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Fading in

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Patient H.M. can form new memories and maintain them for a few seconds before they fade away. From a neurobiological perspective, this amnesia is usually attributed to the absence of memory consolidation, that is, memory storage. An alternative view holds that this impairment reflects that the memory is present but cannot be retrieved. This debate has been unresolved for decades. Here, we will consider some of the arguments that make it so difficult to resolve this issue. In addition, some recent work will be discussed that has gone beyond the shortcomings of previous experimental approaches to strongly suggest that amnesia can be due to a retrieval impairment that can be overcome with a reminder—an example of memories fading in. Finally, this review will suggest some strategies for resolving this debate.

The consolidation of new memories is a time-dependent process, during which the memory trace becomes more resistant to disruption (Ribot 1881; Ebbinghaus 1885). Our use of the term “consolidation” is explicitly aimed at cellular consolidation, which refers to the mechanisms that stabilize changes in synaptic efficacy (Kandel 2001). In the last 20 yr, great progress has been made across levels of analyses in understanding what mediates learning and memory (Davis and Squire 1984; Martin et al. 2000; Kandel 2001; Lisman 2003; Dudai 2004). The dominant approach used in this pursuit—experimental amnesia—entails challenging a candidate mechanism deemed necessary for the stabilization of new memories. The prediction is that if a necessary component of the stabilizing mechanisms is blocked, memory will be impaired on a retention test. Such impairments are called *experimental amnesia*. This approach has indeed led to impressive progress in understanding the mechanisms of consolidation. However, in the past decade, experimental amnesia within the physiological domain has been viewed more and more frequently as an impairment in the stabilization (i.e., storage) of new memories (McGaugh 2000). Amnesia, of course, could be due to the impairment of a multitude of cognitive processes such as learning (i.e., acquisition of new information), memory retrieval, motivation, attention, and so forth. Therefore, it is necessary to identify the nature of amnesia induced by various experimental manipulations. During the 1950s to 1970s, the question was debated of whether retrograde amnesia—which can be induced by agents or events that inhibit the post-training stabilization process—is due to an impairment in memory storage or in memory retrieval (Gold and King 1974; Miller and Springer 1974). Up to now, this issue still has not been completely resolved. Today, it is assumed that intact short-term memory (STM) and impaired long-term memory (LTM) demonstrate that a memory storage process has been blocked. There is not much consideration for the alternate view that the memory might have been stored but cannot be retrieved. As we will discuss, it is possible to interpret every study to date of experimental amnesia equally well as a retrieval deficit.

This article will focus on issues and arguments that surround cellular consolidation, the stabilization of changes in synaptic efficacy that occurs over hours (Dudai and Morris 2000). However, analogous arguments can be applied to the systems level of consolidation, in which the hippocampus is thought to play a time-limited role in memory processing. It will review how re-

covery from amnesia is used to support the retrieval impairment explanation of experimental amnesia and, more importantly, how this phenomenon is unequivocally accommodated by the storage impairment view. We will also outline some of the issues that have prevented reaching a resolution regarding the nature of amnesia. At the end of the article, we suggest some alternative avenues to examine the nature of amnesia, which we hope will stimulate more discussion that will in turn accelerate research on this topic. Perhaps, in this way, this issue can finally be resolved.

Recovery from amnesia

It has been very difficult to resolve whether experimental amnesia is a storage or retrieval deficit because the techniques and rather simple behavioral paradigms that have been used to investigate the issue did not differentiate between these two interpretations. Collectively, these “recovery from amnesia” paradigms entail inducing experimental amnesia by treating animals with an amnesic agent, such as protein synthesis inhibitors or electroconvulsive shock (ECS). Some manipulations involving reminders are typically performed to see whether performance will return or “recover.” The reminder treatments include: waiting (spontaneous recovery) (Serota 1971), performing repeated tests (Zinkin and Miller 1967), exposing animals to the unconditioned stimulus in a different context (reinstatement) (Miller and Kraus 1977), exposing the animals to the conditioned cue (Gordon and Mowrer 1980), and exposing the animals to the conditioning context (renewal) (Sara 1973). These experiments are aimed at testing whether the memory can be retrieved or not. Successful recovery would suggest that the memory was present but inhibited, indicating that the induced amnesia represented a retrieval deficit. If there was no recovery, then, some argue, the amnesia-inducing treatment prevented memory storage.

