and presynaptic side of glutamatergic synapses (Shigemoto et al, 1997). Cells with both AMPA and NMDA receptors display a mixture of fast and slow responses depending on the membrane potential and whether ion channels are blocked by Mg^{2+}.

Acetylcholine (Ach) is a second excitatory neurotransmitter found in the hippocampus (reviewed by Johnston and Amaral, 2004). Presynaptic receptors that respond to Ach modulate the release of glutamate by excitatory synapses. On interneurons, Ach receptors modulate inhibition. In both cases, receptors are ionatropic nicotinic receptors. Additionally, Ach also acts upon metabotropic muscarinic receptors. Presynaptic muscarinic receptors diminish release of glutamate and have an inhibitory effect. On postsynaptic side, muscarinic receptors decrease calcium conductance across the cell membrane producing a postsynaptic depolarization effect. Activation of muscarinic receptors also reduces the hyperpolarization that follows a train of repetitive firings.

Norepinephrine, dopamine, serotonin and several neuropeptides have also been observed to exhibit excitatory effects on hippocampal neurons(Frotscher et al, 1988). Generally, this occurs through G-proteins with the effect being neuromodulatory in nature.

The primary inhibitory transmitter in the hippocampus is GABA (Roberts et al, 1976). The actions of GABA on ionatropic receptors leads to an increased permeability to Cl^- producing a hyperpolarization. GABA additionally decreases postsynaptic input resistance lessening the effectiveness of excitatory inputs. With metabotropic receptors, GABA acts through G-proteins to open both presynaptic and postsynaptic K^+ channels. On the postsynaptic side, GABA produces a slow onset, and slow decay, hyperpolarization of the cell membrane. The presynaptic effect of GABA is to diminish the release of glutamate, as well as GABA.

Synaptic Plasticity in the Hippocampus

It has been observed that with glutamatergic excitatory neurons of the hippocampus, either repetitive trains of synaptic activity or specific parings of presynaptic and postsynaptic firings can produce either an increased responsiveness to similar stimulus conditions (i.e. *long-term potentiation*), or decreased responsiveness (i.e. *long-term depression*). Under differing experimental conditions, the effect may persist for durations ranging from minutes to weeks.

Demonstrations of long-term potentiation begin with an *induction phase*, during which one or more trains of high-frequency (25-200 Hz) stimulation is presented. During the subsequent *expression phase*, test stimuli evoke EPSPs of 50-200% greater amplitude than baseline recordings. In contrast, low-frequency (1-5 Hz) stimulation applied for several minutes results in reduced responsiveness that can persist for over an hour. It has

been shown that long-term potentiation can be effectively induced by supplying brief pulse trains timed to correspond with the naturally occurring theta (4-7 Hz, with 5 Hz being a common target frequency) cycles of the hippocampus EEG (Magee & Johnston, 1997).

The timing of presynaptic activity can determine whether presynaptic activity produces potentiation or depression (Bi and Poo, 2001). Where the postsynaptic action potential occurs 0-20 ms following the presynaptic action potential, potentiation is induced. In contrast, should the postsynaptic action potential precede the presynaptic action potential by an interval up to 100 ms, the effect is to induce a depression of postsynaptic activity.

A primary mechanism underlying long-term potentiation involves NMDA receptors. Specifically, long-term potentiation has been associated with an increased intracellular concentration of Ca^{2+}. This may occur through an influx of Ca^{2+} through NMDA receptors and/or voltage-gated Ca^{2+} channels (Johnston and Amaral, 2004).

Candidate Experimental Paradigms

Biologically Significant Events

Tsien and colleagues (Lin et al 2005; Wittenberg & Tsien, 2002) have demonstrated differential patterns of activation of neurons in the CA1 region of the hippocampus associated with exposure to alternative startle experiences (i.e. air-blow to back of the neck, free-fall while confined within a container and earthquake-like cage shake), and the experience of a given startle experience in alternative environmental contexts (i.e. different containers).

