CA3 and Memory/Review

Using immediate-early genes to map hippocampal subregional functions

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Different functions have been suggested for the hippocampus and its subdivisions along both transversal and longitudinal axes. Expression of immediate-early genes (IEGs) has been used to map specific functions onto neuronal activity in different areas of the brain including the hippocampus (IEG imaging). Here we review IEG studies on hippocampal functional dissociations with a particular focus on the CA3 subregion. We first discuss the cellular functions of IEGs and the brain system interactions that govern their dynamic expression in hippocampal neurons to provide a more solid framework for interpreting the findings from IEG studies. We show the pitfalls and shortcomings of conventional IEG imaging studies and describe advanced methods using IEGs for imaging of neuronal activity or functional intervention. We review the current IEG evidence of hippocampal function, subregional-specific contribution to different stages of memory formation, systems consolidation, functional dissociation between memory and anxiety/behavioral inhibition along the septotemporal axis, and different neural network properties of hippocampal subregions. In total, IEG studies provide support for (1) the role of the hippocampus in spatial and contextual learning and memory, (2) its role in continuous encoding of ongoing experience, (3) septotemporal dissociations between memory and anxiety, and (4) a dynamic relationship between pattern separation and pattern completion in the CA3 subregion. In closing, we provide a framework for how cutting-edge IEG imaging and intervention techniques will likely contribute to better understanding of the specific functions of CA3 and other hippocampal subregions.

The hippocampus lies at the crossroads of information processing in the brain. Information arrives here from most of the cortex as well as from many subcortical regions either directly, or indirectly, via the entorhinal cortex. Activity-dependent lasting potentiation of synaptic transmission, postulated as a mechanism of memory formation in the brain (Hebb 1949), was first observed in the hippocampus (long-term potentiation or LTP; Bliss and Lomo 1973). Place cells, neurons with spatially selective activity, were discovered in the hippocampus (O’Keefe and Dostrovsky 1971) and suggested to form a neural substrate of “cognitive maps” (O’Keefe and Nadel 1978). Indeed, damage to the hippocampus disrupts navigation that requires memory for spatial relationships, but not navigation to a visible cue in a water maze (Morris et al. 1982). These findings suggested that the hippocampus plays a specific role in spatial memory. In humans, damage to the hippocampus results in severe deficits in declarative memory (Scoville and Milner 1957; for review, see also Squire et al. 2004). The hippocampus is also necessary for memory of the context in which events occur (Kim and Fanselow 1992; Anagnostaras et al. 1999; Biedenkapp and Rudy 2007; Lehman et al. 2007; Wiltgen and Silva 2007), consistent with a critical role of the hippocampus in context-rich episodic memory (Vargha-Khadem et al. 1997; Winocur et al. 2007).

The hippocampus consists of distinct subregions along its transversal axis (dentate gyrus [DG], Cornu Ammonis [CA1, CA2, and CA3]), as well as different segments along its longitudinal (septotemporal) axis. The CA3 subregion represents a “bottleneck” of hippocampal processing where information flow converges from >1,000,000 dentate granule cells on only ~220,000 neurons before expanding again on ~400,000 CA1 neurons in a rat (Rapp and Gallagher 1996). A unique, recurrent synaptic connectivity makes the CA3 ideally suited for an autoassociative neural network. Numerous computational models suggested that the putative functions of such a network architecture—including pattern completion, memory encoding, temporary storage, and retrieval (Marr 1971; McNaughton and Morris 1987; McClelland and Goddard 1996; Rolls and Treves 1998; Nakazawa et al. 2002), rapid single-trial learning (Nakazawa et al. 2003), or automatic recording of attended experience (Morris and Frey 1997)—may well serve to encode representations of context of events and episodic memories. Evidence for each of these functions has been obtained using a variety of approaches including lesions, electrophysiological recordings, transgenic mice, and immediate-early gene (IEG) imaging. In addition, different functions have been suggested for different longitudinal segments of the hippocampus (Bannerman et al. 2004). This review describes the contributions of IEG techniques to investigations of hippocampal function and subregional dissociations, with an emphasis on CA3.

IEGs are widely believed to play a critical role in transformatory activity in neural circuits into long-term memories in the brain (Lanahan and Worley 1998; Jones et al. 2001; Guzowski 2002). The molecular mechanisms of their cellular functions and their involvement in synaptic plasticity continue to be a matter of intense research. In an independent approach, IEGs have also been used as activity reporters, making use of their rapid induction by behavioral experience (IEG imaging). We will consider the evidence provided by IEG studies in the context of studies using other approaches that shaped the current ideas in the field.

We start this review by briefly evaluating current evidence of the cellular functions of IEGs and their role in hippocampal synaptic plasticity and memory consolidation to provide back-
ground for interpreting the IEG imaging studies. Then, we provide an overview of the regulation of IEG expression in the hippocampus by behavior and defined brain systems interactions. We describe the basic methods using IEGs for imaging and functional studies, and point out the caveats limiting interpretations of these studies. We then discuss the IEG imaging studies mapping hippocampal function and differences in hippocampal subregional involvement during memory acquisition, consolidation, and retrieval. Several studies reported subregional and temporally selective IEG activation patterns in support of the standard theory of systems consolidation—progressive hippocampal disengagement as memories become increasingly “remote” in time. We review the evidence for systems consolidation derived from IEG imaging studies. Next, we consider studies suggesting possible dissociation of hippocampal function between memory and anxiety. We also present evidence for neuronal ensemble dynamics and network properties of hippocampal subfields CA3/CA1 converging from advanced IEG imaging studies, unit recordings, genetic ablation and theoretical modeling approaches. Finally, we summarize findings from these studies on differential tasks of hippocampal function and outline possible future directions of using the IEG methods to study the respective roles of hippocampal subregions.

