Reversible neural inactivation reveals hippocampal participation in several memory processes

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Studies of patients and animals with brain lesions have implicated the hippocampal formation in spatial, declarative/relational and episodic types of memory. These and other types of memory consist of a series of interdependent but potentially dissociable memory processes—encoding, storage, consolidation and retrieval. To identify whether hippocampal activity contributes to these processes independently, we used a novel method of inactivating synaptic transmission using a water-soluble antagonist of AMPA/kainate glutamate receptors. Once calibrated using electrophysiological and two-deoxyglucose techniques *in vivo,* drug or vehicle was infused chronically or acutely into the dorsal hippocampus of rats at appropriate times during or after training in a water maze. Our findings indicate that hippocampal neural activity is necessary for both encoding and retrieval of spatial memory and for either trace consolidation or long-term storage.

The study of brain dysfunction caused by lesions has revealed evidence that the hippocampal formation and related neocortical structures are involved in various 'types' of memory[1–5]. A logically separate issue concerns the participation of this (or other brain areas) in distinct memory 'processes'—namely encoding, retrieval, storage and consolidation[2,6–9]. Permanent lesions cannot unambiguously dissociate potentially separable memory processes. Lesions made before training would impair later recall were they to disrupt any of these four memory processes; those made after training could, depending on their time of administration, cause deleterious effects by disrupting the integrity of trace storage, time-dependent consolidation processes or retrieval. Reversible temporary inactivation offers an opportunity to isolate the obligatory contribution of hippocampal activity to these processes.

The advantage of using reversible local inactivation is that it should disrupt any memory-related process for which normal neural activity in a brain structure is required, without affecting other memory-related processes engaged at later times[10]. First, with respect to encoding-related and retrieval-related memory processes, we reasoned that chronic inactivation throughout training and acute inactivation during a single retention trial should each be sufficient to disrupt these memory processes independently. We therefore used both drug infusion techniques successively in individual animals. Second, with respect to consolidation processes, we made the reasonable guess that chronic inactivation for seven days, beginning one day after the end of training, might be sufficient to shut down a long-term consolidation process[2]. It is unlikely that neural inactivation beginning one day after several days of training would affect short-term consolidation mechanisms, an assumption tested with relevant control groups. Third, we also recognised the possibility that chronic hippocampal inactivation after training might disrupt the maintenance of altered synaptic weights, widely assumed to underlie information storage in the hippocampus[11,12].

Reversible inactivation of the hippocampus was achieved by blocking fast glutamatergic synaptic transmission using the selective AMPA/kainate receptor antagonist LY326325 (ref. 13). Electrophysiological and two-deoxyglucose methods were first used to validate this method of neural inactivation with respect to its magnitude, time-course and regional extent. We then examined hippocampal participation in these four memory processes using a modified reference memory water maze task designed to maximize the persistence of spatial memory.

Results

Reversible neural inactivation of the hippocampus

LY326325 is a different salt of the better known decahydroisoquinoline compound LY293558 (refs. 13, 14) that blocks the glutamate receptor subunits GluR1–5. It was chosen because its excellent water solubility enabled artificial cerebrospinal fluid to be used as a vehicle for continuous infusion of the hippocampus for up to 14 days. Thus we could avoid the brain damage that would occur with organic solvents such as DMSO, which are required for the initial solubilization of quinoxalinedione AMPA receptor antago-