Unfortunately, the underlying logic of this paradigm does not permit conclusions as to whether amnesia is a storage or retrieval deficit. This is because the only prediction made by the storage impairment view of amnesia (SIVA) is that if the memory is not stored, then performance will not recover. The strongest data supporting this view are cases where nothing changes after a reminder and memory does not recover (Luttges and McGaugh 1967). However, the absence of recovery can hardly be taken as evidence that the memory was blocked from being stored and does not exist in the brain because it is possible that the memory simply cannot be retrieved at the point of testing.

It is, therefore, easier to defend the retrieval impairment view of amnesia (RIVA). Recovery from amnesia is taken as evidence that the memory is always there but cannot be retrieved. If

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there is no recovery from amnesia, it can be argued that the retrieval deficit is too comprehensive to allow memory to recover under the testing conditions (Miller and Springer 1973). Therefore, the logical structure of the retrieval argument a priori accounts for any possible outcome of a recovery manipulation after amnesia induction—this explanation cannot be falsified. Some of the models that have been proposed by proponents of RIVA suggest that consolidation occurs within a few seconds after learning. The subsequent instability of the memory represents the retrieval “route” being organized and stabilized (Lewis 1979; Miller and Marlin 1984; Miller and Matzel 2000). According to these models, it is the stabilization of the retrieval route that post-training amnesic treatments impair.

In conclusion, the central problem of the recovery from amnesia paradigm is that while RIVA can explain any finding and cannot be falsified, SIVA makes a priori negative predictions that cannot be verified. This renders it impossible to decide which explanation accounts most appropriately for the nature of experimental amnesia.

Is recovery uniquely consistent with RIVA?

Although the “absence” of recovery is consistent with both RIVA and SIVA, is the “presence” of recovery sufficient to disprove SIVA and prove RIVA? The short answer to this question is no. Shortly after the early demonstrations of recovery from amnesia, it became clear that the issue was not whether there was recovery, but rather *why* recovery occurred. Some examples of recovery were shown to be consistent with a storage impairment view of amnesia, based on the fact that amnesic treatments never induce complete memory loss. There was always some “small residual trace” on which knowledge could be added or modified. Modification or modulation of the residual trace, in turn, could produce an enhancement in performance, which looked like overcoming retrieval impairment (Cherkin 1972; Gold et al. 1973). For example, Gold et al. (1973) showed that reinstatement could be due to new learning strengthening a weak residual trace, instead of overcoming a retrieval block. If the footshock used for reinstatement was given to non-amnesic animals that were weakly trained in order to match the level of performance of the amnesic animals, then both groups showed the same increase in performance at a subsequent retention test. The reinstatement protocol was ineffective in untrained animals, demonstrating the requirement for some initial baseline behavior. Given the behavioral change in animals that were never amnesic, the increase in behavior induced by the non-contingent footshock could not have been due to overcoming a retrieval impairment. Therefore, an alternative interpretation of reinstatement was that the footshock produced new learning in amnesic animals that had a small residual trace. This position is consistent with SIVA.

Other variables such as the number of tests performed to reach recovery (King and Glasser 1970), strength of conditioning (Quartermain and McEwen 1970), and extent of the behavioral impairment caused by the amnesic treatment (Cherkin 1972; Gold et al. 1973; Davis et al. 1978) were also found to modulate recovery. In the latter case, the probability of recovery was inversely related to the size of the behavioral impairment. Hence, it soon became apparent that recovery from amnesia did not necessarily prove that the animals had overcome a retrieval blockade.