Molecular and cellular functions of IEGs and their role in synaptic plasticity and memory consolidation

Immediate-early genes (IEGs) are rapidly expressed following patterned synaptic stimulation, such as maximum electroconvulsive shock (MECS), high-frequency stimulation inducing LTP, and also after behavioral experience (Guzowski et al. 1999, 2001; Vann et al. 2000a; Hall et al. 2001). IEGs may be categorized into two functional classes: (1) regulatory transcription factors (RTFs), which control transcription of other “downstream” genes, and (2) effector IEGs, which directly influence cellular functions (Llanahan and Worley 1998). By virtue of their ability to regulate transcription, RTFs are well situated to globally regulate cell function and possibly induce metaplastic states (Clayton 2000; Guzowski 2002). In contrast, effector IEGs have a wide range of cellular functions, including those related to (1) cellular growth (BDNF, Narp), (2) intracellular signaling (RheB, RGS-2, and Homer 1a), (3) synaptic modification or other structural changes (Arc, Homer 1a, Narp, tissue plasminogen activator (TPA), BDNF), or (4) metabolism (COX-2) (see Llanahan and Worley 1998; Guzowski 2002; and references therein). Out of the variety of IEGs, only several have been used commonly to map hippocampal activity onto behavior. These are the RTFs c-fos, c-jun, and zif 268 and effector IEGs Arc and Homer 1a. We will briefly summarize data showing a role for the RTF IEGs zif/268 and c-fos in synaptic plasticity and memory consolidation, before giving a detailed overview of the cell biological functions of the effector IEG Arc and its role in memory.

Late-LTP and long-term memory were impaired in various behavioral tasks including the spatial water maze in mice with genetic ablation (knockout) of the zif/268 gene, even though the water maze impairments could be overcome with extended training (Jones et al. 2001). Mice with genetic ablation of the c-fos gene limited to the central nervous system also exhibited impaired LTP, which could be restored, however, by repetitive stimulation. These mice also displayed impaired contextual, but not cued fear conditioning, and impaired memory retrieval in the spatial water maze despite good performance during training (Fleischmann et al. 2003).

In addition to studies using knockout mice, experiments using antisense oligodeoxynucleotides (AS ODNs) also confirmed a role for c-fos in memory consolidation. AS ODNs are short (18–20 bases) synthetic chains of DNA encoding antisense sequence of a particular IEG mRNA that can be delivered with temporal and regional precision into structures such as the hippocampus. Inside the cell, the AS ODNs base-pair with their target mRNA, block its translation, and increase turnover. Intrahippocampal infusions of AS ODNs against c-fos mRNA impaired consolidation of inhibitory avoidance (Guzowski and McGaugh 1997) and spatial water maze training (Guzowski 2002). Intrahippocampal infusion of c-fos AS ODNs also impaired consolidation of socially transmitted food preference (STFP) (Countryman et al. 2005).

The effector IEG Arc (activity-regulated cytoskeleton-associated protein; Lyford et al. 1995; also known as Arg 3.1; Link et al. 1995) has attracted interest in the field of learning and memory for several reasons. Like other IEGs, Arc gene expression is increased dramatically by LTP-inducing stimuli and behavior, and, more importantly, its expression in hippocampus correlates with performance in a hippocampus-dependent task (spatial water maze) (Guzowski et al. 2001). Only principal neurons were found to express Arc mRNA and protein following behavioral experience in hippocampus, neocortex, and striatum (Vazdarjanova et al. 2006). Following induction, Arc mRNA is rapidly transported to activated portions of dendrites (Steward et al. 1998; Moga et al. 2004), and this targeting, as well as behavioral induction, is dependent on NMDA receptor activation (Steward and Worley 2001). Translation of the dendrically localized Arc mRNA can be regulated by BDNF and NMDA receptor activation (Yin et al. 2002) and involves the mTOR pathway (Takei et al. 2004). Together, these data suggest the necessary mechanisms for Arc to modify the function of discrete synapses in activated neurons.

Interaction of Arc with microtubule associated protein 2 (MAP2) was suggested to play a role in cytoskeleton destabilization in studies of transfected primary neurons (Fujimoto et al. 2004). Such destabilization could be important for dendritic remodeling associated with lasting changes in synaptic efficacy. Another in vitro study suggested that Arc interacts with calcium-calmodulin kinase II (CaMKII), and that this interaction promotes neurite outgrowth in cultured neuroblastoma cells (Donai et al. 2003). Arc was also found in NMDA receptor complexes, consistent with a role in LTP and memory (Husi et al. 2000). In the most detailed account of its cellular function, Arc has been shown to facilitate AMPA receptor endocytosis in cultured neurons via interaction with dynamin and specific isoforms of dynorphin (Chowdhury et al. 2006). Accordingly, Arc overexpression reduced APMA receptor-mediated synaptic transmission in organotypic slice culture neurons (Rial Verde et al. 2006). These findings suggested that Arc may mediate homeostatic scaling of synaptic AMPA receptors (Shepherd et al. 2006).