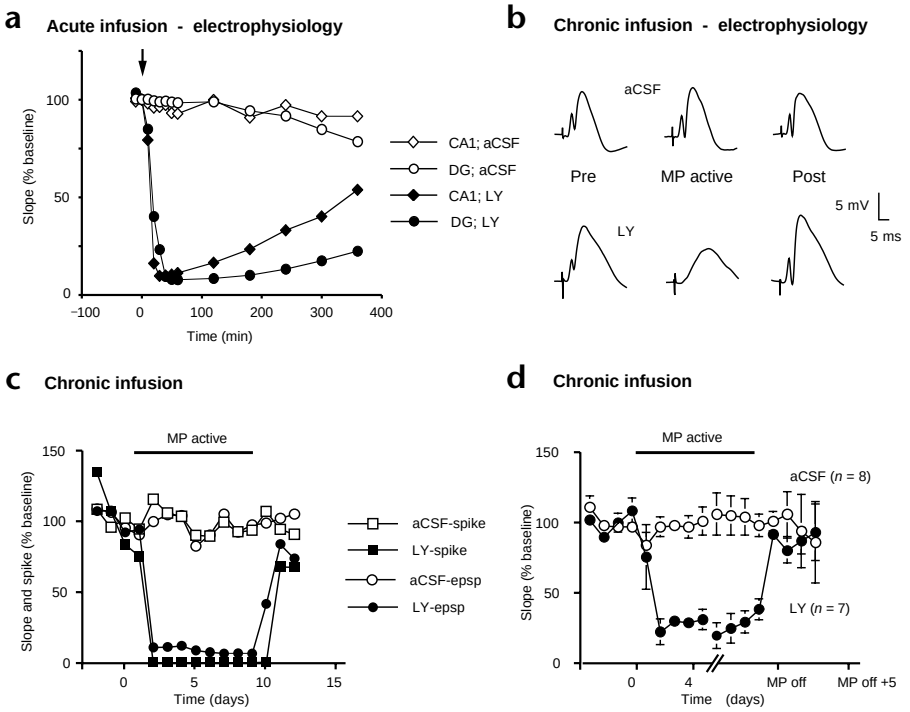

Fig. 1. The AMPA/kainate receptor antagonist LY326325 blocks fast synaptic transmission at perforant path/granule cell synapses. (**a**) Acute field potential recordings in urethane-anesthetized rats in response to stimulation at 0.05 Hz. Filled symbols, LY-treated rats; open symbols, vehicle-treated rats (aCSF). Dentate potentials in response to perforant path stimulation (circles, n = 6, 6); CA1 potentials in response to homotopic stimulation of the contralateral hippocampus (diamonds, n = 8, 8). Data are normalized to a stable baseline (20 min, two data points) and pooled over 10-min periods. Intrahippocampal infusion (arrow) of LY (1 µl, 1.5 mM, over 10 min) caused a decrease in field-potential slope of about 90%, which was maintained with only gradual recovery over 6 hours; vehicle had no effect on baseline signals. (**b**) Chronic recordings from representative freely moving rats. Recording 1 ('Pre') was obtained during baseline (when all animals had continuous vehicle treatment). Recording 2 ('MP active', that is, under LY) shows the reduction in the early rising slope of the extracellular field potential and lack of population spikes. The nature of the slow residual signal remains unknown. Recording 3 ('Post') shows full recovery of potentials after cessation of the micropumps. (**c**) Full time course of representative vehicle and LY micropump-implanted animals. Means for dentate slope and population spike. (**d**) Pooled data from chronic dentate recording experiments (vehicle, n = 8; LY, n = 7; mean ± s.e.). Data were averaged both forward (locked to time to when micropump was changed) and backward (locked to moment when within 20% of normal field-potential was restored). The use of backward averaging was needed to show the abrupt return to normal in all animals when the micropump stopped pumping. (Duration of pumping could vary by ± 1 day.)

nists such as CNQX[15]. We chose a glutamate receptor antagonist over a local anesthetic (such as lidocaine) to avoid disrupting fibers of passage through the dorsal hippocampus. This is analogous to making a neurotoxic rather than an aspiration or electrolytic lesion[16], with the added advantage of reversibility. As this single drug was used throughout, we shall hereafter refer to it as 'LY'.

The extent and time course of temporary inactivation of the hippocampus was examined electrophysiologically in rats. In acute experiments, we monitored either dentate gyrus field potentials in response to perforant path stimulation, or CA1 potentials in response to stimulation of the homotopic contralateral CA1 region. Infusions of LY (1 µl, 1.5 mM) decreased extracellular field potentials by up to 90% with a very gradual recovery over 4–6 hours (**Fig. 1a**). Vehicle infusions had no effect. Dentate population spikes disappeared completely; CA1 stimulation was always below spike threshold.

Chronic LY experiments in awake animals with previously implanted electrodes and intrahippocampal cannulae were then

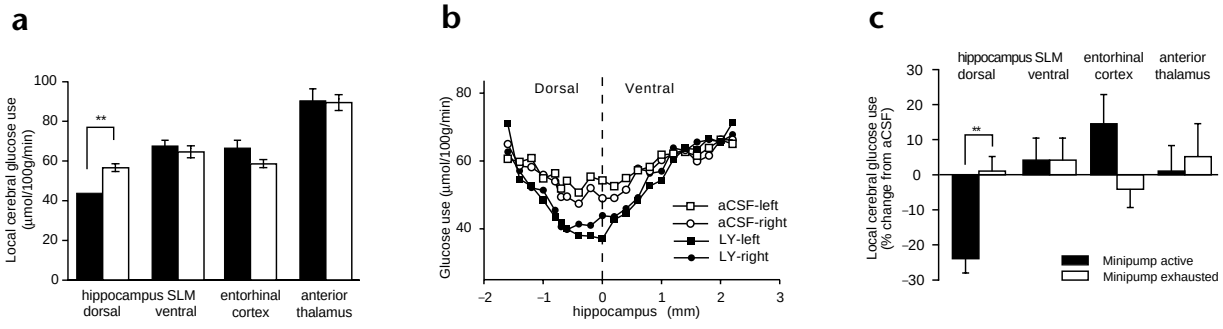

Fig. 2. The AMPA/kainate receptor antagonist LY326325 reduces glucose utilization in the dorsal hippocampus. (**a**) Absolute levels of local cerebral glucose use in 4 memory-related brain areas 4 days after the start of chronic infusion into the dorsal hippocampus. Black bars 0.375 mM LY at 0.5 µl per h, n = 5; vehicle, n = 5; **p=0.002, Student's t-test. (**b**) Local cerebral glucose use in the molecular layer of CA1 along the full longitudinal axis of the hippocampus of both hemispheres. (**c**) Normalized data for relative glucose utilization by vehicle and LY groups when the micropump was active (4 days after start of infusion, as in **a** and **b**) or exhausted (11 days; vehicle, n = 4; LY, n = 5). In this panel only, black and white bars reflect times of glucose measurement after micropump implantation.

