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Grading the gradient: Evidence for time-dependent memory reorganization in experimental animals

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Abstract In humans, damage limited to the hippocampus produces temporally graded retrograde amnesia, with relative sparing of remote compared to recent memory. This observation forms the cornerstone of the idea that as memories mature they are reorganized in a time-dependent manner. In this paper, we evaluate evidence for similar time-dependent reorganization in experimental animals. In the majority of behavioral paradigms examined, these studies provide evidence that memories are gradually reorganized over time, with the hippocampus and cortex playing preferential (although not necessarily mutually exclusive) roles in the expression of recent and remote memory, respectively. This pattern is not observed in all tasks, however. For example, in the water maze hippocampal activity is always necessary for memory expression. Identifying situations when the hippocampus is, and is not, required for remote memory expression will help us to understand hippocampal contributions to memory, and, more generally, whether changes in memory organization lead to qualitative changes in the nature of memory.

Keywords memory consolidation · cortex · hippocampus · remote memory · retrograde amnesia

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In the late part of the 19th century, the French psychologist, Theodule Ribot, described how memory loss after brain insult was often related to the age of the memory: the effect on more recent memories was typically greater than that on older (or more remote) memories [1]. The dissociation has become known as Ribot's law (or Ribot's gradient), and subsequent neuropsychological studies went on to establish a more precise relationship between the locus of brain damage and the gradient. Penfield, Scoville, and Milner [2, 3] characterized memory loss in patients with damage to the medial temporal lobe (MTL, including the hippocampus, and the entorhinal, perirhinal, and parahippocampal cortices) and provided the first evidence that MTL damage preferentially affects recent, but not remote, memories. Later studies of patients with more circumscribed lesions established that damage to the hippocampus, in particular, is responsible for this typical graded amnesia [4–7].

This observation forms the basis of the idea that memories are reorganized in a time-dependent manner. Within this general framework, the relative contributions of different brain regions may vary as a function of memory age. Accordingly, some regions may play important roles in the expression of newly formed (or recent) memory, but their contributions may fade over time. Conversely, other regions may play preferential roles in the expression of older (or remote) memories. Here we shall critically evaluate the evidence for memory reorganization in experimental animal studies. First, we shall consider the

evidence that the hippocampus (including CA fields, dentate gyrus, and subiculum) plays a time-limited role in the expression of memory. Second, we shall consider newer evidence for a preferential role of the prefrontal cortex in the expression of remote memory.

Hippocampal gradients

Hippocampal lesions and graded retrograde amnesia

Two studies in the early 1990s were the first to model temporally graded retrograde amnesia in rodents. In the first, Winocur and colleagues [8] made electrolytic lesions of the dorsal hippocampus at different times after training in the socially transmitted food preference task [9]. Whereas lesions made 1–2 days after training abolished memory for the learned preference, similar lesions made 10 days posttraining were ineffective [8]. In the second study, Kim and Fanselow [10] examined the impact of electrolytic lesions of the dorsal hippocampus on contextual fear memory. Similar to the Winocur study, they found that there was substantial retrograde amnesia when the lesions were carried out 1 or 7 days after training, but hippocampal lesions were ineffective at longer training-surgery delays. The graded nature of these effects supported the idea that the hippocampus is playing a nonessential role in the expression of some forms of remote memory, and these results nicely paralleled observations in human amnesics (albeit on a much shorter timescale).

These initial observations have subsequently been extended in a number of important ways. First, graded amnesia after hippocampal damage has been shown in a number of different tasks, including trace eyeblink conditioning, visual discrimination, inhibitory avoidance, as well as in a subset of spatial tasks. Second, graded amnesia has been observed in a range of species, ranging from mice to monkeys. Third, graded amnesia has been observed after both partial and complete hippocampal lesions (using a variety of lesion techniques). For example, both partial (dorsal hippocampus [8]) and more extensive [11–13] hippocampal lesions produce graded effects on memory in the socially transmitted food preference task. Fourth, graded amnesia has been observed using both within- and between-subject designs. Although one advantage of within subject design is that it more closely mimics the clinical cases, equating the learning experience at two or more time points before surgery is sometimes challenging [14]. This drawback is addressed in studies using between-subject designs (such as in the Winocur [8] and Kim and Fanselow [10] examples above). To date, there are 25 or so published studies showing that damage to the hippocampus preferentially affects recent but not remote memory (Table 1; [8, 10–12, 15–34]). The differential effects of hippocampal damage on recent vs. remote memory essentially recapitulate effects seen in patients, and are consistent with a more global and gradual process of memory reorganization.