Inhibition of Arc protein expression in dorsal hippocampus by local infusion of antisense oligodeoxynucleotides (AS ODNs) disrupted maintenance of LTP, but it did not affect its induction. In addition, intrahippocampal infusion of Arc AS ODNs strongly impaired consolidation of memory for spatial water maze training, without affecting task acquisition or short-term memory (Vazdarjanova et al. 2006). Control infusion of ODNs with the same nucleotide composition but with no homology with the target RNA (scrambled ODNs) had no effect (Guzowski et al. 2000). Similar findings were reported from mice with genetic ablation of the Arc gene (Plath et al. 2006). The mice showed long-term memory impairments with relatively spared acquisition or short-term memory in several explicit and implicit learning tasks including spatial and cued water maze, contextual and cued fear conditioning, conditioned taste aversion, and novel object recognition. They also displayed impaired long-term plasticity with enhanced early- and absent late-LTP and absent stable long-term depression. Together, these find-
ings indicate that Arc plays an essential role in the stabilization of activity-dependent synaptic plasticity and synaptic consolidation (Guzowski et al. 2000; Guzowski 2002; Plath et al. 2006).

Although not functionally tested at present, the effector EGF Homer 1a may also play a role in modification of hippocampal synaptic plasticity and memory consolidation. Homer 1a is a synaptically regulated isoform of the Homer 1 gene (Brakeman et al. 1997; Bottai et al. 2002). Neurons expressing Homer 1a following a behavioral experience are the same population that express Arc (Vazdarjanova et al. 2002)—these two dendritic effector proteins are coexpressed in the same neurons following experience. Overexpression of Homer 1a in dissociated hippocampal neuronal cultures decreased the density and size of dendritic spines, the size of PSD-95 clusters, the number of NMDA receptor clusters, and the level of surface AMPA receptors, and also diminished postsynaptic AMPA and NMDA receptor synaptic currents (Sala et al. 2003).

At this point, it is worth emphasizing an important distinction between synaptic and systems consolidation. Generally, the term “memory consolidation” refers to transformation of newly formed memories not from an initially weak and disruption-sensitive state into more permanent and persistent long-term memories. This process, however, may occur at several levels. Synaptic consolidation occurs on the level of synapses or neurons, and it results in transformation of short-term memories to long-term memories (hours to days or even months) (McGaugh 2000; Frankland and Bontempi 2005). Systems consolidation occurs between different brain systems, particularly between hippocampus and neocortex, and it results in long-lasting memories (months to lifetime) (Squire and Alvarez 1995). Although primarily implicated in synaptic consolidation, IEGs may act to support continued synaptic strengthening during overtraining, retrieval, or off-line processing in the course of systems consolidation (Ribeiro and Niccolés 2004). Throughout this review, by “memory consolidation” we mean the synaptic process, unless explicitly mentioning “systems consolidation.”

In conclusion, the functional studies targeting RTF IEGs and Arc demonstrate a critical importance of IEG expression for long-term memory formation and implicate IEG expression in coupling neuronal activity with lasting changes in synaptic efficacy underlying long-term memory (Tischmeyer and Grimm 1999; Guzowski et al. 2000, 2001; Jones et al. 2001; Guzowski 2002; Davis et al. 2003). Thus, IEG imaging methods may reveal not only which brain regions are active during a specific behavior, but also which areas might be undergoing neural plasticity.

Regulation of hippocampal IEG expression by behavior and brain systems interactions

IEGs are induced in the hippocampus after behavioral experience in a novel environment (Arc; Guzowski et al. 1999; Vazdarjanova et al. 2002); in a water maze (Arc, zif268, c-fos; Guzowski et al. 2001); on a radial arm maze (c-Fos; Vann et al. 2000a); or in cued, but not cued, fear memory retrieval (zif268; Hall et al. 2001). The proportions of Arc-expressing neurons in CA1 and CA3 after exploration of an environment, the selectivity of the activated ensembles to specific spatial contexts (Guzowski et al. 1999; Vazdarjanova and Guzowski 2004), and the reliability of Arc expression after repeated experience in the same environment (Guzowski et al. 2006) strongly resemble the characteristics of location-specific activity of hippocampal place cells, suggesting that neurons expressing Arc after exploration of an environment are place cells that expressed a firing field in that environment (Guzowski et al. 2004). Both location/context-specific neuronal activity and IEG expression are likely to derive their characteristics from the activity patterns of inputs to the hippocampus.

The hippocampus receives input, either directly or indirectly, from much of the rest of the brain. Highly processed cortical input arrives via the perforant path projection from the entorhinal cortex. The main subcortical input comes to the hippocampus from medial septum via the fornix, and emotional and motivational modulatory input comes from the amygdala (Amaral and Witter 1995; Píkkaiainen et al. 1999). Unilateral lesions of entorhinal cortex abolished behavioral induction of Arc expression in both ipsi- and contra-lateral dentate granule cells and to a lesser extent also in the CA3 and CA1 fields (Temple et al. 2003). This finding points to the physiological importance of commissural projections to the dentate gyrus either directly from the contralateral entorhinal cortex or from the mossy cells in the polymorphic layer of the contralateral dentate gyrus (Amaral and Witter 1995). Behavioral induction of Arc reappeared with a time course consistent with reinnervation, but baseline expression remained somewhat reduced (Temple et al. 2003). These data are consistent with the entorhinal cortex providing the major input to the hippocampus.