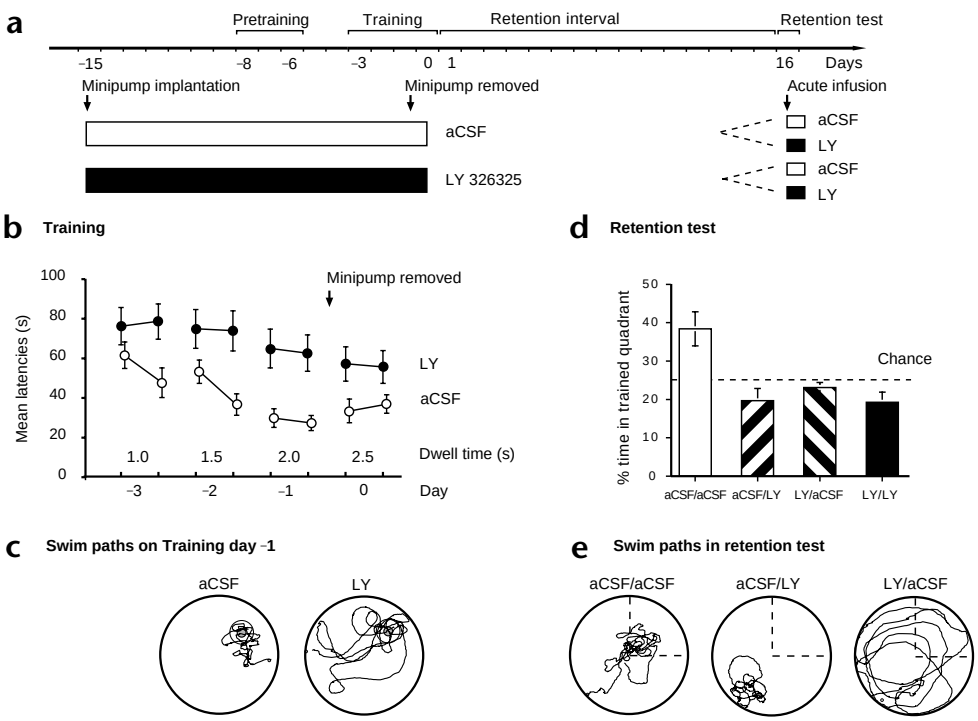

Fig. 3. Hippocampal inactivation disrupts encoding- and retrieval-related processes of spatial memory. (**a**) Experimental design showing chronic infusion of vehicle (open panel) or LY (black) over 14 days, followed after 16 days, by acute infusions of either vehicle or LY. Day 1 was always the first day of the retention period after the end of training. Training was conducted over 4 days (day −3 to day 0), with the 14 day minipump removed on day −1 (see Methods). (**b**) Escape latencies of groups trained during continuous chronic infusions of vehicle or LY (vehicle, $n = 13$; LY, $n = 11$; mean ± s.e.; $F_{1,22} = 9.31$, $p < 0.01$). (**c**) Representative paths taken by a vehicle- and an LY-treated rat on day −1 of training. Note the direct path of the control animal and efficient search at the correct location; the path of the LY-treated animal is more circuitous. (**d**) Proportion of time spent during the 60-s retention test in the 'correct' quadrant of the pool that had formerly contained the escape platform. The groups differed significantly (mean ± s.e.; $F_{3,20} = 8.04$, $p < 0.001$). Subsequent comparisons using Dunnett's test showed the vehicle/vehicle group was significantly better than the other three groups, which did not differ from one another ($p < 0.05$). (**e**) Representative paths taken during memory recall by a vehicle/vehicle, LY/vehicle and vehicle/LY rat. Note that the animal trained under vehicle but tested under LY shows focused search, but at an inappropriate location in the pool, whereas the animal trained under LY but tested under vehicle fails to show a focused searching strategy (dotted line within pool, training quadrant).

used to determine the time course of temporary inactivation and recovery over several days. Stable baselines were first obtained during continuous unilateral infusion of vehicle from a 14-day osmotic minipump. After 10–12 days, this was replaced with a 7-day micropump containing 0.375 mM LY or vehicle, and field potentials were monitored daily. A representative LY-treated rat (**Fig. 1c**) showing both field-potential slope and population spikes reveals that dentate field potentials 'switched off' over one day and then 'switched on' again seven to eight days later. As the exact moment of switching on varied from animal to animal, averages were obtained by aligning data both with respect to when the micropump containing LY was implanted and separately with respect to the point at which field potentials returned to within 20% of normal. The resulting 'split plot' reveals the abrupt decrease in fast synaptic transmission, which remained inhibited throughout the infusion period (with some animals showing a slow late potential; **Fig. 1b**) and then the relatively abrupt return to baseline levels in individual animals within one day of micropump exhaustion (**Fig. 1d**). Signals in vehicle controls remained at baseline throughout.

The spatial distribution of the effect of intrahippocampal infusion was examined by measuring function-related glucose use with [^{14}C]2-deoxyglucose autoradiography (2-DG)[17]. Peripheral administration of LY293558 reduces glucose utilization throughout the brain[18], but more localized changes should occur during chronic intracerebral infusion. Animals were implanted with bilateral cannulae into the hippocampus connected to seven-day micropumps and examined with standard 2-DG techniques after either 4 days (during drug infusion) or 11 days (after micropumps were exhausted).

At 4 days, local cerebral glucose utilization was reduced by 23% in the stratum lacunosum moleculare of the dorsal but not ventral hippocampus (**Fig. 2a**). No changes were observed in other memory-related areas (entorhinal cortex, anterior thalamus) or in over 20 other brain areas examined (data not shown). The maximal reduction in glucose use occurred along the dorsal part of the longitudinal axis adjacent to the implant site (**Fig. 2b**), with an equivalent decrease in both hemispheres. At 11 days (micropump exhausted), glucose use returned to levels indistinguishable from those of vehicle-treated animals (**Fig. 2c**). Thus, intrahippocampal infusion of LY reversibly decreases fast synaptic transmission in a spatially restricted manner so that dentate granule cells no longer fire action potentials for at least seven days but function in an apparently normal manner thereafter.

Memory encoding and retrieval

To examine whether reversible hippocampal inactivation could affect the encoding or retrieval of memory, we treated animals with vehicle or LY during training and/or during retention using a 2 × 2 factorial design (**Fig. 3a**). We disrupted the dorsal hippocampus during training (to determine if this affected encoding-related memory processes) and disrupted it during a later retention test (to determine if this affected retrieval). These manipulations were done symmetrically, which would not be possible with permanent lesions.