For these studies, a computer–controlled mechanical device generated a controlled simulation of the three startle experiences with each animal exposed to seven repetitions of each startle experience. Recordings were measured from a 96-channel electrode array positioned within the CA1 pyramidal cells of the hippocampus. Depending on the animal, this arrangement allowed recordings from 138-260 individual CA1 units. To estimate the firing rates of individual neurons, four measures were obtained:

(1) f_{pre} ; firing rate for 500ms prior to onset of startle experience
(2) $f_{poststartle}$; firing rate for 500ms from onset of startle experience
(3) f_o ; global mean response frequency

and the neural response, R_n, calculated as follows:

$$R_n = (f_{poststartle} - f_{pre}) / (f_o + f_{pre})$$

Most of the CA1 units measured exhibited significant changes in their firing rates in response to the startle experiences, although there were some CA1 units for which there

were no changes in their firing rates. In general, changes in firing rates could be divided into four response modes:

(1) Transient increase (13.5% of all unites recorded)
(2) Transient decrease (1.9% of all unites recorded)
(3) Prolonged increase (31.7 % of all unites recorded)
(4) Prolonged decrease (1.4% of all unites recorded)

Transient changes in firing rate could be as short as 250 ms, whereas prolonged changes could extend up to 40s in duration. Summed across all units recorded, the ratio of units that showed an increase in firing relative to a decrease in firing was ~14 to 1. With little exception, CA1 units exhibited the same response mode to a given startle experience. Some of the units responded to all three startle experiences, whereas other units only responded to a specific startle experience. Furthermore, CA1 units were observed that responded to two of the three startle experiences, but not the third, with some of these CA1 units responding the same to each startle experience and others responding differently.

These findings suggest that for a specific type of biologically significant experience, a recurrent pattern of response occurs in CA1 units. Across a population of CA1 units, the change in firing rate for a given unit will have similar temporal dynamics for each exposure to a given experience, although different units will exhibit different dynamics. A given unit may respond to more than one type of experience, with the dynamic response for any pair of experiences being similar or different. Thus, it may be hypothesized that it is the combination of CA1 units, and the experience-specific dynamic response of each unit, that differentiates various types of biologically significant experiences.

Further investigation using the above paradigm presented a given biologically significant event (i.e. air-blow to back of the neck and free-fall while confined within a container) to animals in different environmental contexts (i.e. different containers). It was observed that some CA1 units responded similarly to the event in each environmental context, however other CA1 units responded in one environmental context, but not others. Thus, it appears that CA1 coding of events combines the type of experience with the environmental context, suggesting an integrated representation of both facets of events.

For the analysis underlying the above findings, each trial was represented as a feature vector in which dimensions corresponded to the individual CA1 units. The values assigned to each dimension corresponded to neural response (i.e. R_n) measurements. Non-responsive CA1 units were excluded from the analysis based on a criterion threshold of $R>0.5$. Applying this threshold, approximately 35% of the CA1 units were considered to be responsive. Multiple discriminant analysis was computed to find the maximally discriminating subspace. When individual startle experiences were plotted as points within this space, clearly defined clusters emerged that differentiated each type of startle experience from each other, as well as from a rest condition. These patterns did not change when previously excluded non-responsive units were added to the analysis

suggesting that non-responsive units are not a component of the neural coding of experiences.

Finally, classifiers were trained to categorize events corresponding to the clusters identified for each type of startle experience. When assessed with a unique set of trial data (i.e. data not utilized in training the classifiers), classifiers exhibited an approximately 90% accuracy in categorizing experiences. Furthermore, it was demonstrated that this level of accuracy could be achieved using only the ten CA1 units most responsive to each type of startle experience. This suggests the potential to accurately classify experiences based on detailed measurement of CA1 neural units.

A sliding-window of 1s width was used to chart the trajectory of neural responses within the MDA-defined subspace for the duration of the experiment. It was observed that the neural response would begin in the area corresponding to the rest state. Then, with the onset of a startle event, the neural response would generally follow a planar trajectory into the appropriate space for the startle experience, after which the neural response would return to the area corresponding to the rest state. Moreover, between trials, it was observed that the neural response would intrinsically return to areas corresponding to startle experiences, following direction-specific paths with time profiles and geometric shapes characteristic of paths observed with the actual startle experiences.

Functional Representation of Space

Several studies have established that the pyramidal cells of the hippocampus exhibit "place fields" whereby units respond with their maximum firing rate when the animal is in a specific area of the environment (O'Keefe & Nadel, 1978). These findings have been interpreted to indicate that there is a internalized model of the external environment coded within the neural units of the hippocampus. More recent findings (Moser & Paulsen, 2001) have shown that the extent of neural units that respond to a given location is proportionate to the functional significance of different locations (e.g. disproportionately larger numbers of units should respond to places in which a food reward is delivered).