Unilateral fornix lesions abolished the behavioral induction of Arc expression only in the ipsilateral DG, CA3, and CA1, but not in the contralateral hemisphere (Temple et al. 2003). Similarly, bilateral lesions of the fornix abolished behavioral induction of Arc expression in all three subfields bilaterally and prevented spatial, but not cued, water maze learning (Fletcher et al. 2006). In the same study, the fornix lesions did not affect baseline expression or induction of Arc expression by high-frequency stimulation of the medial perforant pathway. In other studies, fornix lesions were also shown to block learning-dependent increases in the phosphorylation of CREB (Taubeufeld et al. 1999), increases in c-Fos protein (Vann et al. 2000b), and expression of C/EBP β and δ mRNA (Taubeufeld et al. 2001) in the hippocampus. Fornix lesions eliminate theta rhythm in dorsal hippocampus (Rawlins et al. 1979), severely disrupt hippocampus-dependent learning, and alter, but do not eliminate, location-specific activity of hippocampal place cells (pyramidal neurons in CA3 and CA1, also called complex-spike cells; Miller and Best 1980; Shapiro et al. 1989). Noneselective lesions of medial septum eliminate hippocampal theta (Rawlins et al. 1979), impair spatial working memory on the radial arm maze, and mildly alter the physiology of hippocampal place cells without changing their locotorial specificity (Leutgeb and Mizumori 1999). Inactivation of the medial septum also eliminated hippocampal theta rhythm, disrupted spatial learning (Mizumori et al. 1990), and reduced (50%) place cell activity of CA3 neurons, leaving CA1 place cells intact (Mizumori et al. 1989). On the other hand, selective cholinergic lesions of the medial septum by 192 Ig-Saporin, which reduces but does not eliminate theta (Lee et al. 1994), spared water maze learning and behavioral induction of Arc expression by exploration of a novel environment (Fletcher et al. 2007). Together with our observation that inactivation of medial septum abolishes the behavioral induction of Arc expression in CA1 and CA3 subregions of the hippocampus (T. Miyashita, S. Kubik, and J.F. Guzowski, unpubl.), these findings support the conclusion that hippocampal theta rhythm is critical for behavioral induction of Arc expression. In the absence of theta rhythm, desynchronized activity is not able to trigger plastic changes in synaptic transmission necessary for activity-dependent IEG transcription and memory consolidation. In this sense, Arc expression may not automatically follow neuronal activity such as expression of a place field, which, unlike Arc expression, is maintained in the absence of theta.

The link between long-term memory formation and Arc expression in the hippocampus suggests that Arc is involved in...
learning-related plasticity rather than in mere neuronal activity. On the other hand, Arc RNA is faithfully induced by repeated exploration of a familiar environment over many days, and only declines dramatically after repeated exposures during a single day (Guzowski et al. 2006). These findings may suggest that hippocampal neurons continue to update their representations (and express Arc) during repeated exposures to a familiar environment over multiple days. Alternatively, sustained increases in Arc protein level may be required for learning, whereas transient Arc transcription may simply reflect neuronal activity. In support of this view, memory-enhancing β-adrenergic activity in the basolateral amygdala (BLA) increased, and memory-impairing BLA blockade with lidocaine decreased, Arc protein level in dorsal hippocampus without affecting Arc mRNA levels in rats trained in inhibitory avoidance (McIntyre et al. 2005). In support of the conclusion that IEG expression in hippocampal neurons, whether at mRNA or protein levels, can be modulated by activity in the BLA, Huff and colleagues demonstrated that BLA inactivation with muscimol attenuated increases in Arc and c-fos mRNA in hippocampus after contextual fear conditioning, but not after place exploration (Huff et al. 2006). It should be noted that a critical difference between the Huff and McIntyre studies was the time of BLA inactivation, with Huff et al. (2006) using pre-training infusions and McIntyre et al. (2005) using post-training infusions. In this context, it is important to remember that the term "IEG activation" must be examined carefully as it does not describe a unitary molecular process, but rather a regulated series of processes including transcriptional regulation, post-transcriptional processing of mRNA, mRNA trafficking and targeting, translational regulation, post-translational regulation, and control of protein degradation.

In conclusion, we interpret the available findings to suggest that the combination of place cell activity associated with expression of a firing field, NMDA receptor activation, and theta rhythm provides a sufficient signal to activate IEG transcription in the hippocampus, but that neuregulatory inputs, such as those from the amygdala, can alter downstream steps in functional IEG expression.

Using immediate-early genes to map and dissect hippocampal subregional functions

As discussed above, IEG expression is believed to mediate learning-related changes in synaptic efficacy. Therefore the levels of IEG transcription (mRNA) are believed to faithfully reflect strong neuronal activation. This idea formed the basis of IEG imaging—using IEG expression to map neural activity in brain circuits. The major advantage of this approach is the ability to evaluate activity simultaneously in many different brain regions with cellular resolution.

Conventional IEG imaging

In its conventional form, IEG imaging uses either immunohistochemistry or in situ hybridization to detect IEG protein or mRNA, respectively. Densitometry of in situ autoradiography provides relative signal differences between brain regions such as between CA3 and CA1 and between different behavioral conditions (Hess et al. 1995a,b; Gall et al. 1998; Kelly and Deadwyler 2002, 2003). Fluorescent or chromagenic immunohistochemical detection allows comparing proportions of IEG-positive cells (Colombo et al. 2003; Weitemier and Ryabinin 2004). As noted above, IEG protein levels are subject to both transcriptional and post-transcriptional regulation. In this sense, IEG imaging studies that detect RNA or protein provide complementary, not identical, information, and the detection method used should be kept in mind when interpreting the findings from IEG studies. Because mRNA levels are more directly connected to the inducing event, IEG RNA detection methods provide a more direct readout of neuronal activation. In addition, the half-life of IEG mRNA is also shorter, yielding higher signal-to-noise ratio for some IEGs like Arc or zif268 (Guzowski 2002). In contrast, steady-state protein levels reflect integration of multiple cellular processes and modulatory signals, and, as such, may be more closely associated with neuroplastic changes engaged by distinct behaviors.