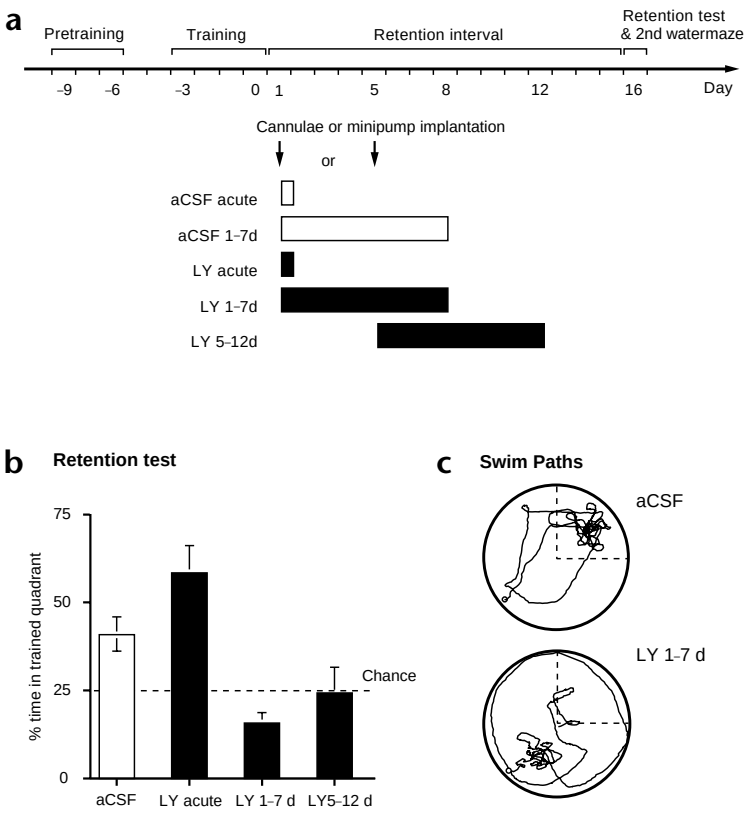

Fig. 4. Chronic but not acute inactivation of the dorsal hippocampus interferes with a post-training memory process. (**a**) Experimental design shows spatial training by normal animals followed by the five different treatments beginning one or five days later. The retention test was always conducted 16 days after the end of training. (**b**) Retention test. The two vehicle control groups did not differ and were combined into a single group ($n = 17$). The 5 groups differed overall (means ± s.e.; $F_{4,43} = 7.76$, $p < 0.0001$). Subsequent comparisons using Dunnett's test showed the two groups treated with LY for 1–7 days ($n = 8$) and 5–12 days post-training ($n = 8$) were impaired relative to the vehicle group ($d = 2.30$, $p < 0.05$). The vehicle-treated group showed unexpectedly poorer recall than the group treated acutely with LY ($n = 8$) at 1 day after training ($d = 2.32$, $p < 0.05$). (**c**) Representative paths taken by the animals treated for seven days after training with vehicle or with LY. Note that both animals were trained before infusion, and the retrieval test was conducted after the micropumps were exhausted. Only the vehicle animal searched preferentially in the training quadrant (dotted line).

Rats were trained on a variant of the open-field water maze in which the escape platform rises to within 1.5 cm of the surface of the water if and only if the animal is both accurate (< 20 cm) and persistent (increasing from 0.5 to 2.5 s) in searching at the correct location[19]. An automated tracking system, calculating the animal's location in real time, triggered the release of the 'Atlantis' platform at appropriate times. Following pretraining and 4 days of spatial training, retention was tested after a 16-day interval.

During training, chronic LY caused a clear deficit in spatial performance (**Fig. 3b**). Paths taken on the third day of spatial training (Day –1, just before the micropumps were disconnected; Methods) showed a representative control rat taking a direct path to the platform area and then remaining there until the hidden platform rose. The LY-treated animal was more circuitous (**Fig. 3c**). The issue is to establish whether this impaired performance is a memory deficit, with memory subdivided into encoding-related and retrieval-related processes. To address this, we subdivided the vehicle and LY training groups before the retention test. Half the animals in each group were given acute intrahippocampal infusions of drug or vehicle (1.5 mM LY or vehicle, 1 µl over 5 min, 1 h before testing). Animals treated with vehicle during both training and retention performed well, whereas those treated with LY in both phases performed poorly (**Fig. 3d** and **e**). The new findings made possible by the use of reversible inactivation are from the group treated with LY during training but tested under vehicle (enabling any effect of hippocampal inactivation on encoding-related processes to be identified) and from the group treated with vehicle during training but tested under LY (enabling a role in memory retrieval to be identified). Both these groups performed at chance. An analysis of variance showed that the four groups differed overall, with planned comparisons revealing the vehicle/vehicle subgroup to be significantly better than each of the other three during memory recall ($p < 0.01$).

The paths taken during the retention test (**Fig. 3d**) differed among groups. Rats trained with LY but tested with vehicle tended to swim all over the pool. However, rats trained with vehicle but tested with LY showed the appropriate searching strategy but failed to execute it in an appropriate location. This was analyzed by computing the maximal time spent in any quadrant of the pool. The mean percentage times for the vehicle/vehicle, vehicle/LY and LY/vehicle groups respectively were 40.9 ± 2.9%, 42.4 ± 3.1% and 31.6 ± 0.9% ($F_{2,16} = 4.27$, $p < 0.05$). Subsequent orthogonal comparisons showed that maximal quadrant time in the vehicle/vehicle group did not differ from that in the vehicle/LY group($F < 1$), whereas the mean for these two groups was higher than that of the LY/vehicle group($F_{1,16} = 9.39$, $p < 0.01$). This dissociation suggests the localized searching strategy is not acquired during hippocampal inactivation, perhaps because the animal cannot encode where the platform is located[20], but that it can be performed during retrieval, provided it has been learned earlier, even if the animal cannot remember where to search.