The "watermaze" has been an effective paradigm for studying the functional representation of space within the hippocampus. With this paradigm, an animal is placed in a water-filled pool and must swim to keep its head above water. Typically, the pool contains one or more submerged platforms that provide the animal a perch to escape the water. Generally, placed in this environment, an animal will vigorously explore the pool until it locates the submerged platform. During this exploration, it is believed that an internalized model of the environment is established that may be used on subsequent trials to quickly navigate to the platform. Accordingly, over a series of trials, the amount of time the animal explores the environment will quickly diminish such that eventually no matter where the animal is placed within the pool, they will quickly navigate to the submerged platform. Various studies have shown that animals can utilize a variety of cues to acquire a model of this environment (e.g. markings on the walls of the pool, geometrical relationships between landmarks) and can readily adapt their model if the

spatial layout of the environment is altered from one series of trials to the next (Moser & Paulsen, 2001.

The traditional watermaze is not methodologically suited for studying the proportionate representation of space within the hippocampus because after the first few trials, an unequal distribution of time is spent in different regions of the maze since animals only traverse those regions along a path from their start position to the platform. To overcome this problem, Moser and colleagues (Hollup et al, 2001)) have utilized an annular watermaze. This is a circular, doughnut-shaped, watermaze in which the animal must swim one or more times around the maze before the platform is made available, at a variable or fixed location. This configuration assures that the animal traverses the extent of the watermaze at least once during each trial. Cues (i.e. simple markers) placed outside the watermaze provide a basis for the animal's spatial orientation.

In studies by Moser and colleagues, electrodes were implanted in the CA1 region of the dorsal hippocampus, midway between the CA3 and subiculum. This implant consisted of a vertical, cannula-enclosed wire that provided multiple leads 0.3-0.5 mm above one another. To locate the desired CA1 units, the animal was placed in a 2.5 x 4.0 m room and allowed to either rest on a sawdust-filled platform or walk abut the enclosure. During this time, electrodes were lowered in approximately 50 μm steps until multiple cells were recorded spiking with an amplitude greater than 100-150 μV. At each lead, a differential was measured with respect to a more shallow reference lead and spikes identified when the signal amplitude exceeded a threshold manually adjusted to be three times the noise level of the corresponding channel.

Cluster-based algorithms were utilized to attribute spikes to individual cells on the basis of voltage and temporal criterion. Pyramidal cells were distinguished from interneurons based on the extracellular action potential (>0.3 msec), firing pattern (complex spikes) and low average firing rate. This allowed a total of 132 CA1 pyramidal units to be distinguished. Spikes attributed to a single neuron were autocorrelated to assess the quality with which clusters isolated individual units and identify complex spikes, whereas spikes attributed to adjacent neurons were cross-correlated to assure the early and late spikes of a complex-spike burst were not mistakenly assigned to different clusters. The average firing rate for a CA1 unit was calculated by dividing the total number of spikes by the duration of the recording period.

Analyses of neural activity utilized data from 60s epochs beginning once the animal was placed in the watermaze and ending prior to the platform becoming available. During this time, animals typically swam 4-5 circuits of the watermaze, in their preferred swimming direction. The average firing rate for CA1 units was 1.46 Hz, which was readily distinguished from interneurons which had an average firing rate of 12.1 Hz.

To assess place fields, the annular watermaze was divided into 6 segments of 60°, and the firing rate determined for each CA1 unit, discarding trials in which the animal did not swim in their preferred direction. Each CA1 unit was assigned a field location based on the segment in which the maximum average firing rate was recorded for that unit. Across

a series of trials, most CA1 units (70%) exhibited a stable place field (i.e. maximally responded to a single segment of the watermaze), with there being units that responded to each segment of the watermaze. In contrast, interneurons fired over all segments of the watermaze exhibiting no trial-to-trial selectivity.

A disproportionate number of CA1 units responded to the segment with the platform (n=27), than other segments (n=8-14), with the second greatest number of units (n=14) responding to the segment immediately preceding the segment with the platform. Subsequent analysis found a similar disproportionate representation when the watermaze was further divided into 12 segments of 30°. Furthermore, both analyses revealed that substantially fewer units responded to the segment immediately following the segment with the platform. Thus, prior to the platform being made available, the animal would swim the circumference of the watermaze with the number of CA1 units responding to each segment increasing as they approached the segment in which the platform would appear with the maximum number in that segment. Then, once passed the segment in which the platform would appear, fewer CA1 units responded as the animals traversed the subsequent segments. In contrast to these findings, in separate analysis in which the location of the platform was randomly varied, a roughly proportionate number of CA1 units responded to each segment of the watermaze. Finally, it should be noted that once the platform was made available, there was a precipitous reduction in the number of responding CA1 units (i.e. <30% of the number that responded while swimming), although some units exhibited an increased firing rate once the animal had climbed onto the platform.