Doubts about the specificity of the IEG response to task-related learning stimulate the use of nonlearning controls. However, matching controls for all behavioral parameters except learning in order to subtract the "learning signal" brings significant problems. For example, latent learning occurring in the control animals (Rapp et al. 1987; Packard and McGaugh 1996) may preclude finding significant learning-related increase in IEG expression (Guzowski 2002), particularly in the hippocampus, which has been implicated in automatic encoding of ongoing experience (Morris and Frey 1997). Indeed, spatial, (hippocampus-dependent) and cued (hippocampus-independent) learning in the water maze caused equivalent increases in hippocampal expression of IEGs Arc, c-fos, and Homer (Frankland et al. 2004). This problem is also known in human fMRI studies where substantial activity in the hippocampal region during control "rest" states obscures specific signal during task conditions (Stark and Squire 2001).

Moreover, changes in which neurons are active, rather than how many of them, are likely to carry specific information within neural codes. Conventional IEG imaging methods cannot resolve this problem because they can only detect IEG expression at a single time point, limiting behavioral comparisons to between-subject design. Learning-specific signal obtained in this way may be easily overlooked in regions with high coding sparseness (low proportion of simultaneously active neurons) such as dentate gyrus or CA3. This may be the reason that some conventional IEG imaging studies reported learning-related changes only in CA1, but not CA3 or dentate gyrus (Hall et al. 2001; Frankland et al. 2004; Mavie1 et al. 2004).

catFISH

The catFISH technique (cellular compartment analysis of temporal activity by fluorescence in situ hybridization; Guzowski et al. 1999) circumvented several of the problems noted above by enabling assessment of activity of the same neurons during two distinct behavioral epochs. This advance was achieved using the dynamic regulation of transcription of the IEG Arc (Link et al. 1995; Lyford et al. 1995) and rapid translocation of its mRNA from nucleus to soma and dendrites (Steward et al. 1998; Guzowski et al. 1999). Briefly, ongoing transcription of Arc RNA induced by the behavioral epoch immediately (2–10 min) preceding the animal’s death can be detected as bright intranuclear foci (Arc-INF), whereas activation induced 20–25 min earlier can be seen as diffuse somatodendritic staining (Arc-cyto). A variant of this method, Arc/Homer 1α catFISH, combines hybridization to two IEGs, Arc and Homer 1a, which are coincided in the same population of neurons (Vazdarjanova et al. 2002). The activity at the recent time point is again detected as Arc INF, but the activity at the remote time point is now detected as Homer 1α intranuclear foci (H1α-INF). The different time course of detection of the two IEGs arises from using a riboprobe for the 3’ UTR (untranslated region) of the primary transcript of Homer 1α (40 kb from the start site), which does not occur until 25 min after the inducing episode because of the limited elongation rate of the RNA polymerase II (Bottai et al. 2002). Therefore, neurons active during 2–10 min before the animal’s death show Arc-INF, whereas neurons active ~25–30 min earlier will display Arc-cyto and/or Homer 1α INF.
zif268 mRNA expression in CA1 was observed following exposure to novel individual items did not (Wan et al. 1999). The increase was most prominent in CA1, whereas the dentate gyrus and subiculum displayed a decrease in c-Fos activity (Wan et al. 1999). On the other hand, Jenkins et al. (2004) found equivalent increases across hippocampal subregions (CA1, CA3, dentate gyrus) in the most rostral portion of the hippocampus, but no effect in subiculum or remaining portions of the hippocampus (Jenkins et al. 2004). Novel combinations of familiar visual cues in familiar locations attenuated induction of c-Fos activity in the retrosplenial cortex and the CA1 of the dorsal hippocampus with no change in the CA3, dentate gyrus, or ventral hippocampus (Amin et al. 2006). However, no effects of cue recombination were found on the expression of Zif268 (Amin et al. 2006). In addition, increased zif268 mRNA expression in CA1 was observed following retrieval of contextual, but not cued, fear conditioning (Hall et al. 2001).

Although these studies yielded conflicting results concerning differences between the hippocampal subregions, they support a role of the dorsal hippocampus in spatial and contextual learning and memory, in agreement with aforementioned studies (Guzowski et al. 1999, 2000, 2001; see above). They are also consistent with the idea that the hippocampus is a part of a “configural” or “structural” learning and memory system including fornix, anterior thalamic nuclei, mammillary bodies, and retrosplenial cortex (Aggleton and Pearce 2001), which may form the core of a context-rich episodic memory system (Aggleton and Brown 1999; Winocur et al. 2007).

Memory acquisition, consolidation, and retrieval

Hippocampal subregions have been suggested to functionally segregate between processes of memory acquisition, consolidation, and retrieval. Studies using selective lesions of hippocampal subregions suggested that CA3 is preferentially involved in acquisition and CA1 in retrieval of memory for contextual fear (Lee and Kesner 2004b). Similarly, disconnection between DG and CA3 disrupted encoding, not retrieval, of spatial navigation tasks (Hebb-Williams maze; Lee and Kesner 2004a; Jerman et al. 2006).
High-resolution fMRI revealed that regions “early” in the hippocampal circuit (DG, CA3, and CA2) were active during episodic memory formation, whereas regions “later” in the circuit (subiculum) were active during recollection (Eldridge et al. 2005). Subregion-specific infusions of the NMDA receptor antagonist APV suggested that NMDA receptors in CA3 are involved in updating spatial memory in a non-matching-to-place task on a radial arm maze; whereas NMDA receptor function in CA1/DG is primarily involved in maintenance of memory over longer delays (Lee and Kesner 2002). Mice with CA3-specific genetic ablation of the NR1 subunit of the NMDA receptor showed an impairment in a spatial working memory water maze task (delayed matching to place, DMP) and impaired spatial tuning of CA1 place cells during initial exposure to a novel environment (Nakazawa et al. 2003). In a different study, these mice also displayed transient impairment of contextual memory after a brief, but not after an extended exposure (Cravens et al. 2006). Local deletion of the NR1 subunit in CA3 in ~30% of dorsal hippocampus disrupted learning of paired associations if both components (an odor and a context) were novel, but not expression of learned associations, context recognition, or learning to a previously learned novel context (Rajji et al. 2006). These studies suggested that CA3, via its NMDA-dependent plasticity, is involved in rapid, one-trial encoding of complex associations involved in spatial or contextual stimuli.

