Schemas and Memory Consolidation
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dissociative electrophilic alkylation of the double bond in IPP by the allylic cations generated from DMAPP or GPP (*23*). By analogy, for biosynthesis of irregular monoterpenes, we suggest that a related dissociative electrophilic alkylation of the double bond in DMAPP by the dimethylallyl cation results in a protonated cyclopropane intermediate. This species can be deprotonated to give CPP or rearrange to a tertiary cation, which can in turn be deprotonated to give LPP. Alternatively, the tertiary cation can cyclize to give a cyclobutylcarbinyl cation that can then be deprotonated to give MPP or LPP. Formation of any specific product would be controlled by the ability of the enzyme to stabilize a specific intermediate along the reaction coordinate through dipolar and electrostatic interactions and to facilitate the selective removal of protons. The stereochemistries of the products result from the conformations of the two bound substrate molecules before the reaction. Only minor changes in the relative positions of the substrates are required to accommodate the formation of the different products.

This scenario provides an attractive mechanism for the evolution of the isoprenoid pathway through gene duplication and random mutagenesis of the duplicate genes to give new proteins, one of which is constrained to retain its original function, whereas the other is free to acquire a new activity. The isoprenoid fold first seen in the *E*-selective chain-elongation enzyme avian FPPase (*12*) has also been found in the cyclopropanation enzyme squalene synthase (*13*) (sterol biosynthesis) and several different terpenoid cyclases (*14*) along with aspartate-rich motifs involved in binding allylic diphosphate substrates, indicating that the enzymes evolved from a common ancestor. Phylogenetic correlations suggest that the cyclopropanation enzyme phytoene synthase (carotenoid biosynthesis) also has an isoprenoid fold. Our discovery that chimeric enzymes from FPPase and CPPase catalyze branching and cyclobutanation reactions suggests that WT enzymes with these activities also share this common ancestor.

References and Notes

1. K. Gunawardena, S. B. Rivera, W. W. Epstein, *Phytochemistry* **59**, 197 (2002).
2. A. Zhang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 9601 (2004).
3. C. H. Heathcock, B. L. Finkelstein, T. Aoki, C. D. Poulter, *Science* **229**, 862 (1985).
4. M. B. Jarstfer, D. L. Zhang, C. D. Poulter, *J. Am. Chem. Soc.* **124**, 8834 (2002).
5. B. S. J. Blagg, M. B. Jarstfer, D. H. Rogers, C. D. Poulter, *J. Am. Chem. Soc.* **124**, 8846 (2002).
6. H. C. Rilling, W. W. Epstein, *J. Am. Chem. Soc.* **91**, 1041 (1969).
7. L. J. Altman *et al.*, *J. Am. Chem. Soc.* **94**, 3257 (1972).
8. J. D. Berkowitz, J.-L. Giner, T. Andersson, *J. Nat. Prod.* **63**, 267 (2000).
9. B. A. Bierl-Leonhardt, D. S. Moreno, M. Schwarz, J. Fargerlund, J. R. Plimmer, *Tetrahedron Lett.* **22**, 389 (1981).
10. C. D. Poulter, *Phytochem. Rev.* **5**, 17 (2006).
11. L. C. Tarshis, M. Yan, C. D. Poulter, J. C. Sacchettini, *Biochemistry* **33**, 10871 (1994).
12. J. Pandit *et al.*, *J. Biol. Chem.* **275**, 30610 (2000).
13. D. W. Christianson, *Chem. Rev.* **106**, 3412 (2006).
14. S. B. Rivera *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4373 (2001).
15. A. Hemmerlin, S. B. Rivera, H. K. Erickson, C. D. Poulter, *J. Biol. Chem.* **278**, 32132 (2003).
16. D. Iwata-Reuyl, S. K. Math, S. B. Desai, C. D. Poulter, *Biochemistry* **42**, 3359 (2003).
17. H. K. Erickson, C. D. Poulter, *J. Am. Chem. Soc.* **125**, 6886 (2003).
18. H. V. Thulasiram, C. D. Poulter, *J. Am. Chem. Soc.* **128**, 15819 (2006).
19. K. Alexander, W. W. Epstein, *J. Org. Chem.* **40**, 2576 (1975).
20. M. Soucek, L. Dolejs, *Collect. Czech. Chem. Commun.* **24**, 3802 (1959).
21. G. Popjak, J. Edmond, S.-M. Wong, *J. Am. Chem. Soc.* **95**, 2713 (1973).
22. L. J. Altman, R. C. Kowerski, D. R. Laungani, *J. Am. Chem. Soc.* **100**, 6174 (1978).
23. J. M. Dolence, C. D. Poulter, in *Comprehensive Natural Products Chemistry*, O. Meth-Cohn, Ed. (Elsevier, Oxford, UK, 1999), vol. 5, pp. 18473–18500.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5821/73/DC1
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Electron Impact (EI) Mass Spectra
Chemical Ionization (CI) Mass Spectra
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Schemas and Memory Consolidation