Arguments were also put forward to reconcile spontaneous recovery from consolidation blockade induced by protein synthesis inhibition (Flexner et al. 1965; Quartermain and McEwen 1970; Serota 1971; Quartermain et al. 1972; Squire and Barondes 1972) and ECS (Cooper and Koppenaal 1964; Kohlenberg and Trabasso 1968; Young and Galluscio 1971) with a storage deficit

interpretation of amnesia. Squire and Barondes (1972) reported spontaneous recovery from cycloheximide-induced amnesia 3 d after training, and suggested that the initial impairment was due to a blockade in memory storage. They further proposed that the underlying reason for recovery was due to a novel memory system that expresses behavior over a delay of some days. This memory system was posited to work independently of the traditional systems. Similarly, the incubation effect (Bindra and Cameron 1953), in which levels of responding increase with time in aversive paradigms, also represents the strengthening of a residual trace. This can be a mechanism for the residual trace to reach the threshold for the expression of behavior with time.

We propose another more contemporary interpretation for the memory recovery observed in hippocampus-dependent tasks, which are commonly used to show recovery from amnesia. The hippocampus has been shown to perform pattern completion when only degraded or incomplete input is provided (Nakazawa et al. 2002). It is plausible to assume that if an amnesic treatment leaves a residual memory trace (i.e., a partial or degraded one) in a simple paradigm, such as inhibitory avoidance, the hippocampus might, by virtue of pattern completion, recreate an activity pattern that approximates the original one in a future retention test. This would produce apparent “spontaneous recovery,” although the amnesia reflected storage impairment.

Another set of findings shows that activation of the catecholamine system before tests of LTM can reverse amnesia induced by a protein synthesis inhibitor (anisomycin and cycloheximide) or cholinergic receptor blocker (scopolamine) (Quartermain and Leo 1988; Quartermain et al. 1988). These effects are difficult to interpret in terms of a general enhancement caused by relearning or motivation. Instead, catecholamine is believed to enhance memory by modulating its strength (McGaugh 2000). Even in this case, SIVA might still be able to argue that the catecholamine system is modulating or enhancing the small residual trace. However, invoking modulation in a post hoc manner makes the storage impairment interpretation of amnesia almost as difficult to falsify as the retrieval impairment view. It is now clear why in the vast majority of cases, recovery from amnesia can be consistent with either RIVA or SIVA.

There is no evidence in the recovery from amnesia studies supporting the storage view of amnesia that cannot also be supportive of the retrieval position. Furthermore, recovery is not on its own a sufficient condition to permit the conclusion that amnesia was due to a retrieval impairment. The critical question has been *why* recovery occurs, rather than whether recovery is observed or not. For example, does the memory recover because a retrieval impairment has been overcome? Or, does it recover because a residual trace has been strengthened through new learning, possibly pattern completion, or modulation?

Onward to the past

The demonstration that consolidated memories, when reactivated, can undergo another time-dependent memory stabilization process called reconsolidation (Nader et al. 2000; Sara 2000; Nader 2003) has renewed interest in the nature of experimental amnesia. Cellular reconsolidation states that reactivation of the synapses that contribute to a consolidated memory representation can induce another time-dependent stabilization process. However, reconsolidation does not bring any new behavioral techniques to bear on whether amnesia is due to a storage or retrieval impairment. Several studies have already begun to test whether the post-reactivation amnesia reported in reconsolidation studies is due to a retrieval or storage impairment (Vianna et al. 2001; Anokhin et al. 2002; Debiec et al. 2002; Bozon et al. 2003; Child et al. 2003; Pedreira and Maldonado 2003; Eisenberg

and Dudai 2004; Fischer et al. 2004; Lattal and Abel 2004; Salinska et al. 2004; Suzuki et al. 2004). Unfortunately, these studies use the same recovery from amnesia paradigms that, as discussed above, have failed in the past to reveal the nature of amnesia. Currently, the majority of studies take recovery as sufficient evidence for amnesia being a retrieval impairment and do not consider the alternative interpretations that are consistent with SIVA. For example, one study (Lattal and Abel 2004) reported spontaneous recovery in animals that were administered anisomycin after reactivation, but not in animals that were administered the drug after initial learning. The authors suggested that the post-reactivation anisomycin impairment was due to a retrieval impairment. However, the amnesia in the post-reactivation group was weaker than that in the post-learning group, and the magnitude of amnesia is a factor known to influence the probability of recovery (Davis et al. 1978). Similarly, a recent finding (Power et al. 2006) showed spontaneous recovery from a relatively weaker amnesia in avoidance task, compared with no spontaneous recovery from a relatively stronger amnesia in contextual fear conditioning (Debiec et al. 2002). Although these are reported in different behavior tasks, we want to point out that the difference in baseline amnesia is an important factor to predict recovery as what has been reported before—the greater the amnesia, the lower the probability of recovery (Davis et al. 1978). In addition, the study by Power et al. used multiple retention tests on the same animals, which is also a factor affecting the probability of recovery (King and Glasser 1970). Because of the observed spontaneous recovery, these investigators would seem to support RIVA as an explanation of the post-reactivation amnesia. However, if one applies that same standard to amnesia for new learning, which also shows recovery under these conditions (Zinkin and Miller 1967; Squire and Barondes 1972; Davis et al. 1978), then presumably they would have accepted RIVA for that outcome as well.² In fact, given that consolidation and reconsolidation have different parameters (Lee et al. 2004), if recovery were to occur, then differential recovery should not be surprising.