Greater c-fos mRNA expression was found in CA3 than in CA1 during initial acquisition of an odor discrimination task, but it was equivalent in rats that only learned a nose-poking response for water reward (Hess et al. 1995a). Interestingly, whereas odor discrimination per se does not depend on the hippocampus (Kaut and Bunsey 2001; Jonasson et al. 2004), the nose-poking protocol required alternating between multiple nose-poking sites, somewhat resembling hippocampus-dependent alternation (Olton et al. 1979; Aggleton et al. 1986). In a follow-up study (Hess et al. 1995b), rats that only explored the testing environment for the first time showed greater c-fos expression in all hippocampal fields than rats overtrained in the odor discrimination. Unlike in the previous study, the c-fos expression was highest in CA1 in both groups (Hess et al. 1995b). These patterns of CA1 or CA3 dominance were suggested to reflect different modes of hippocampal function. The CA3 dominance observed during initial odor discrimination learning may be associated with switching between associative memory retrieval and new learning in CA3. Alternatively, it may reflect suppression of pre-potent response of such as the previously learned nose-poking response (Hess et al. 1995a; Gall et al. 1998). The CA1 dominance observed during exploration and overtrained discrimination may be associated with ongoing spatial processing. Parallel functional modes manifested as different balance of activation between the hippocampal subfields might reflect functional dissociation between memory and anxiety, but the majority of the available evidence place this functional dissociation along the septotemporal hippocampal axis (Bannerman et al. 2004; see “Analysis of CA3/CA1 Ensemble Dynamics Using Catfish” section, below).

A transient learning-specific increase in Arc mRNA expression in CA1 and CA3 was shown in rats performing an operant lever-pressing task (Kelly and Deadwyler 2002, 2003). The Arc activity was generally larger in CA1 than CA3, and it was more persistent in CA1 compared to home cage controls (Kelly and Deadwyler 2003), but not pseudotrained controls (Kelly and Deadwyler 2002). These findings somewhat resemble the CA1 dominance during exploration in the study of Hess et al. (1995b). The more transient activation in CA3 is consistent with its role in initial learning. It is not clear, however, how the lever-pressing task relates to hippocampal function and whether the activity observed in the hippocampus reflects formation of memory for the task, incidental spatial encoding, or an independent process of “automatic recording of attended experience” (Morris and Frey 1997).

The above IEG imaging studies seem to suggest that the CA3 subregion of the hippocampus is preferentially involved in initial learning. While this is in agreement with lesion (Lee and Kesner 2004a,b; Jerman et al. 2006) and fMRI data (Eldridge et al. 2005), there are several constraints to this interpretation. First, latent hippocampus-dependent learning of a spatial location can occur as animals are trained in hippocampus-independent tasks (Rapp et al. 1987; Packard and McGaugh 1996). Second, the use of behavioral tasks without an obvious requirement for hippocampal function renders it difficult to interpret how increased activation in CA3 is involved in learning the task. Third, other IEG studies (Gusev et al. 2005; Smith et al. 2007) reported persistent, rather than transient increase in IEG expression in CA3. Fourth, all of the above studies used conventional imaging methods with limitations relating to control groups and between-subject design as mentioned above. It is possible that the specific contributions of different subregions to hippocampal function are interdependent and orthogonal to the stages of memory formation so that the subregions are differently activated by different task demands rather than during different stages of memory. In conclusion, conventional IEG studies as a whole have been equivocal with regard to specific subregional functions in different stages of memory. Instead, the IEG activity observed in the hippocampus in hippocampus-independent tasks (Hess et al. 1995a,b; Guzowski et al. 2001; Kelly and Deadwyler 2002, 2003) supports the idea that hippocampus engages in continuous recording of ongoing experience (Morris and Frey 1997).

IEG imaging studies of systems consolidation

Several studies have shown elevated IEG expression in the hippocampus following recent, but not remote, memory retrieval in support of a time-limited involvement of the hippocampus in declarative long-term memory (standard theory of systems consolidation; Squire and Alvarez 1995). Increased zif268 RNA was found in CA1 of rats tested for retrieval of contextual conditioned fear 24 h, but not 28 d, after training (Hall et al. 2001). C57Bl/6 mice trained in the contextual fear paradigm showed increased levels of Zif268 and c-Fos proteins in the CA1 after recent (1 d) but not remote (36-d) memory retention test relative to nonshocked controls (Frankland et al. 2004). Zif268 expression was increased after recent (1 d), but not remote (30 d), memory test in the CA1 of mice trained in a reference memory task on a five-arm radial maze relative to nonlearning controls. Lidocaine infusion into the hippocampus abolished the Zif268 immunoreactivity in CA1 and impaired retrieval of the recent, but not remote memory (Mavil et al. 2004). Unfortunately, this report does not make clear if the task could have been solved using a response strategy, in which case a shift from hippocampal-dependent place to caudate-dependent response strategy could be expected to occur between the recent and remote time points (Packard and McGaugh 1996). In addition, persistent Zif268 activation was observed in hippocampus 30 d after training in a working memory paradigm (Mavil et al. 2004), indicating that a lack of demand for hippocampal function could have caused the diminished hippocampal activation in the reference memory version of the task.