Evidence for hippocampal gradients from convergent approaches

One of the strengths of the above data set is that graded effects were observed in such a diversity of tasks, including tasks that differ greatly in terms of their stimulus properties, performance demands, and motivation. However, a potential weakness of this data set is the reliance on a single technique to establish the gradient. Lesions, like any technique, have a number of advantages and inherent limitations. In particular, the fact that memories can survive hippocampal damage at remote time points does not necessarily imply that the hippocampus would not have been engaged in an intact animal. In this regard, therefore, demonstrations of graded effects using alternate approaches are especially valuable, and have strengthened the idea that circuits supporting memory are reorganized in a time-dependent manner. Two main classes of approaches have been applied.

First, mouse-genetic approaches. Tsien and colleagues generated mice in which the NR1 subunit of the *N*-methyl-D-aspartate (NMDA) receptor in CA1 region of the hippocampus could be deleted in an inducible manner [28]. Mice with normal NMDA function were trained in two hippocampus-dependent learning tasks: the Morris water maze and contextual fear conditioning. Only suppressing NMDA receptor function in the week immediately after training, rather than at later time points, blocked the establishment of remote memories. Like the lesion studies, these experiments emphasize the importance of maintaining the integrity of the hippocampal trace in the days after training and indicate that recent memories are preferentially sensitive to a disruption of hippocampal function. Perhaps what is most striking is that two dramatically different types of hippocampal manipulations—nonreversible anatomical lesions and reversible molecular lesions—both produce very similar graded effects on memory.

Second, brain mapping approaches. The (^{14}C)2-deoxy-glucose approach can be used to monitor changes in metabolic activity across brain regions [35]. Taking advantage of this technique, Bontempi and colleagues [36] were the first to comprehensively map time-dependent changes in memory organization in mice, and in particular show that recall of recent, but not remote, spatial discrimination memory was associated with activation of the dorsal hippocampus. Subsequent studies have used the expression of immediate early genes (such as *c-fos* and *zif268*) as markers of neuronal activity to examine the role of the hippocampus in recent and remote memory [13, 24,

Table 1 Hippocampal (and/or extrahippocampal) graded amnesia

Behavioral paradigm	Species	Brain target	Extent of damage	Method	Outcome	References
Socially acquired food preference	Rat	Dorsal hippocampus	Partial	Electrolytic	Deficit at 2 days, no deficit at 10 days	Winocur, [8]
		Dorsal and ventral hippocampus	Extensive	Neurotoxic	Deficit at 1 and 2 days, no deficit at 5 and 10 days	Winocur et al., [12]
		Hippocampus and subiculum	Extensive	Electrolytic	Deficit at 1 day, no deficit at 30 days	Clark et al., [11]
		Hippocampus	Extensive	Electrolytic	Deficit at 1 day, no deficit at 21 days	Ross and Eichenbaum, [13]
Object discrimination	Monkey	Hippocampal formation (hippocampus, subiculum, entorhinal and parahippocampal cortices)	Extensive	Aspiration	Deficit up to 4 weeks	Zola-Morgan and Squire, [34]
Contextual fear conditioning	Rat	Dorsal hippocampus	Partial	Neurotoxic	Deficit at 1 and 28 days, no deficit at 100 days	Maren et al., [23]
				Electrolytic	Deficit at 1, 7, and 14 days, no deficit at 28 days	Kim and Fanselow, [10]
					Deficit at 1 day, no deficit at 50 days	Anagnostaras et al., [15]
					Deficit up to 14 days	Ward et al., [32]
					No deficit at 45 days	Debiec et al., [18]
	Mouse	CA1 field of hippocampus	Partial	Mutation: inducible CA1- specific knockout of NMDA receptors	Memory probed at 30 days, deficit when mutation active from day 1 to 14 posttraining, no deficit when active from day 22 to 29	Shimizu et al., [28]
Contextual and cued fear conditioning	Mouse	Hippocampus and other forebrain regions	Extensive	Mutation: inducible forebrain-specific disruption of αCaMKII function	Memory probed at 30 days, deficit when mutation active from day 1 to 7 posttraining, no deficit when active from day 8 to 28	Wang et al., [31]
Spatial two-choice discrimination	Mouse	Entorhinal cortex	Extensive	Neurotoxic	Deficit up to 4 weeks	Cho et al., [16]
		Dorsal hippocampus	Partial	Electric stimulation	Deficit up to 4.5 weeks	Laurent-Demir and Jaffard, [22]
Spatial multiple choice discrimination	Mouse	Dorsal hippocampus	Partial	Inactivation	Deficit at 1 day, no deficit at 30 days	Maviel et al., [24]
	Rat	Entorhinal cortex	Extensive	Electrolytic	Deficit up to 4 weeks	Cho and Kesner, [17]
		Dorsal hippocampus	Partial	Electrolytic	Deficit up to 32 days	Ramos, [26]
Water maze spatial learning	Rat	Perirhinal cortex	Extensive	Aspiration	Deficit at 2 days, no deficit at 28 days	Glenn et al., [19]
		Temporoammonic path (projection)	Complete	Electrolytic	Deficit up to 21 days	Remondes and Schuman, [27]
	Mouse	CA1 field of hippocampus	Partial	Mutation: inducible CA1-specific knockout of NMDA	Memory probed at 30 days, deficit when mutation active from day 1 to 14 posttraining, no deficit	Shimizu et al., [28]