Similarly, there are studies showing reinstatement as evidence for amnesia being a retrieval impairment (Fischer et al. 2004) without considering the alternative interpretation from the perspective of storage impairment (Gold et al. 1973). Indeed, some scientists who were part of proposing the novel reinterpretation of reinstatement seem to have had a conceptual change of heart in the debate and are now taking recovery from amnesia from post-reactivation-induced amnesia, including reinstatement, as sufficient evidence that the initial amnesia was due to a retrieval impairment (Power et al. 2006; Prado-Alcala et al. 2006).

To move beyond the conceptual stalemate, we suggest not investing any more time in trying to understand whether amnesia is a retrieval or storage processes by using simple behavioral paradigms in conjunction with the recovery from amnesia protocols. Rather, we suggest that (1) new, falsifiable models of retrieval be developed; and (2) protocols capable of positive predictions for the blockade of memory storage be designed. These

approaches will help differentiate between the a priori predictions of SIVA and RIVA. In the following section, we will propose some tentative suggestions that may move research closer into the right direction and that may initiate a dialogue on how to best approach these issues.

Can a neurobiological description of consolidation identify the nature of amnesia?

In the past decade, there have been attempts to unravel the cellular and molecular mechanisms of memory consolidation using a reduced preparation modeling simple behaviors. This research has, for example, identified that local protein synthesis is required for maintaining the strengthened connections between sensory and motor synapses, and that this process involves the activation of cAMP-dependent gene transcription (e.g., Yin et al. 1994; Bailey et al. 1996). These studies led to the proposal that these cellular and molecular correlates of consolidation were the mechanisms by which memories are stored in the brain. As no other neural connection is involved in this reduced preparation, the conclusion appears to be straightforward that the observation site is the storage site and that, therefore, these changes necessarily reflect storage mechanisms. Moreover, it is generally assumed that in cellular consolidation the memory retrieval is simply the read-out of the changes in plasticity induced by learning. Therefore, a “retrieval” mechanism is not required at this level of analysis.

In general, this evidence and evidence of this kind provide strong support for SIVA. It is admittedly difficult to imagine an alternative interpretation. However, RIVA can easily incorporate this data. For example, models of memory that are based on amnesia being due to a retrieval impairment posit that memory consolidation occurs very quickly, typically within 1 sec. After that, the subsequent lability of the memory represents the stabilization of retrieval routes (Lewis et al. 1969; Miller and Matzel 2000). From this perspective, the cellular changes occurring during the post-training period are correlates of retrieval route stabilization instead of memory trace storage.

This argument is not limited to molecular studies and applies to neural recording studies as well, in which post-learning neural activity signatures are linked to a memory storage mechanism. Miyashita et al. (1998) showed that training with paired visual stimuli can induce a pair-encoding pattern of neuron responses in the inferotemporal cortex. If the input signal is blocked by entorhinal and perirhinal cortex lesions, these neurons lose the pair-encoding property. The investigators suggest these neurons are able to “represent” the long-term memory. Although it is not stated explicitly, these neurons could be storing the memory.