Arc mRNA expression in CA1, CA3, DG, subiculum, and entorhinal cortex all along the septotemporal axis of the hippocampus was compared in rats trained in the water maze and tested for retention either 24 h or 1 mo later in three nonrewarded probe trials (Gusev et al. 2005). Both groups showed robust place responses with different, but nonsignificant trends.
Rats in the 24-h retention group were slightly worse in the third probe, whereas animals at the long retention interval actually improved over the three probes, perhaps suggesting some kind of "reminder" effect. CA3 displayed the most persistent Arc learning-specific signal at the remote retention test, as compared to swimming controls yoked for time. This learning-specific signal disappeared completely in CA1 and ventral hippocampus. Generally, the learning-specific signal dramatically declined between recent and remote retention interval in all hippocampal subfields. This result was interpreted as a decline in hippocampal involvement in declarative memories over time, in support of the standard theory of systems consolidation (Gussev et al. 2005).

The interpretations of these IEG studies supporting systems consolidation, however, have some serious limitations. First, memory for the water maze does not undergo systems consolidation because hippocampal dysfunction always results in impairment (Sutherland et al. 2001; Clark et al. 2005; Martin et al. 2005; Broadbent et al. 2006; Teixeira et al. 2006). It has been argued that this is also the case for contextual fear and possibly even other types of hippocampus-dependent learning because hippocampal lesions impaired recent as well as remote memory for contextual fear (Lehman et al. 2007). The most parsimonious account of these discrepant results suggests that the spared contextual fear at remote retention intervals (Kim and Fanselow 1992; Anagnostaras et al. 1999; Hall et al. 2001; Frankland et al. 2004) is actually a consequence of forgetting leading to generalization of fear to nontraining contexts (Rudy et al. 2005). Pre-exposure to the training context prevented generalization of fear to a nontraining context during the test, demonstrating that poor contextual memory was really the cause of generalization of the fear response (Biedenkapp and Rudy 2007; Wittegen and Silva 2007). Second, the increase in learning-specific signal (see discussion above) observed after recent memory retrieval (Frankland et al. 2004; Maviel et al. 2004) may reflect an ongoing process of synaptic consolidation associated with recent learning. However, this process should have been completed by the time of the remote memory recall, and therefore IEG expression was not required. Alternatively, the transitory increase in activity observed in CA1 may be a correlate of new learning, whereas remote memory retrieval is mediated by the persistent activity in CA3. Slower development of hippocampal representation in the non-training context may be a correlate of new learning, whereas remote memory retrieval is mediated by the persistent activity in CA3. Similarly, training in a spatial working memory task on a radial arm maze induced greater c-Fos activation in the dorsal hippocampus (Vann et al. 2000a). Also, robust behavioral induction of Arc expression by exploration of a novel environment (Guzowski et al. 1999) or by training in a water maze (also c-fos and zif268; Guzowski et al. 2001) was observed in the dorsal hippocampus.

In support of functional dissociation along the septotemporal axis, spatial rearrangement (Jenkins et al. 2004) or novel configuration of familiar stimuli (Amin et al. 2006) increased the number of c-Fos-positive neurons in the dorsal, but not the ventral, hippocampus. Similarly, training in a spatial working memory task on a radial arm maze induced greater c-Fos activation in the dorsal hippocampus (Vann et al. 2000a). Also, robust behavioral induction of Arc expression by exploration of a novel environment (Guzowski et al. 1999) or by training in a water maze (also c-fos and zif268; Guzowski et al. 2001) was observed in the dorsal hippocampus.

Analysis of CA3/CA1 ensemble dynamics using catFISH

Structural differences between hippocampal subfields have been recognized already by early (Ramón y Cajal 1893; Lorente de No
1934) as well as modern neuroanatomists (Amaral and Witter 1989, 1995). Ideas of their differential functions were formulated in numerous computational models (Marr 1971; McNaughton and Morris 1987; McClelland et al. 1995; Rolls and Treves 1998; O’Reilly and Rudy 2001; Rolls and Kesner 2006). Despite this history, demonstrations of functional differentiations within the hippocampus remained scarce, until recently. Rats with selective lesions of the CA3 region were impaired in spatial discrimination across all spatial separations (Gilbert and Kesner 2006), whereas rats with lesions of the dentate gyrus showed a separation gradient, and rats with CA1 lesions performed at control levels (Gilbert et al. 2001). These results suggested that the dentate gyrus is responsible for spatial pattern separation, and the CA3 is necessary for spatial working memory encoding. Mice with NMDA receptor subunit NR1 knockout specific to pyramidal neurons in CA3 showed memory impairment when only a subset of the original training cues was present, supporting the idea that CA3 performs pattern completion in an autoassociative network (Nakazawa et al. 2002). Accordingly, rats with selective lesion of the CA3 showed impairment in spatial pattern completion in a delayed matching-to-place task (Gold and Kesner 2005).