Table 1 (continued)

Behavioral paradigm	Species	Brain target	Extent of damage	Method	Outcome	References
				receptors	when active from day 22 to 29	
		Medial entorhinal cortex, pre- and parasubiculum	Partial	Mutation: inducible disruption of αCaMKII function	Deficit when mutation active 0–21 days, no deficit when mutation active 22–42 days	Yasuda and Mayford, [75]
Trace eyeblink conditioning	Rabbit	Hippocampus	Extensive	Aspiration	Deficit at 1 day, no defict at 30 days	Kim et al [21]
	Mouse	Dorsal hippocampus	Partial	Aspiration	Deficit at 1 day, no deficit at 28 days	Takehara et al., [30]
	Rat	Dorsal hippocampus	Partial	Aspiration	Deficit up to 14 days, no deficit at 28 days	Takehara et al., [29]
Inhibitory avoidance	Rat	Dorsal hippocampus	Partial	Inactivation	Deficit at 1 day, no deficit beyond	Quillfeldt et al., [25]
				Inactivation or NMDA antagonist	Deficit at 1 day, no deficit beyond	Izquierdo et al., [20]
Visual discrimination	Rat	Fornix or perirhinal cortex or both	Extensive	Electrolytic	Deficit up to 3 weeks	Wiig et al., [33]

37]. Similar to the initial study by Bontempi and colleagues, these studies showed that the hippocampus was preferentially activated after expression of recent, but not remote, five-arm maze [24], contextual fear [37] and socially transmitted food preference [13] memories. Therefore, together with the mouse-genetic and lesion-based approaches, these brain mapping studies support the idea that the circuits supporting these memories evolve over time, with the hippocampus playing a time-limited role in memory expression. The brain mapping studies go perhaps one step further to show that not only might the hippocampus be not necessary, but it might not even be engaged, during the expression of remote memory (at least in the tasks studied here). In human imaging studies, the situation is less clear-cut. For semantic memories, there is evidence for reduced hippocampal contribution as a function of time. However, for episodic memories, retrieval may engage the hippocampus at both recent and remote time points (especially when the material being recalled is detailed in nature; for detailed discussion of these issues see [38, 39]).

Examples of ungraded amnesia

Extrahippocampal damage

One set of situations where graded amnesia is typically not observed is when significant damage occurs beyond the hippocampus (see Table 2; [40–54]). For example, perirhinal or combined perirhinal/postrhinal lesions have ungraded effects on water maze, object discrimination [45], and contextual fear [54] memories, respectively. These observations parallel similar observations in human patients where the length of the gradient is related to the extent of damage. When damage is limited to the CA1 region of the hippocampus in humans retrograde amnesia may span only 1–2 years. However, when damage is more extensive (also including the perirhinal, entorhinal, and parahippocampal cortices) amnesia may cover decades. Damage beyond the MTL is typically associated with ungraded retrograde amnesia, perhaps because sites of permanent storage are affected [55–58]. These studies suggest that cortices adjacent to the hippocampus play more extended roles in the consolidation process.