Trace storage or long-term consolidation

We then examined whether temporary hippocampal inactivation could disrupt processes occurring after training but before recall. Various treatments immediately after training (for example, electroconvulsive shock, drugs, brain stimulation) cause deficits or enhancements of several types of memory tasks[21–24]. These are thought to affect a short-term consolidation process that is vital for encoding traces into long-term memory. We focused on a longer-term consolidation process, in which the hippocampal formation and other structures of the medial temporal lobe are implicated[2,25]. Long-term consolidation is thought to involve the interaction of hippocampal and neocortical networks and so require fast synaptic transmission.

Unlike the previous experiment, in which the training and retention phases were conducted under various combinations of drug or vehicle, training in this experiment occurred before treatment, and the memory retention test occurred after micropump exhaustion, with the hippocampus again working normally. Acute or chronic inactivation happened in between. One day after the end of spatial training, the animals were divided into five groups matched with respect to their training performance (**Fig. 4a**). Two

groups were given intrahippocampal cannulae for acute LY or vehicle infusions administered four hours later, two received bilateral seven-day micropumps infusing into the dorsal hippocampal formation, and the fifth group was left undisturbed until five days later, when they were also implanted with bilateral seven-day micropumps containing LY. An additional caudate-infusion group was also trained, but its results are considered separately (below). The retention test was conducted 16 days after the end of training in all groups.

The results showed a highly significant difference in retention between groups (**Fig. 4b** and **c**). The two vehicle control groups did not differ and were combined. These controls remembered the former location of the platform well, swimming repeatedly over the correct location. In contrast, groups given seven-day infusions of LY into the dorsal hippocampus, whether beginning one or five days after training, showed poor memory recall. Once again, the LY-treated animals showed a tendency to remember the strategy of localized searching, albeit at an inappropriate location. Animals given acute infusions of LY in which the duration of hippocampal inactivation is short-lived (**Fig. 1a**) showed, if anything, a trend toward better memory than controls. These findings suggest that the integrity of normal neural activity in the hippocampal formation is necessary for later retrieval for at least five days after training.

Memory-process specificity

The memory-process specificity of this deficit in storage or long-term consolidation was explored in several ways. The first step was to check that it was indeed independent of any effect of LY on retrieval (encoding-related processes being necessarily excluded because the animals had been normal during training). To do this, the learning and retention of a second water maze task was tested on the same day that retention had been tested in the previous experiment (using the same animals). We reasoned that the encoding and retrieval phases of this additional task should be conducted as quickly as possible after the previous experiment because any residual hippocampal dysfunction caused by seven days of temporary inactivation might be transient. Accordingly, immediately after the retention test (Day 16, **Fig. 4a**), all five groups were given six trials of training in a second water maze in a separate room and then tested for retention five hours later (that is, within the domain of long-term memory, but not requiring long-term consolidation). All groups learned rapidly at the same rate (**Fig. 5a**) and did not differ in their memory of the platform location five hours later (**Fig. 5b**). Thus, if there is any 'residual hippocampal dysfunction' after temporary inactivation, it does not affect encoding or retrieval. The basis of the deficit in the previous experiment must be in some other memory-related process.

Two other specificity tests were conducted. First, we double-checked whether chronic intrahippocampal infusion could be causing residual hippocampal dysfunction in encoding or retrieval-related processes. Two new groups of animals were given chronic infusions beginning and ending before the start of all training. Starting 9 days after the micropumps were exhausted, the groups that had been treated with LY or vehicle showed equivalent rates of learning (data not shown) and good recall 16 days later (**Fig. 6a**). Second, anatomical specificity was examined using the same consolidation design (**Fig. 4a**), but with chronic bilateral LY infusion into the caudate. (This group was, in practice, trained at the same time as those infused into the hippocampus, but it addresses a logically distinct issue and is therefore considered separately.) Caudate-treated animals showed good memory recall, performing significantly better than the group infused with LY into the dorsal hippocampus over the same time period and no differently from vehicle controls (**Fig. 6b**). Thus, chronic inactivation of the dorsal hippocampus but not the caudate after training can impair retention of hippocampal-dependent spatial memory by disrupting a memory process other than encoding or retrieval.

Discussion

These findings imply an obligatory but independent participation of hippocampal neural activity in encoding- and retrieval-related processes of one type of memory—spatial memory—and its participation in either long-term consolidation or storage processes. We consider our method of inactivation and these memory processes in turn.

Reversible inactivation of neural activity

Our use of both acute and chronic infusion of a selective glutamatergic antagonist revealed that it is possible to 'switch off' the dorsal hippocampus for varying periods, and for it then to 'switch on' again and apparently work normally thereafter. The electrophysiological data showed residual low-amplitude field potentials during LY infusion but no population spikes; evoked potentials returned when the micropumps were exhausted. The return to near-normal levels is intriguing, as homeostatic changes in AMPA receptor number and efficacy may occur during inactivation. In culture, long-term AMPA receptor inactivation causes a reversible, quantitatively scaled increase in synaptic strength[26,27]. If this reversible homeostatic plasticity were also to occur *in vivo* during chronic infusion, resumption of baseline field potentials would be expected. The 2-DG data revealed an anatomically specific decrease in function that also returned to normal after infusion. Dynamic