However, RIVA could argue that, despite the correlation in changes between the neuronal and behavioral responses, the neurons are encoding information for retrieval and that there is an alternative mechanism for memory storage, which is left intact after the lesion. The same argument applies to all similar correlational studies (e.g., studies that correlate synaptic or neural changes to memory). In conclusion, these diverse approaches may not be helpful in dissociating SIVA and RIVA.

It is very informative to have a complete description of the post-training modification that represents the neural correlates of memory. However, we are concerned that if the nature of amnesia is not resolved at the behavioral level, then the same arguments could be made across levels of analyses and the arguments can become post hoc. The different positions could simultaneously claim that the cellular correlates of the post-training stabilization period represent storage or retrieval mechanisms. Ultimately, the question is whether there is any inherent reason

²Because of all of these issues surrounding amnesia and the lack of consensus on its nature, K.N. took an agnostic view of amnesia (Debiec et al. 2002). Given that consolidation and reconsolidation are both time-dependent memory stabilization processes and there are many commonalities in mechanism, then it seemed parsimonious to view the nature of amnesia for new learning and reactivated memories as being the same. Furthermore, K.N. is open to amnesia being either a storage or retrieval impairment. Amnesia for new information is typically discussed in terms of a blockade of consolidation. Therefore, in line with this tradition, K.N. has discussed amnesia for reactivated memories as a blockade of reconsolidation. This is admittedly a slightly confusing position, but the other options were a view that cannot be proven and one that cannot be refuted.

why the growth of a synapse, or any other correlate of memory stabilization, is uniquely consistent with a storage mechanism of memory.

Ways forward

Exploiting the different predictions between SIVA and RIVA

SIVA can reinterpret reinstatement as new learning adding onto a residual trace. One line of approach that has been used to differentiate between RIVA and SIVA is to test if a reminder treatment, which would decrease behavior in control animals, could lead to recovery from amnesia. If this could be demonstrated, then it is unlikely that the reminder could have caused recovery by new learning because the new learning should decrease, not increase performance. There are some such demonstrations. For example, reminding animals with a CS presentation that leads to extinction in control animals (lower levels of responding) can actually induce recovery (higher levels of responding) in animals made amnesic with ECS (Gordon and Mowrer 1980). This is challenging for an interpretation of recovery based on the idea of new learning. However, the possibility that new learning may contribute to the recovery from amnesia cannot be completely ruled out.

Another demonstration of this approach is a recent study that differentiates between RIVA and SIVA (de Hoz et al. 2004). In this study, a more complex behavioral protocol is used to assay specific information about spatial location in the water maze in conjunction with an amnesic manipulation that does not allow for new learning. These two changes from the traditional approach make the conclusions of this study very powerful. After the animals were trained to find a hidden platform in a quadrant of a pool, they were made amnesic by partial or complete lesions of the hippocampus. The authors used probe trials as a reinstatement procedure with platform positions either in the same quadrant as during training (SAME) or in the opposite quadrant (DIFF). Reinstatement was observed in both SAME and DIFF groups if the animals were partially lesioned, although the effect size was smaller than in the sham-lesioned animals. SIVA can use learning summation to explain reinstatement in the SAME group because the new spatial information provided during reinstatement was consistent with the old information regarding the platform location. However, SIVA cannot easily explain the reinstatement in the DIFF group because new information acquired during reinstatement was in conflict with old information represented by the residual memory. This should have impaired, as opposed to improved, performance. By using a behavior that is richer in the information content being acquired in conjunction with an amnesic treatment that prevents new learning, the investigators have shown a new approach to differentiate SIVA and RIVA. It would be interesting to test if the amnesia in a water maze, induced by modern genetic tools such as inducible knockouts, would also show reinstatement when the reminder platform is presented in the opposite quadrant to the trained one as performed in this study.

Patient H.M., who, in 1953, received resection of his medial temporal lobe to control intractable epilepsy, cannot maintain new memories, as they soon “fade away” during the day (Milner et al. 1968). H.M. had incomplete lesions of his medial temporal lobes, which are similar to the animal subjects with partial hippocampus lesions. The data by de Hoz et al. (2004) suggest that memories can also “fade in” if a proper reminder is provided.