Water maze studies

There are other important exceptions that cannot be accounted for in terms of extrahippocampal damage. By far the most common (and widely recognized) instance where hippocampal lesions produce ungraded retrograde amnesia is in studies using the water maze (Table 2). In the water maze, rodents are trained to navigate to a submerged escape platform using an array of extra maze cues. Studies have consistently shown that hippocampal lesions disrupt the expression of spatial memories regardless of the interval

Table 2 Hippocampal (and/or extrahippocampal) ungraded amnesia

Behavioral paradigm	Species	Brain target	Extent of damage	Method	Outcome	References
Water maze spatial learning	Rat	Dorsal hippocampus or subiculum	Partial	Neurotoxic	Flat RA up to 14 weeks	Bolhuis et al., [40]
		Dorsal and ventral hippocampus and part of subiculum	Extensive	Neurotoxic	Flat RA up to 14 weeks	Mumby et al., [45]
		Dorsal hippocampus	Partial	Subchronic neuronal Inactivation	Flat RA up to 16 days but memory not probed at longer delay	Riedel et al., [47]
		Perirhinal cortex	Extensive	Electrolytic	Flat RA up to 2 weeks	Mumby and Glenn, [46]
		Dorsal and ventral hippocampus	Extensive	Neurotoxic	Flat RA up to 14 weeks	Sutherland et al., [48]
		Dorsal or dorsal and ventral hippocampus	Partial or extensive	Neurotoxic	Flat RA up to 6 weeks	Martin et al., [44]
		Dorsal or dorsal and ventral hippocampus	Partial or extensive	Thermocoagulation	Flat RA up to 9 or 14 weeks	Clark et al., [43]
		Hippocampus	Extensive	Thermocoagulation	Flat RA up to 114 days	Clark et al., [42]
		Dorsal hippocampus	Partial	Inactivation	Flat RA up to 30 days	Broadbent et al, [41]
	Mouse	Dorsal hippocampus	Partial	Inactivation	Flat RA up to 30 days	Teixeira et al, [49]
Spatial task in cross maze	Rat	Hippocampus	Extensive	Neurotoxic	Flat RA up to 9 months	Winocur et al., [50]
Spatial task in complex maze	Rat	Hippocampus	Extensive	Neurotoxic	Flat RA up to 14 weeks	Winocur et al., [65]
Object recognition	Rat	Dorsal and ventral hippocampus	Extensive	Neurotoxic	Flat RA up to 5 weeks	Gaskin et al., [52]
Object discrimination	Monkey	Rhinal (entorhinal + perirhinal) cortex	Extensive	Aspiration	Flat RA up to 16 weeks	Thornton, [53]
	Rat	Dorsal and ventral hippocampus	Extensive	Neurotoxic	Flat RA up to 14 weeks	Sutherland et al., [48]
Visual (scene) discrimination	Monkey	Fornix	Extensive	Transection	Flat RA up to 6 months	Gaffan, [51]
Contextual fear conditioning	Rat	Perirhinal and postrhinal cortices	Extensive	Neurotoxic	Flat RA up to 100 days	Burwell et al, [54]

between training and surgery. This is the case even after extensive pretraining in the water maze [42], partial lesions of hippocampus [43, 44], when extended delays between training and surgery are used [43], when water mazes with reduced spatial complexity are used [43] or when reminders to aid retrieval are used [44]. Similarly, reversibly inactivating the dorsal hippocampus disrupts the expression of both recent and remote water maze memory in rats [41] and mice [49]. Consistent with these lesion and inactivation studies, brain mapping approaches indicate that the hippocampus may be activated during the expression of both recent and remote water maze memory [49] (but see [59]). It is particularly important to note here that the absence of a hippocampal gradient does not simply imply that circuits supporting water maze memories are not reorganized over time. Indeed, inactivation of the anterior cingulate cortex (ACC) disrupts the expression of remote, but spares recent, water maze memory [49]. These results indicate that the anterior cingulate cortex is recruited into circuits supporting water maze memories over time and are consistent with the idea that water maze memories undergo reorganization.

To understand why the water maze studies are a consistent exception, two accounts have been proposed. First, the hippocampus may be important for the expression of spatial memories that require animals to navigate through space, as in the water maze. In such tasks, hippocampal activity may be required to continuously integrate both idiothetic (self-motion cues) and allothetic (e.g., distal visual cues) information to successfully navigate to the escape platform (for discussion, see [43, 44]). This performance account is

consistent with the standard view of consolidation [60, 61], that is, memories are initially encoded in hippocampal-cortical networks, but gradual strengthening of connections between cortical regions allows memories to eventually become independent of the hippocampus at remote time points. Accordingly, removal of the hippocampus does not abolish the spatial memory, but interferes with the expression of this memory, in part, by affecting the animal's ability to integrate idiothetic and allothetic information.