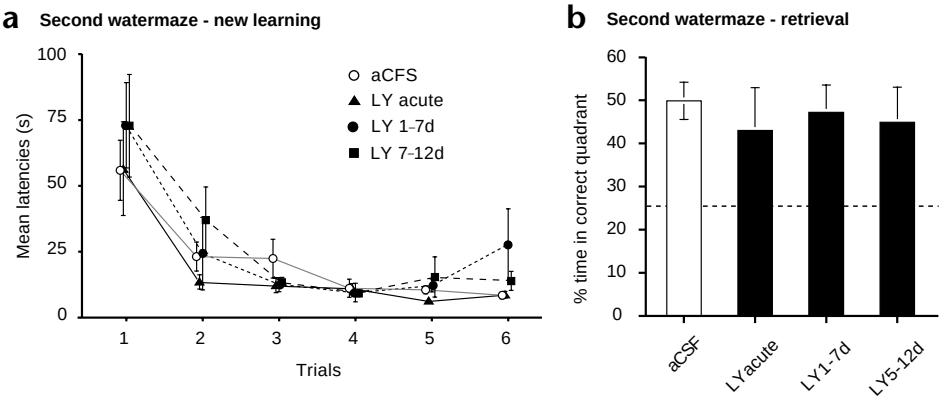

Fig. 5. Chronic LY interferes with a post-training memory process but does not cause any residual impairment of encoding or retrieval. (**a**) Animals that had completed the previous experiment (**Fig. 4**) were also trained in a second water maze in a different room using a standard reference memory procedure to a fixed hidden platform. The groups did not differ in rate of learning ($F = 1.80$, $p > 0.10$). (**b**) All groups showed an equivalent tendency to swim in the training quadrant in the retention test 5 h later ($F = 1.97$, $p > 0.10$).

changes in glucose utilization predominantly reflect nerve terminal activity[28]. The remainder relates to ion fluxes and transport, which can be manipulated only after the inhibition of all electrical activity. In this study, the 23% decrease in glucose utilization in the dorsal hippocampus during LY infusion is similar to that observed after the suppression of synaptic transmission by pentobarbital[29,30]. Increasing the dose of LY might have further reduced the field-potential magnitude, 2-DG utilization or the volume of hippocampus affected, but at the risk of increasing extra-hippocampal diffusion. The primary site of action of our chronic LY infusions was the dorsal hippocampal formation, inclusive of areas CA1–CA3 and the dentate gyrus. The ventral (temporal) hippocampus was relatively unaffected, but this may be unimportant for the present learning task because similar spatial tasks are impaired more by dorsal than ventral neurotoxic lesions[31]. These electrophysiological and 2-DG data calibrate our claim that there would have been little or no residual cognitive function of the hippocampus during LY infusion but that normal function should return subsequently.

Encoding and retrieval

Turning to the dissociation between encoding and retrieval, we found that the only animals to perform above chance during recall were those given vehicle in both phases of training. This suggests that hippocampal activity is essential for both encoding- and retrieval-related memory processes—at least over a 16-day retention period. If LY had only impaired motor performance during training, masking normal memory encoding, the LY/vehicle animals should have performed well during the final retention test. LY treatment cannot, however, have only affected encoding-related processing because the vehicle/LY group was also impaired. The differential search patterns of these two groups are intriguing because, if hippocampal neurotransmission is involved in encoding, the vehicle/LY group would have been in a position to develop a localized search strategy during training, whereas the LY/vehicle group would not. However, the finding that the vehicle/LY group searched in a spatially localized way during the memory test, but in inappropriate locations, suggests that normal hippocampal activity is necessary to retrieve location information but is unnecessary to retrieve and execute the swimming strategy. Strategy information is most likely encoded and stored elsewhere. With respect to alternative explanations, the poor performance of the LY/LY treated group argues against state dependency. The localized searching of the vehicle/LY group argues against LY causing any motivational deficit during retrieval, and it therefore seems unlikely that motivation would be impaired during encoding either. An attentional account of chronic hippocampal inactivation remains possible, if implausible, and could be examined using tasks requiring vigilance or sustained attention[32].

We envisage that spatiotemporal patterns of glutamatergic or neuromodulatory activity in the hippocampus necessary to encode information into long-term memory must differ from those later required to retrieve it[33,34]. AMPA/kainate receptor blockade during encoding would necessarily prevent NMDA receptors being stimulated sufficiently to trigger the activity-dependent modifications of synaptic weights that constitute trace storage[11,12]. The integrity of transmission mediated by AMPA/kainate receptors in the hippocampus also seems to be necessary for retrieval. In contrast, NMDA receptor activation is necessary for encoding but not retrieval[35,36].

Consolidation or storage

We also found that memory traces are fragile to chronic reversible hippocampal inactivation starting one to five days after training and lasting seven days. Control experiments set various constraints on the interpretation of this finding. First, the impairment induced by chronic LY displays a measure of anatomical specificity. As predicted from rodent lesion studies (for example, ref. 37), hippocampal but not caudate inactivation causes retrogade amnesia for spatial memory. Second, inactivation for the limited period of 4–6 hours, beginning 24 hours after the end of training, did not impair memory. (If anything, it was paradoxically enhanced.) This is consistent with previous studies of acute post-training drug infusion in other tasks in which effects are generally seen at much shorter intervals[38]. Interference with short-term consolidation mechanisms is thereby excluded. Third, a residual deficit in a retrieval-related memory process once the hippocampus has switched back on was also excluded by the successful training of LY-treated animals in a second water maze task on the same day that they revealed impaired memory of the first task and by the null effects of reversible inactivation before all training in the main task. Thus, hippocampal neural activity is required for a memory-related process other than encoding or retrieval, which lasts for at least five days after the end of this training protocol.