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Memory encoding occurs rapidly, but the consolidation of memory in the neocortex has long been held to be a more gradual process. We now report, however, that systems consolidation can occur extremely quickly if an associative "schema" into which new information is incorporated has previously been created. In experiments using a hippocampal-dependent paired-associate task for rats, the memory of flavor-place associations became persistent over time as a putative neocortical schema gradually developed. New traces, trained for only one trial, then became assimilated and rapidly hippocampal-independent. Schemas also played a causal role in the creation of lasting associative memory representations during one-trial learning. The concept of neocortical schemas may unite psychological accounts of knowledge structures with neurobiological theories of systems memory consolidation.

The concepts of "mental schema" and "mental models" as frameworks of knowledge are now well established (*1*, *2*), with implications for story recall, deductive inference, and education (*3*, *4*). For example, the memory of grammatically correct but semantically unusual prose passages is substantially better when subjects have an activated and relevant mental framework with which to understand them (*5*). An everyday experience for working scientists is remembering complex new information in an academic seminar. Our ability to do so depends as much on our possession of an appropriate mental schema as on the communicative skill of the speaker in logically conveying his or her message. In the absence of such mental frameworks, we are unable to follow scientific inferences in a talk and have the phenomenological experience of being functionally amnesic for its content a surprisingly short time later.

Curiously, this fundamental idea about memory has had little impact in neuroscience. Selective activation of a specific region within the posterior parietal cortex occurs in human subjects when, having been given relevant pictorial information earlier, they correctly interpret unusual textual information that would otherwise be incomprehensible (*6*). Animal studies have rarely considered the issue of what the animal itself brings in the way of knowledge to a learning situation, with the exception of studies of spatial and relational memory (*7*–*9*). This is partly because most experiments are conducted with "experimentally naïve" animals, and also because the creation of a mental schema is difficult to map precisely onto concrete neuroscience concepts such as anatomical connectivity or synaptic plasticity. The present experiments test the idea that the schema concept is directly relevant to the neural mechanisms of systems memory consolidation (*10*–*12*).

Experiments on schema learning. We trained rats to learn several flavor-place associations concurrently, using different flavors of food (flavor cues) and sand wells (place cues) located within a familiar testing environment called an "event arena" (*13*). The task was to learn which

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flavor was in which location such that, when cued with a specific flavor in start boxes at the side of the arena, the animals would be rewarded for going to the correct location by receiving more of that same food (i.e., "cued recall"). They should be able to recall that banana-flavored food is at one location, bacon-flavored food at another, and so on (Fig. 1, A and B). Such paired-associate learning is likely to be mediated by the hippocampus initially (*14*–*16*), with long-term storage of paired-associate memory traces eventually consolidated in the neocortex (*17*, *18*). This makes this paradigm ideal for looking at the temporal dynamics of systems memory consolidation (*10*, *12*, *19*, *20*), a process widely held to be quite slow. Additionally, the use of location as one member of each paired associate allowed the animals to learn each association as either an isolated declarative "fact," in which spatial information is generally considered as no different from other kinds of information (*10*), or as some kind of mapping of flavors to arena locations, resulting in the formation of a spatial or relational framework (*7*, *21*).

After habituation, the animals were started from one start box of the arena (at north, south, east, or west) on all six trials of a session. A different start box was used for each session. A trial began when the rat was given a cue flavor in the start box. Upon entering the arena, the animal was confronted by six sand wells (Fig. 1, A and B) of which only one contained flavored food—the same flavor given as a cue in the start box (*22*). The animals visited and sometimes dug at incorrect sand wells, which did not contain food on that particular trial, until they found the correct one. On each trial, the animals would retrieve the first of three buried food pellets, return to the start box to eat it, and then run back to the correct sand well to collect and transport the second and third pellets. One hour later, the second trial began with a different cue flavor in the start box and a different sand well baited. There were six trials per session, with the next session run 48 hours later (*23*).