The second account has its roots in multiple trace theory [62, 63]. In this model, it is argued that hippocampal-dependent and hippocampal-independent memories are fundamentally different. The expression of detailed, context-dependent memories always depends on the hippocampus, regardless of their age. In contrast, memories may persist in the absence of the hippocampus, but these hippocampal-independent memories are necessarily less detailed, context-free, and more gist-like [62, 64, 65]. Accordingly, the water maze is a special case because a precise, detailed spatial representation of the environment is essential for normal performance. Lack of access to such a precise spatial representation in hippocampal-damaged animals therefore results in impaired performance.

Designing experiments that distinguish between these two possibilities has proven to be quite tricky. One strategy has been to develop spatial tasks that place reduced navigational demands on animals. However, even tasks with minimal navigational demands (such as the oasis maze and annular maze) are associated with flat gradients [43]. Only when navigation is eliminated—for example, in tasks where animals must use spatial information to discriminate between arms in a maze [16, 17, 24, 26]—is performance spared at remote time points after hippocampal disruption. This dissociation suggests that the hippocampal activity contributes to navigational performance.

A second strategy has been to provide extensive preoperative experience [65]. This is motivated by the finding that remote spatial memory is spared after large medial temporal lobe damage in a number of patients (T.T. [66], E.P. [67], and K.C. [64]). To mimic this situation, in one study rats were reared in a "rodent village" for 3 months [65]. At the completion of the 3 months, rats were trained on a specific spatial problem within the village environment before receiving lesions of the hippocampus. Remarkably, hippocampal-damaged rats performed as well as controls when tested postoperatively in the trained task. However, some perturbations of the spatial environment (e.g., village rotation, room change), but not others (e.g., substitution of distal cues) produced deficits in hippocampal-damaged rats [65]. These findings parallel those in some of the patients described above. For example, T.T. was previously a London taxi driver. After bilateral hippocampal damage he showed relatively intact performance navigating main routes through London, but navigation was impaired in non-main routes. This suggests the interesting possibility that hippocampal-dependent and hippocampal-independent representations of the spatial environment may differ in important ways, and is consistent with multiple trace theory, which predicts that hippocampus-dependent memories are richer in detail compared to hippocampus-independent memories. In the rodent village study [65], whether the spared spatial memory is less precise, or just weaker, is less clear. Furthermore, the lesions were incomplete and so it is not clear whether this less robust representation of the village is supported by extrahippocampal structures, or by residual ventral hippocampal tissue. To resolve this issue it will be important to examine whether complete hippocampal lesions produce equivalent effects in the village task and extend these sorts of analyses to nonspatial tasks.

Cortical gradients

The hippocampus is only one of several potential windows on what is likely a more global process of memory reorganization. By focusing on cortical contributions to the development and expression of remote memory, recent mouse-genetic and brain-mapping approaches have further strengthened the idea that circuits supporting different types of memory are reorganized over time (Table 3; [24, 29, 37, 49]).

First, mouse-genetic studies. During consolidation, the strengthening of cortico-cortical connections is thought to be crucial for cortical memories to gain independence from the hippocampus [68]. Therefore, disrupting cortical plasticity should hinder the establishment of remote hippocampal-independent memories, and result in premature forgetting at extended retention delays. This prediction was tested using mice that are heterozygous for a null mutation of α-calcium/calmodulin kinase II [69]. These mice have global deficits in cortical plasticity, but normal hippocampal plasticity [70–73]. Accordingly, they have normal memory at short retention delays (1–3 days), but pronounced forgetting at longer delays (10–50 days). Similar findings in mice that overexpress a dominant-negative mutant form of p21-activated kinase [74] also suggest that normal cortical plasticity is essential for the development of remote (hippocampal-independent) memories.