At first sight, this finding suggests that hippocampal neural activity is necessary for the long-term consolidation of spatial memory traces. This interpretation is consistent with evidence for time-limited changes in glucose utilization in the hippocampus reflecting the energy demands of the putative consolidation process[39] and with lesion studies in humans and animals showing temporal gradients of retrograde amnesia[40–45]. However, an outstanding puzzle about this literature is that retrograde amnesia "…gradients do not provide a direct measure of the time required for the consolidation of long-term memory" (p 550, ref. 22). Specifically, two separate conceptions of long-term consolidation should be distinguished.

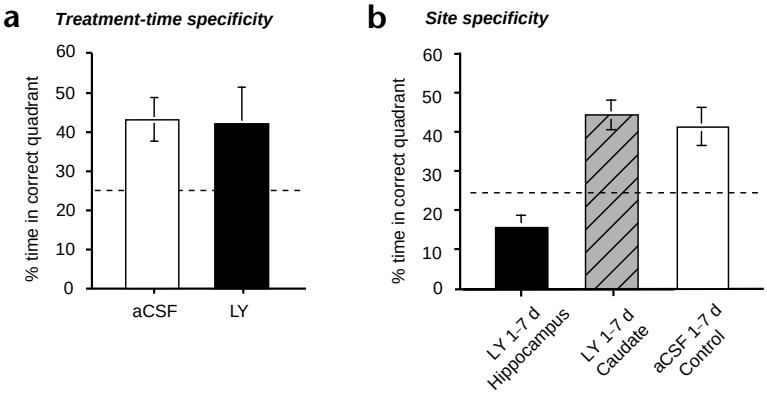

Fig. 6. The specificity of the effects of post-training hippocampal inactivation. (**a**) Treatment-time specificity was examined by implanting 7-day micropumps with either LY (n = 8) or vehicle (n = 8) and allowing them to infuse and become exhausted before the start of all training. Nine days later, the pretraining and spatial training protocol was conducted as in Fig. 4a. Both groups learned at an equivalent rate (F < 1; data not shown). Retrieval, tested 16 days later, showed equivalent levels of recall (F < 1). (**b**) Site specificity was examined by comparing LY infusions into the hippocampus (n = 8) and into the striatum (n = 8) one to seven days after spatial training. Only the intrahippocampal infusions impaired memory relative to the pooled vehicle controls (p < 0.01). Mean ± s.e.

One is that memory traces take time to be stabilized within one or more brain areas such that they can then persist for the lifetime of the animal; the other is that long-term consolidation is a network process through which traces stored in one brain area become resistant to brain damage elsewhere to which they are initially sensitive. These are distinct but not mutually exclusive ideas, excepting that the stabilization concept allows for the possibility that there is significant long-term storage of certain kinds of information in hippocampus (for example, context information) and that neural activity there always remains necessary for its retrieval[46].

Comparison of the effects of reversible inactivation and irreversible lesions provides one way of distinguishing these two accounts, specifically in a task such as the water maze in which a flat retrograde amnesia gradient is sometimes observed after permanent lesions. (See Table 2 in ref. 46.) Stabilization may still be occurring within hippocampus, even though hippocampal neural activity remains necessary both for retrieval and for aspects of performance during navigation, path-integration, etc. Unfortunately, our efforts to find the duration of stabilization/consolidation by extending the retention interval and delaying the start of inactivation until long after training have so far been unsuccessful because of poor memory baselines in vehicle-treated controls after retention intervals of up to 60 days. Other tasks may be more appropriate.

Without a temporal gradient, we are obliged to recognize the possibility that LY infusion, rather than affecting stabilization/consolidation, may disrupt the integrity of storage sites in the hippocampus for long-term spatial or contextual memory 'traces'[1,46]. Specifically, AMPA/kainate receptor blockade for seven days might disrupt spatially distributed patterns of synaptic weights, irrespective of how long after training it occurs. A possible mechanism would be the breakdown of the quantitative scaling of homeostatic plasticity. Quantitative scaling implies that the relative efficacy of different synapses is sustained in the face of partial AMPA receptor blockade and its cessation. Were this to occur *in vivo* also, the disruption to trace storage should be minimal. However, quantitative scaling may break down when sustained for several days, such that, when the micropump became exhausted, normal levels of field-potentials, neural excitability and glucose utilization would resume but the spatial pattern of altered synaptic weights might have been 'scrambled'. Immunogold labelling, ligand-binding and LTP experiments should be conducted to examine this idea rigorously.

Conclusion

In humans, functional imaging studies have taken us beyond classical lesion techniques to enable the identification of human brain structures differentially active during encoding and retrieval[47]. The hippocampus can be activated during encoding and retrieval[48,49], and these memory processes may occur preferentially at different points along its longitudinal axis[50]. In animals, reversible chronic inactivation of fast synaptic transmission complements neuron recording and other techniques by establishing, for each of several types of memory, whether the functional integrity of a brain area is necessary for a specific memory process.