We began by examining the impact of neurotoxic hippocampal lesions made before training (experiment 1). After 13 sessions, sham-lesioned animals were digging less frequently at incorrect sand wells before going to the correct one, whereas the hippocampal-lesioned animals did not improve. A single nonrewarded probe trial was then scheduled, which started with the provision of a cue flavor in the start box. The sham-lesioned animals spent significantly more time digging at the cued location than at the other five incorrect sand wells, whereas the hippocampal-lesioned animals were at chance (Fig. 1C; see tables S1 and S2 and figs. S1 to S3 for the lesions and full experimental design). The lesions were extensive, leaving minimal residual tissue throughout the longitudinal axis of the hippocampus (Fig. 1D).

To investigate the properties of paired-associate learning and its consolidation in more detail (experiment 2), we trained normal animals in a similar way. Probe tests, other controls, and novel context training were scheduled at various stages before and after making sham or hippocampal lesions (fig. S4). Using the same paired-associate layout as in experiment 1, we examined acquisition of sand-well choice behavior during training. A "performance index" was calculated, and this index improved monotonically across sessions (Fig. 2A). In nonrewarded probe trials, preferential digging at the correct location rather than the other five locations increased from chance levels at the outset of training to a highly significant preference for the cued location (Fig. 2B). To exclude the possibility that an olfactory cue in the correct sand well guided choice performance on training days, we conducted a single session of six trials in which the daily protocol was unchanged, except that no cue flavors were offered in the start box. Choice performance fell to chance (Fig. 2A, session 18), returning to above chance on the next normal session. The possibility of cryptic olfactory guidance by cues on the arena near the correct sand well was also ruled out in a later session by physically rotating the arena through 90° after the third trial of a session and back to its normal orientation after the third trial of a second session on the next day. The sand wells and intramaze cues were relocated such that their places relative to the distal room cues remained the same. Arena rotation had no effect (fig. S4, A3). With a different start box used in each session, it would appear that the animals can visually perceive their own location relative to the intra- and extramaze landmarks and use allocentric memory representations to identify the correct goal location among the six available sand wells.

If the animals develop a neocortical associative schema for this task, and if this is activated when the animals enter the apparatus, it might aid the encoding of new paired associates and their rapid assimilation into the schema. A single training session of six trials was given (Fig. 2A, session 21) in which paired associates (PAs) 1 and 6 were replaced by two new PAs, 7 and 8, hidden at two nearby locations; PAs 2 to 5 were trained normally. Note that PAs 7 and 8 received only one rewarded trial each. The inset of Fig. 2C shows how the new PAs were located near those of the now-closed sand wells. A nonrewarded probe trial was given 24 hours later to test memory for the new associates. Preferential digging was observed at the correct cued location in the arena relative to the new noncued location (i.e., less digging at location 8 for those animals on a PA7 trial, and vice versa) and to any of the original locations (PAs 2 to 5; Fig. 2D). The rapid acquisition of new PAs in a single trial, and their retention over 24 hours, are indications that the prior learning of an associative schema may aid the encoding, storage, and/or consolidation of new PAs. In contrast, animals trained on a similar one-trial task, but with novel PAs each day, showed consistent forgetting over 90 min (*13*).

Time course of memory consolidation. Hippocampal or sham lesions were then made 24 hours later—a much shorter time after training of the new flavors (48 hours) than is usually thought necessary for systems consolidation to be completed (*24*–*27*), and shorter than the usual time scale of differential changes in the patterns of glucose use or immediate early gene activation between hippocampus and neocortex after learning (*19*, *28*). After recovery from surgery, a series of nonrewarded probe tests (with