One of the limitations of the first generation of knockout and transgenic approaches is that gene function is altered throughout development. More recent studies have used mice where the expression of a dominant active form of α-CaMKII can be regulated in a temporally specific manner [31, 75]. For example, Tsien and colleagues [31] trained these mice in contextual fear conditioning and tested them a month later. They found that forebrain overexpression of the

Table 3 Cortical graded amnesia

Behavioral paradigm	Species	Brain target	Method	Outcome	References
Water maze spatial learning	Mouse	Anterior cingulate cortex	Inactivation	Deficit at 30 days, no deficit at 1 day	Teixeira et al, [49]
	Mouse	Forebrain	Mutation: Disruption of p21-activated kinase activity	Deficit at 21 days, no deficit at 1 day	Hayashi et al, [74]
Spatial multiple choice discrimination	Mouse	Prefrontal, anterior cingulate cortex	Inactivation	Deficit at 30 days, no deficit at 1 day	Maviel et al, [24]
Contextual fear conditioning	Mouse	Anterior cingulate cortex	Inactivation	Deficit at 18 and 36 days, no deficit at 1 and 3 days	Frankland et al, [37]
			αCaMKII$^{+/-}$ mutation	Deficit at 36 days, no deficit at 1 day	Frankland et al, [37]
Trace eyeblink conditioning	Rat	Medial prefrontal cortex	Aspiration	Deficit at 28 days, not 1 day	Takehara et al., [29]

mutant α-CaMKII in the week immediately after training, but not thereafter, blocked the formation of remote memory. Similarly, pharmacological inhibition of NMDA receptor function in the prefrontal cortex in the week immediately after training, but not thereafter, blocks the formation of remote trace eyeblink conditioning memory [76]. These studies not only identify a window during which hippocampal–cortical interactions are critical for the successful development of remote memories, but also begin to provide hints into molecular mechanisms underlying reorganization. They suggest that reactivation of networks of neurons supporting a memory induces a round of NMDA/α-CaMKII-dependent synaptic modifications. These modifications may incrementally alter the network, and many iterations of this process may eventually lead to wholesale changes in circuits supporting memory [68, 77, 78].

Second, brain mapping studies. Bontempi and colleagues [36] were the first to systematically compare activation patterns after expression of recent and remote memory throughout the brain. In the spatial discrimination task, they found that activation patterns depended on the age of the memory: Whereas the recall of recent spatial memories was associated with activation of the hippocampus and entorhinal cortex, the recall of remote spatial memories was predominantly associated with activation of cortical regions including the prefrontal, frontal, anterior cingulate, retrosplenial, and temporal cortices. This study provided the first brain-wide perspective on memory reorganization, and suggested that remote memories may be supported by distributed cortical circuits.

This time-dependent shift toward greater levels of cortical activation has now been observed in a number of other tasks using immediate early gene expression as an index of neuronal activation [13, 24, 37, 49]. For example, expression of a remote socially transmitted food preference is associated with activation of the orbitofrontal cortex [13], expression of a remote five-arm maze memory is associated with activation of the anterior cingulate, prelimbic, and infralimbic cortices [24], and expression of a remote water maze memory is associated with activation of the anterior cingulate cortex [49]. Similarly, expression of remote contextual fear memory was associated with activation of a number of prefrontal cortical regions, including the prelimbic, infralimbic, and anterior cingulate cortices [37]. Most importantly, this activation was absent in α-CaMKII$^{+/-}$ mice with deficits at this remote time point. This suggests that activity in these regions is playing a key role in the expression of remote memory [37].

The above experiments examined gene expression induced by memory expression (i.e., online memory reactivation). In a related series of studies, Ribeiro and colleagues [79, 80] have examined the induction of *zif268* during offline states (i.e., various stages of sleep) after behavioral exploration and long-term potentiation (LTP) induction. For example, after rats had explored a novel environment, upregulation of Zif268 was observed in the hippocampus and various cortical regions such as the piriform and frontal cortices during subsequent sleep [79]. Similarly, LTP induction in the dentate gyrus in awake, behaving rats led to upregulation of Zif268 in various cortical regions such as the entorhinal, auditory, somatosensory and frontal cortices during subsequent sleep [80]. Importantly, tetracaine-induced inactivation of the hippocampus before the onset of rapid eye movement (REM) sleep blocked the upregulation of Zif268 in these cortical regions. This indicates that gene expression in the cortex

might be under the control of the hippocampus and, therefore, cortical remodeling depends on hippocampal activity at least in the first several hours after training [80].