Differential Representation of Different Types of Information within the Hippocampus

Based on the remapping that occurs within hippocampal place fields in response to changes in a previously learned environment, Moser and colleagues hypothesized that different types of information are represented differently within the hippocampus (Leutgeb et al, 2005). It was noted that depending on experimental conditions, the remapping of place fields could involve either (1) changes in magnitude, (2) shifts in position or (3) emergence oo abolishment of individual place fields. In general, it was proposed that two types of representations occur simultaneously and are reflected in the differential remapping of place fields. In *rate remapping*, the locations of place fields remain the same, however there are changes in firing rates. Differential representations based on different firing rates were suggested as the mechanism by which memories are stored for different experiences within the same location. In contrast, *global remapping* involves both changes in the distribution of place fields as well as the associated firing rates. It was argued that this remapping allowed similar experiences in different locations to be distinguished from one another.

The above hypotheses were based on research in which a vertical, cannula-enclosed wire was implanted in the dorsal hippocampus, similar to the arrangement described in the preceding section. Summing across ten animals, 487 cells were recorded from CA1 and 330 from CA3.

In the first experimental condition, animals were placed in the same room on each trial, however the room was varied with respect to its shape (circular or square) and color (black or white). Analysis of recordings utilized *rate maps* in which a series of grids were constructed to represent the physical space. Each of these grids were populated with firing rates recorded for an individual neural cell when the animal was in the corresponding location of the physical space. This produced a three-dimensional data structure in which the x and y axis mapped to the x and y dimensions of the physical space and the z axis represented individual cells. Using this data structure, a series of population vectors were derived that each consisted of the distribution of mean values along the z-axis for a point location. The entire set of population vectors for a given trial of the experiment provided the basis for trial-to-trial ensemble comparisons.

For the first experimental condition in which each trial was recorded in the same room, but the shape and color of the space were varied, there was a significant spatial correlation in firing for both CA1 and CA3 units. This correlation suggests a consistent neural representation of the physical space that persists despite changes in the layout and appearance of the space. In contrast, while the mapping of location to firing remained constant from trial-to-trial, the distribution of firing rates changed, particularly with the CA3 units. The median rate of change between the black and white conditions for CA3 units was 3.9x, whereas the difference between the square and circle conditions was 6.9x. Based on this finding, it was hypothesized that differential firing rates serve as the means for representing different experiences within a given spatial environment.

With the second experimental condition, animals were placed in geometrically identical enclosures of the same color, however data was recorded in different rooms. In this condition, both the firing locations and firing rates varied, with there being a 10x difference observed for CA3 units. Thus, it was proposed that the neural representations of different locations vary with respect to both the make-up of neural units forming the ensemble and the firing rates of those neural units.

Representation of Prospective and Retrospective Memory

Concepts concerning the representation of memory within the hippocampus generally involve an integration of memory for locations (i.e. place fields) and memory for experiences, or episodic memory. Investigations by Ferbinteanu and Shapiro (2003) have examined the manner in which the integration of memories for locations and experiences give rise to prospective (i.e. capability to anticipate future states) and retrospective (i.e. capability to recollect related past states) memory processes.

These experiments utilized a "+" shaped maze. Opposite arms of the maze were designated as the start locations, with the two remaining arms of the maze alternating as the goal locations. An animal was placed in the maze at the start location, traveled to the intersection, and turned either left or right to locate the goal (i.e. food reward). Thus, on each trial, the animal made one of four possible journeys: (1) from north to east, (2) from north to west, (3) from south to east and (4) from south to west.

"Journeys" were distinguished from routes in that a journey was defined on the basis of the start and end position, and could be accomplished following multiple routes (e.g. an animal could go directly from the start to the end following an L trajectory, or could first turn and travel a short distance into the wrong arm of the maze before turning around to enter the correct arm).

The experiment involved five rats for which electrodes were implanted within CA1. To identify place fields, the maze was divided into a 28 x 28 array and the firing rate of individual units identified for each grid unit by dividing the total number of spikes by the time spent in the grid unit. Place fields were observed throughout the maze with some focused on a specific arm of the maze and others responding to more than one arm. Three hundred and seventy-eight complex spiking cells with distinct waveforms were identified. Of these cells, 278 had place fields within a single arm of the maze, with 153 on the start arms of the maze and 125 on the goal arms. For purposes of analysis, these numbers were increased by including cells that responded to more than one arm (e.g. responded to both the east and west arm) providing a total of 525 cells, with 240 in the start arm and 285 in the goal arm.