Methods

***In vivo* electrophysiology.** This work was undertaken under the auspices of UK Home Office Project and Personal Licences held by the authors and designated laboratories. Male Lister hooded rats were anesthetized with urethane (acute experiments, 1.5 g per kg) or tribromoethanol (chronic experiments, 10 ml per kg). Using standard stereotaxic techniques, we implanted teflon-coated platinum-iridium electrodes (75 μm) to the appropriate depths for perforant path (AP –7.5 and L –4.0 mm; left hemisphere) or homotopic CA1 (AP –3.5 and L 2.0 mm) bipolar stimulation, and dentate (AP –3.5 and L –2.0 mm) or CA1 (AP –3.5 and L –2.0 mm) monopolar recording. Throughout surgery, all animals were placed on a heating blanket to maintain body temperature at 36.2 ± 0.2°C. In acute experiments, a stable baseline (20 min) was first obtained in response to electrical stimulation consisting of biphasic pulses with 100 μs half-width delivered at 0.05 Hz. Either vehicle or LY (1.0 μl of a 1.5 mM solution, concentration varied in pilot studies) was then infused through a stainless steel cannula (AP –4.5 and L –3.0 mm) attached by rubber tubing to a Hamilton syringe in a syringe driver. Recordings continued for another six hours. In chronic experiments, an infusion cannula was inserted into the hippocampus at AP – 4.5 and L –3.0 from which a catheter led to a minipump. Dental cement and stainless steel screws (one connected to the ground electrode) secured these to the skull. The minipump (ALZA2002) containing vehicle was placed in a cavity below the skin of the neck, and the length of tubing attached was calculated to contain fluid for 20 hours (at a pumping rate of 0.5 μl per hour). After a recovery period of 7–10 days, the awake animals were placed in a recording chamber where they could move about freely while electrically connected via a swivel commutator to signal-processing equipment. Daily recordings (3 days) included both input/output curves using stimulation varying from 100 to 1000 μA (100 μs half-width, 0.1 Hz) and 10-min baselines at a fixed stimulus intensity (50–70% of maximal response) with markers time stamped to positions on the trace. Under anesthesia, the minipump was then replaced with a micropump (ALZA1007, 0.5 μl per h for 7 days) containing either vehicle or LY (0.375 mM). Daily recordings continued in the same way for another 12 days.

Deoxyglucose measurements. Rats previously implanted (4 days or 11 days earlier) with intrahippocampal cannulae (AP –4.5 and L ±3.0 mm) and 7-day micropumps on both sides of the brain were anesthetized with halothane, and polyethylene cannulae were inserted into the right femoral vein and artery to allow the injection of [^{14}C]2-deoxyglucose and the sampling of blood, respectively. The cannulae were then passed subcutaneously and externalized at the nape of the neck. After at least 2 hours recovery, an intravenous pulse of 50 μCi [^{14}C]2-DG (specific activity 55.0 mCi per mol, Amersham Life Science) in 0.7 ml saline was injected over 30 s. Timed arterial blood samples (approximately 100 μl) were drawn at fixed time points over the next 45 minutes and concentrations of [^{14}C]2-DG and glucose in the blood samples determined. The rats were overdosed with euthatal 45 min later, and their brains removed and frozen. These were cut serially (3:10) into 20-μm thick coronal sections and autoradiograms generated by exposing the brain sections with medical X-ray film (Biomax™ MR film, Eastman Kodak Company), together with a series of precalibrated [^{14}C]-methyl methacrylate standards. Local rates of glucose utilization were determined with quantitative densitometric analysis using a computer-based densitometer (MCID, Imaging Research). Data from the 4- and 11-day groups were analyzed separately using 2-tailed Student's unpaired *t*-test.

Spatial learning. In the main task, rats were trained in an open-field water maze and tested for their memory of the escape platform location 16 days later. We used the 'Atlantis platform'[19], in which the polystyrene platform only became available from the bottom of the pool if the animal swam to and stayed within a 20 cm radius of the correct location for a predetermined and experimentally controllable dwell time. The platform then rose until its top surface is 1.5 cm below the water surface. This procedure encouraged highly focused searching. Pretraining (3 days, 6 trials per day; dwell time, 1 s on days 1 and 2, 1.5 s on day 3) consisted of approach to a visible hanging target above the location of the platform with the pool surrounded by curtains to occlude extramaze cues. Spatial training (4 days, 10 trials per day, dwell times of 1 s on days 1 and 2, 2 s on day 3 and 2.5 s on day 4) involved no hanging cue, and the curtains were drawn back to reveal extramaze cues. Swim paths and the times required to mount the hidden platform were monitored and stored on-line for later analysis. In the consolidation experiment, animals were assigned to groups matched with respect to their performance during the last training day. A single 60-s probe trial examined retention 16 days after the last training session. Surgery to implant acute infusion cannulae or micropumps was done at various times before or after the end of training as stated in the text. In the

encoding/retrieval experiment, all groups had their 14 day minipumps removed after training on day –1 (rather than day 0) so that any residual drug would be cleared within the next 24 h and would not interfere with any long-term consolidation process taking place after acquisition trials were completed.

All groups in the consolidation experiment were also trained in a second water maze located in a different laboratory on the retrieval test day (Day 16). There were 6 trials with a 30-s intertrial interval to a standard hidden platform in a fixed location, and a retention test (platform absent) 5 hours later. In the animals trained after the minipumps were exhausted, micropumps were inserted in both groups before all behavioral training in the main task. The group given bilateral caudate infusions was actually trained at the same time as the hippocampus-infused consolidation group.

Surgical procedures. Chronic bilateral implantation of cannulae and minipumps was done under general anesthesia (tribromoethanol) using standard stereotaxic techniques. Intrahippocampal cannulae (stainless steel, 26 gauge, L shaped) were connected via flexible polyethylene tubing to micropumps (ALZA 1007D). For hippocampal infusion, the cannulae tips were lowered to –3.0 mm below dura at coordinates of AP –4.5 and L ± 3.0 mm. For animals that received chronic and acute infusions at different times, the acute guide cannula (24 gauge) was soldered to the rostral end of the chronic one (26 gauge) with both tips level, so that the drug infused similar locations in the hippocampus. For caudate infusions, the coordinates were AP +0.7, L ±2.8, D –4.5 mm.

Histology. After termination of experimental procedures, brains of all animals were removed and histologically processed using a Nissl stain for verification of electrode and cannula placement. All data from a small number of animals with cannula misplacements or brain infections were discarded.

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