Although these brain-mapping studies provide evidence that different cortical regions play increasingly important roles in the expression of memory as a function of time, one difficulty in these studies is the choice of an appropriate control group. For some types of tasks (e.g., fear conditioning), there is relative consensus as to what an appropriate control is (e.g., no-shock group, immediate-shock group). However, in tasks such as the water maze the situation is much less clear, and controls that are overly conservative will lead to a high false-negative rate (e.g., swimming or visible platform controls). In contrast, less conservative controls (e.g., home cage) are likely to produce a high false-positive rate. A second issue is that immediate early genes such as *c-fos* and *zif268* are only indirect markers of activation. Whereas the expression of these genes is tightly correlated with levels of neuronal activity [81], differences in endogenous expression patterns across brain regions may limit their utility in brain-wide mapping studies. A third issue is that reorganization may not necessarily result in an overall increase in activity in a given region. For example, there is no overall increase in gene expression in the parietal cortex after recall of remote, compared to recent, spatial discrimination memory. However, closer examination showed that activation shifted from the deep cortical layers to more superficial layers over time [24]. These observations highlight the complexities of defining reorganization and raise the issue of what level of anatomical resolution is appropriate (or even feasible) for this type of analysis [82]. Keeping these caveats in mind, there is nonetheless convergent evidence from lesion and inactivation studies that prefrontal cortical regions preferentially contribute to the expression of remote memories.

A number of studies have now shown that disrupting prefrontal cortical function preferentially affects remote compared to recent memories (Table 3). In the first such study, Takehara and colleagues [29] showed that lesions of the prefrontal cortex (including the anterior cingulate cortex and prelimbic cortex) preferentially affected month-old trace eyeblink conditioning memories. (In this case, recent trace eyeblink memories were also affected, albeit to a lesser degree). Similarly, pharmacological inactivation studies have highlighted the role of the anterior cingulate cortex in the expression of remote contextual fear [37], spatial discrimination [24] and water maze [49] memories. Together these studies establish a causal role for these regions in the expression of remote memory, and are consistent with the idea that memories are reorganized in a time-dependent manner. However, to date there are relatively few studies, and many issues remain. For example, are these effects localized to a specific prefrontal cortical region (e.g., the anterior cingulate cortex) or do different regions support different types of remote memory? Are these regions storing remote memories, or merely coordinating their retrieval? Perhaps most central is the issue as to whether activation of prefrontal regions during remote memory recall depends on the age (or remoteness) of the memory or more effortful recall. Because older or more remote memories may be weaker, it is possible that increased activation of prefrontal regions reflects greater effort required to access a partially degraded memory trace [83].

Summary and future directions

Graded retrograde amnesia after hippocampal damage in patients forms the cornerstone of the idea that memories for certain types of information are reorganized in a time-dependent manner. However, these neuropsychological studies inevitably involve relatively few patients, and there is necessarily a lack of control over what is learned and when (because of the retrospective nature of these analyses), and over the size and extent of the lesion (combined with, in many cases, limited anatomical information). The superior level of control available in experimental animal studies (e.g., control of what is learned and when, extent of lesion, temporally and spatially specific overexpression of mutant genes), therefore, offers a huge opportunity to better define and understand how memories are reorganized over time. This brief review has highlighted what these studies have told us, but the fact that many issues remain unresolved suggests that this opportunity has not yet been fully exploited.

When hippocampal gradients occur

What we know A relatively conservative reading of the above experiments indicates that hippocampal gradients exist. At least in the majority of conditions and paradigms, these studies show that the hippocampus is preferentially activated during the expression of recent memory, and memory consolidation is preferentially affected by hippocampal manipulations in the days immediately after training (but not thereafter). These effects have been demonstrated using a range of different tasks, and importantly, a wide variety of approaches—including neuroanatomical lesions, brain-mapping and mouse-genetic manipulations. Insofar as these studies show that the circuits supporting the expression of recent and remote memory differ, they are generally consistent with the broad notion of reorganization. This conclusion that hippocampal gradients exist is not necessarily even a controversial one: Both standard models of consolidation [60, 61], as well as alternative accounts [62,

63], posit reorganization as a central process and both can readily account for hippocampal gradients.

What we do not know Whereas these studies successfully recapitulate the observations in human patients, it is less clear whether they provide any additional insights into the mechanisms underlying reorganization. Two issues in particular stand out. First, when hippocampal gradients are observed, the length of the gradient may vary dramatically across studies. For example, estimates of temporal gradients in studies of contextual conditioning vary between 28 and 100 days in rats [10, 15, 23]. This variability indicates that the relationship between a number of factors (e.g., intensity of learning, type of learning, type of lesion) and gradient length is poorly understood, and more systematic examination of how these factors impact on gradient length are needed.