Early recording studies of hippocampal place cells found few differences between CA3 and CA1 place fields (Muller et al. 1987), with experimenters sometimes even pooling the data. However, the presence or absence of intranuclear foci for large volume and relatively diffuse staining with DAPI, were assessed for activated in both epoch 1 and epoch 2. For quantitative analyses of being exposed to two similar environments, and the A/B group being exposed to the same environment twice, the A/A group was different from groups A/A and A/B, but not with each other, justifying their collapse into a single group. The sequence of the rats’ exposure to environments in epochs 1 and 2 defined the behavioral group, with the A/A group being exposed to the same environment twice, the A/A group being exposed to two similar environments, and the A/B group being exposed to two completely different environments. (A) Confocal projection image of Arc/Homer catFISH in area CA3 from an A/B rat; (red) Arc RNA; (green) H1a RNA; (blue) nuclei. (Green arrow) Points to an H1a+ neuron, which was activated only during behavioral epoch 1; (red arrow) points to an Arc+ neuron, which was activated only during behavioral epoch 2; (yellow arrow) points to an Arc/H1a double+ neuron that was activated in both epoch 1 and epoch 2. For quantitative analyses of image stack data, only putative neuronal nuclei, characterized by their large volume and relatively diffuse staining with DAPI, were assessed for the presence or absence of intranuclear foci for Arc or H1a. (B) Analysis of similarity scores of rats from the different behavioral conditions. The similarity scores are single measure derived from the raw staining class data in which individual neuronal nuclei are classified as Arc+, H1a+, Arc/H1a double+, or negative. The derivation of the similarity score is described in detail elsewhere (Vazdarjanova and Guzowski 2004) and provides a normalized measure of overlap between neuronal ensembles active in the two behavioral sessions. A similarity score of 1 indicates a complete overlap of the neuronal ensembles activated by epoch 1 and epoch 2, whereas a similarity score of 0 indicates no overlap beyond that predicted by chance. Each symbol denotes data from an individual rat. In both CA3 and CA1, the similarity scores from rats of the A/A group were significantly different from those of the A/A and A/B groups (P < 0.0001). Notably, the similarity score of the A/A rats was greater in CA3 as compared to CA1 (P = 0.001). The similarity score in the A/A condition in CA1 was significantly different from chance (P = 0.02) and greater than in CA3 (P = 0.015). Note the discontinuous pattern of overlap in the A/A, A/A, and A/B groups for region CA3 as compared to region CA1, which is more graded and linear in nature. (C) The within-rat ratio of CA3 to CA1 activity was strongly correlated across behavioral epochs and was not influenced by the nature of epoch 2. The fact that the CA3 and CA1 activity was not correlated across rats (data not shown), but that the CA3/CA1 within-rat ratio was correlated across sessions, suggests that the balance of CA3 to CA1 activity is highly specific to each individual rat. This individual difference in the intrinsic hippocampal CA3/CA1 network dynamic could play a role in determining the capacity of individuals for spatial navigation or mnemonic functions. This figure is based on data and figures from Vazdarjanova and Guzowski (2004) (Copyright 2004 by the Society for Neuroscience).
ploration session (epoch 2). Nearly the same ensembles were active in the CA3 of rats exposed to the same environment twice (A/A condition; Fig. 1B), but dramatically different CA3 ensembles were active in two different environments (A/B condition; Fig. 1B). The response of CA3 ensembles was relatively insensitive to minor changes of the environment (A/A’ condition; Fig. 1B). These changes included different objects (Aobj), different spatial configuration of familiar objects (Aconfr), or different distal stimuli (Ab), and because the ensemble responses to those changes did not differ, they were pooled into the minor change category (A/A’). The CA1 ensembles were also highly similar in the same environments (A/A condition; Fig. 1B), but in contrast to CA3, they were not entirely independent in different environments (A/B condition; Fig. 1B), suggesting that the CA1 ensembles represented sensory similarities between the environments or handling procedures. The CA1 ensemble responses across different conditions (A/A, A/A’, A/B) were more linearly related to the changes in the environment (Fig. 1B). The CA3 ensembles activated in different environments were statistically independent, indicating orthogonalization by an active pattern separation process, whereas a pattern completion process led to reactivation of the same representation if the change was sufficiently small. Dynamic tension between these two processes in CA3 was responsible for the nonlinear responses of CA3 ensembles (Fig. 1B; Vazdarjanova and Guzowski 2004). A competitive neural network in the dentate gyrus was most likely responsible for the pattern separation process (Rolls and Treves 1998). The sudden orthogonalization of CA3 representations resembles global remapping in unit recording studies, whereas more subtle differences may be encoded by rate remapping without changing the encoding ensemble (Leutgeb et al. 2005). The proportions of active neurons corresponded to the electrophysiological findings (Guzowski et al. 2004; Vazdarjanova and Guzowski 2004) and to the theoretical models (Marr 1971; Rolls and Treves 1998). The sparseness was greater in CA3 (≈18%) than in CA1 (≈35%), but far greatest in the dentate gyrus, where exploration induced Arc in only ≈2% of granule cells in the dorsal blade and practically in none in the ventral blade (Temple et al. 2003; Chawla et al. 2005). In addition, CA3 and CA1 activity levels were not correlated within a session, arguing against unitary hippocampal activation across subregions. On the other hand, the balance of CA3/CA1 activity was highly correlated across sessions within an animal, indicating a possible source of inter-individual variance (Mayford et al. 1996; Mansuy et al. 1998) may help to disentangle the contributions of IEG expression in individual subregions to different stages of memory formation.

In closing, the IEG studies provide robust evidence for a role of the hippocampus in declarative learning and memory, possibly via continuous encoding of ongoing experience. The IEG studies are consistent with the idea that the hippocampus is functionally differentiated along the septotemporal axis. They also demonstrated different neural network properties of CA1 and CA3 subregions and showed evidence of competition between pattern separation and pattern completion in CA3. Taken together with findings from lesion, genetic, and recording experiments, IEG studies provide support for the role of CA3 in rapid encoding and retrieval of complex memory representations, such as those involving spatial and contextual information.

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