Journey-dependent units were common. These units responded to a location in the maze (i.e. exhibited a place field) only for a specific journey. For example, a unit might respond to locations in the east arm only after starting in the north arm of the maze. Here, activity was specific to a journey consisting of a beginning and end, with activity being retrospective in that it was contingent upon the location in which the animal started. Of the cells with place fields in the goal arm 67% were journey-dependent. In contrast, other units exhibited the opposite pattern where location-specific firing was contingent on the destination. For example, activity might occur in the south arm only for journeys in which the destination was the west arm. These units responded in a prospective manner in that their activation was a function of the anticipated end state for a given journey. Of the cells with place fields in the start arm, 57% were journey-dependent. Additionally, journey-dependent cells responded similarly regardless of the trajectory taken on a given trial, or whether the animal initially entered the wrong arm of the maze.

Long-Term Potentiation in Memory Acquisition

Whereas the previously described experimental paradigms have addressed the representation of memory within the hippocampus, an alternate area of research concerns the formation of hippocampus memories. It has been generally accepted that memory formation involves some form of long-term potentiation whereby activation of neural units alters their chemical and structural make-up to create an enhanced likelihood of the unit responding if presented similar conditions. Research by Buzsaki and colleagues (Dragoi, et al, 2003) has illustrated the system-level characteristics of long-term potentiation.

To establish place fields, animals were exposed to a rectangular track which they ran in either a clockwise or counterclockwise direction. Activity was recorded from 229 CA1 units and 173 CA3 units and place fields identified, with many exhibiting directional-

dependence (e.g. unit only responded when the animal entered a specific area of the track traveling in a clockwise direction). Long-term potentiation was induced by delivering a series of high frequency pulses (i.e. trains of 10 pulses delivered at 200 Hz) immediately after removing the animal from the test track.

Long-term potentiation was assessed by measuring the response evoked by a low frequency stimulus (10-20 pulses at 0.1 Hz) recorded in CA1 and CA3 units. The response to long-term potentiation varied across units with some exhibiting increased and others decreased responsiveness. One neuron which had exhibited a place field for the same area in both clockwise and counterclockwise runs prior to long-term potentiation, acquired a new place field in counterclockwise runs while retaining the same place field in clockwise runs. In contrast, a second neuron with a similar response prior to long-term potentiation had no change in its place fields. With a third neuron, prior to long-term potentiation there was only a place field for the counterclockwise direction. After, long-term potentiation this neuron exhibited a different place field that appeared for both the clockwise and counterclockwise directions. Then, after 5.5 hours when activity had returned to levels observed before 1ong-term potentiation, there was a reinstatement of the previous place field. The latter finding was interpreted to suggest that synaptic weights are a function of the strongest input to a given unit and once the residual effects of long-term potentiation weaken, the original weightings may re-emerge.

In general, of the cells that responded to long-term potentiation, the majority showed a unidirectional shift in place fields (e.g. only in the clockwise direction). This indicates that the representation of different contexts by a given neural unit--in this case, different directions--is controlled by different inputs to that neuron. Consequently, activity in input units corresponding to one direction may have caused a shift in the unit's place field for that direction, but left the place field for the opposite direction unchanged.

For cells with overlapping place fields (i.e. two units that exhibited place fields for the same area), 30% dissociated after long-term potentiation responding with place fields to different areas. Based on this observation, it was proposed that long-term potentiation effects the functional connections between neural units. This occurred with there being no significant change in the overall firing rate of units indicating a balance between units that increased and decreased their firing rates. Furthermore, long-term potentiation did not alter the size of areas represented by place fields.

The magnitude of changes in place fields was quantified on the basis of the distance between the area originally represented by the place field and the area represented after long-term potentiation. A positive correlation was found between the magnitude of the evoked response recorded for units and the magnitude of changes in place fields This suggest that due to either a greater convergence of input units on a given neuron and/or a greater plasticity to long-term potentiation, increasing responsiveness to long-term potentiation produces more significant changes in representations following long-term potentiation.

Finally, there was a consideration of the relationship between the strength of response exhibited by units in-field (i.e. when the animal was within the area represented by a place field) and the magnitude of changes in place fields following long-term potentiation. There was a negative correlation such that units showing stronger responses in-field were more resistant to changes to their place field. Similarly, it was found that units with slower firing rates in-field appeared more susceptible to long-term potentiation than units with faster firing rates. These observations suggests the strength of place field representations may vary with their persistence being positively related to the strength of their representation.

Conclusion

This report has provided a summary of relevant research cocnerning the anatomical structure, connectivity and cellular neurophysiology of the hippocampus as it pertains to memory storage and retrieval. The intent is that these materials will provide a reference for future activities in which nano-scale techniques are developed for studying memory processes through in vivo measurements.

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