Second, what is the role of memory reactivation in reorganization? The general idea is that memory reactivation is the driving force behind reorganization [68]. Within this framework, reactivations in either online situations (e.g., during explicit recall) or offline situations (e.g., during sleep) provide an opportunity for modifying a memory trace, and many iterations of this process will eventually lead to dramatic changes in the way memories are organized at the systems level. Consistent with the idea that reactivation plays a key role are recording studies showing that patterns of brain activity associated with earlier activity are selectively replayed during sleep [84]. However, establishing a causal relationship between reactivation and reorganization has been more difficult. To establish this, one would need to show that selectively blocking replay prevents time-dependent changes in the organization of memory. Alternatively, increasing replay (e.g., by using explicit reminders) might accelerate reorganization.

When hippocampal gradients do not occur

What we know There are several examples of ungraded amnesia after hippocampal damage (and it is likely that these flat gradients are underrepresented in published literature). In some instances, flat gradients are associated with extrahippocampal damage (e.g., perirhinal cortex). However, there are a significant number of examples that cannot be accounted for in terms of extrahippocampal damage. The most common (and widely recognized) instance is in the water maze where lesion, inactivation, and brain-mapping studies suggest that the hippocampus is always necessary for the expression of spatial memory. It is also important to recognize that the absence of hippocampal gradients in the water maze does not imply that water maze memories do not undergo reorganization because the finding that the anterior cingulate cortex (ACC) plays a preferential role in the expression of remote water maze memory suggests that circuits supporting water maze memories evolve over time [49].

What we do not know The key question here is not whether hippocampal damage is sometimes associated with flat gradients—in the case of the water maze there is widespread agreement that this is the case. Rather, it is whether flat gradients can tell us anything about hippocampal contributions to memory expression, and, more generally, the relationship between memory reorganization and memory quality. The finding that hippocampal manipulations tend to have greater impact in spatial tasks with greater navigational demands suggests that hippocampal activity contributes to navigational aspects of performance. However, the rodent village paradigm also raises the possibility that hippocampal-dependent and hippocampal-independent spatial memories differ qualitatively [65]. In these sorts of paradigm it will be important to establish explicit predictions about what a less precise or gist-like memory might look like, and extend these sorts of studies to examine nonspatial memories.

Cortical gradients

What we know We described accumulating evidence that different cortical regions play increasingly important roles in the expression of memory as a function of time. Again, this conclusion is based on experiments using a variety of tasks, and importantly, a wide variety of approaches. These initial results are generally consistent with the broad notion of reorganization, and, more specifically, a role for the prefrontal cortex in the expression of remote memory.

What we do not know It is perhaps surprising that localized inactivation of prefrontal cortical regions would have such profound effects on the expression of remote memory, given that it is widely thought that remote memories are supported by a much broader, distributed network. This suggests that some regions within this network (such as the ACC) may be more critical for function than others. Two goals are therefore important: first, to comprehensively identify regions supporting remote memory; second, to understand how the architecture of this network impacts on its function. Regarding the former, our current picture of how remote memories are organized is still very much incomplete. For example, the analysis of six cortical regions in the Frankland et al. study [37] represents only 2–3% of total forebrain volume, and brain-wide analyses are necessary to comprehensively map networks supporting remote contextual fear and other memories. Regarding the latter, the analysis of other complex systems may provide some clues [85, 86]. Any network—whether an airline

route map, a protein interaction network, or the anatomical connectivity of the cortex—can be described as a collection of nodes (in this case, brain regions) connected by links (in this case, afferent and efferent projections). An emerging theme is that many real-world networks, from metabolic and protein interaction networks to the World Wide Web [86, 87], have a "scale-free" structure [85]. The defining feature of such networks is that whereas the vast majority of nodes have few links, a small number of nodes are highly connected, and the distribution of linkages in such a scale-free network is then best described by a power law. In general, scale-free networks are robust, as the majority of localized perturbations will, more often than not, affect less connected nodes. However, the functional integrity of the network is vulnerable to targeted lesions of highly connected "hubs". The inactivation experiments raise the possibility that the ACC (and other prefrontal regions) occupy hub-like positions in networks supporting remote memory [88].

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