This principal experimental approach could be adapted for simple behaviors. What will be needed is to prevent new learning from occurring during the reinstatement trials and test whether

reinstatement still occurs. For example, new learning of auditory fear conditioning requires NMDA receptor activation in the basolateral amygdala (BLA) (Rodrigues et al. 2001). Intra-amygdala infusions of ifenprodil, an NR2B antagonist, only affect performance when they are given prior to training. These infusions are without effect when given prior to a LTM test, demonstrating that ifenprodil does not induce a retrieval impairment. Furthermore, post-training infusion of ifenprodil also has no effect on the acquisition of auditory fear conditioning, demonstrating that ifenprodil is not affecting the post-training stabilization period. Thus, the simplest interpretation of the nature of ifenprodil's impairment is that it induces a learning deficit.

Protein synthesis inhibition in the BLA immediately after training induces amnesia (Schafe and LeDoux 2000; Maren et al. 2003). Although reinstatement has not been reported for amnesia of auditory fear conditioning (Duvarci and Nader 2004), for the sake of argument, let's assume it has. If reinstatement is due to new learning, then NMDA receptor blockade in the BLA prior to reinstatement will prevent the memory from recovery in amnesic animals because no new learning could be added to the residual memory trace in the BLA. This, of course, assumes that the mechanisms mediating new learning are similar to the original learning, which can be empirically verified. For NMDA receptors in the amygdala in fear conditioning, they are required for both initial learning and subsequent fear learning (Lee and Kim 1998). Therefore, blocking the mechanisms that mediate new learning in this memory system can test the new-learning interpretation of reinstatement. In order for this approach to be successful, the dose of the compound that prevents learning needs to be optimal to ensure as little learning as possible; any residual learning could potentially summate with the residual trace that remains after induction of amnesia, and thus enhance performance. We believe this design is a reasonable first approximation to understand the nature of amnesia.

Understanding the mechanism mediating retrieval

Another strategy that might lead to a resolution of the debate on the nature of amnesia is to fully describe the cellular mechanisms that mediate retrieval itself, as others have suggested (Quartermain et al. 1988; Sara 2000; Dudai 2006). A significant number of experimental amnesia studies are being performed by blocking intracellular processes that contribute to cellular consolidation. If we had a complete description of the molecular changes that occur at the synaptic level to mediate the expression of a long-term memory, then we could empirically determine whether they have been compromised by a prior amnesic treatment. This in turn would permit experiments that allow falsification of the retrieval impairment interpretation of amnesia. Experimentally, this would be accomplished by infusing some amnesic agent prior to a LTM test and taking performance during the LTM test as the dependent measure. Examples of this approach include work demonstrating that MAPK and PKA in the hippocampus are necessary for the expression of inhibitory avoidance (Szapiro et al. 2000, 2002).

The advantage of studying the mechanisms that mediate retrieval of a memory during a retention test is that we can study the retrieval processes independently of any contributions from memory storage (Quartermain et al. 1988). For example, infusions of the AMPA antagonist CNQX into the amygdala prior to a long-term retention test performed days after training, when cellular consolidation is complete, blocks the expression of a conditioned fear response (Kim et al. 1993). This effect cannot be reinterpreted as a storage impairment. Thus, by examining expression mechanisms that are active during a long-term retention test, we can study memory expression in the absence of

storage. In contrast, mechanisms that are blocked after new learning and then lead to amnesia could, as discussed above, mediate either a memory-retrieval or storage function.

Summary

Significant advances have been made in understanding the molecular basis of the post-training memory stabilization period by using experimental amnesia as a measure for consolidation blockade. Historically, there was, however, no resolution of the question of whether amnesia reflects a storage or retrieval impairment of memory. Therefore, it remains logically possible that the post-training molecular mechanisms that, when blocked, lead to amnesia, are actually reflecting the stabilization of retrieval processes. Currently, it appears that there are too many studies using protocols and paradigms that have failed in the past to reveal the nature of amnesia. We suggest that an effective strategy to advance our understanding of amnesia is based on three approaches: (1) the development of new protocols, as has been done for the water maze, that can differentiate between SIVA and RIVA; (2) providing a complete description of the retrieval mechanisms of long-term plasticity at the cellular level; and (3) establishing a method to positively demonstrate that a memory has not been stored.

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