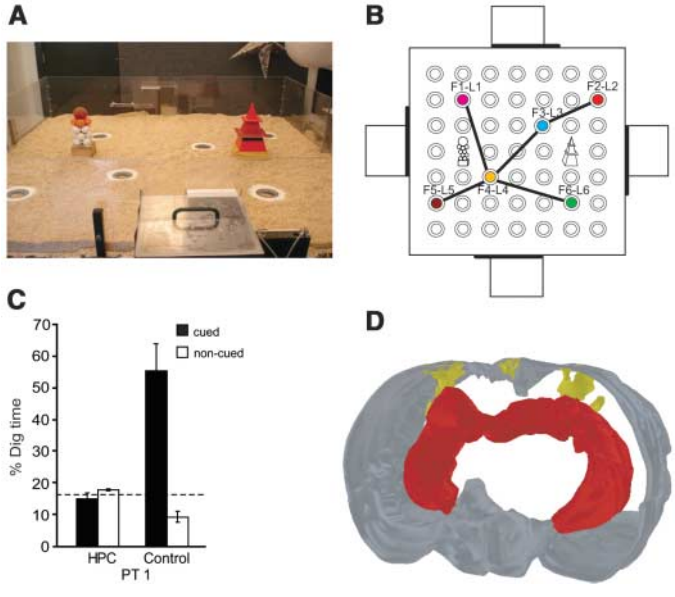

Fig. 1. Paradigm for hippocampal-dependent paired-associate (PA) learning. (**A**) The large event arena (1.6 m by 1.6 m) contains a 7 × 7 grid of locations at which sand wells can be made available and four surrounding start boxes. After being given a cue flavor in a start box, the animals recall the spatial location with which it is associated, and run into the arena to that location to secure more of that flavor of food. (**B**) The spatial arrangement of the six PAs and the "schema" this constitutes (F, flavor; L, location). (**C**) Preferential digging during a nonrewarded probe test [probe trial 1 (PT1)] by sham-lesioned but not hippocampal (HPC)-lesioned animals (*ns* = 6). Groups $t = 5.25$, df = 10, $P < 0.001$; sham versus chance, $t = 5.01$, df = 5, $P < 0.005$; HPC versus chance, not significant (n.s.). (**D**) A three-dimensional reconstruction of the volume of hippocampus lesioned in a representative rat (red), together with typical overlying cortical damage (yellow). The gray region represents the transparent volume of the rat brain.

interpolated training days using the original flavor-place pairs) was given to examine memory for the original schema and the two new PAs. These consisted of separate tests of the original PAs 2 to 5 and new PAs 7 and 8, each repeated once across a series of four sessions to enable both PA7 and PA8 to be tested in all animals. The hippocampal-lesioned group not only could successfully recall the original PAs learned over the previous month (Fig. 2D) but also, remarkably, could remember the newly acquired pairs PA7 and PA8. Because the lesions were near-complete (~90%; see Fig. 1D and fig. S2B), these two findings imply that (i) the memory traces for these PAs must be stored outside the hippocampus, probably in the neocortex; and (ii) consolidation of new associates whose acquisition is mediated by the hippocampus takes place within 48 hours.

To be more confident of these claims, it was essential to establish that the learning of further new PAs still required the integrity of the hippocampus in these same animals. Accordingly, immediately after this series of postoperative probe tests, we conducted a single six-trial training session with PAs 2 to 5 of the original schema, but with PAs 7 and 8 now replaced by sand wells containing two new flavors in nearby locations in the arena (PAs 9 and 10; Fig. 2E). The probe test conducted 24 hours later showed that sham-lesioned animals could readily learn and recall these new pairs, whereas the hippocampal-lesioned group could not. Thus, the one-trial acquisition of new PAs in this paradigm in experienced animals was still blocked by hippocampal lesions. Hence, it is unlikely that any relearning took place after the hippocampal lesions during the earlier series of four probe tests that had examined remote memory (the interpolated training was restricted to the well-trained PAs 2 to 5). The effective cued recall of the new PAs 7 and 8 introduced before the lesion must therefore reflect rapid, successful systems consolidation.

Although the animals appear to have acquired an associative schema reflecting the mapping of flavors to places in the arena, an alternative might be a response-based "win-stay, lose-shift" inference strategy in the manner of a learning set (*29*). It is not entirely clear how such a procedural strategy could be applied in this context, with six choice locations and only one trial per day to each cued location. However, as procedural strategies are generally context-independent, this account would predict that the learning of an entirely new set of six PAs in a new context would occur very quickly. In contrast, the schema hypothesis requires that a new schema be gradually learned. The same animals of experiment 2 were first trained on a new set of PAs in the same event arena (fig. S7) and then in a novel event arena in a different room with new intra- and extramaze landmarks, new flavors, and a distinct spatial geometry to the new set of sand wells (Fig. 3, A and B). Acquisition again took

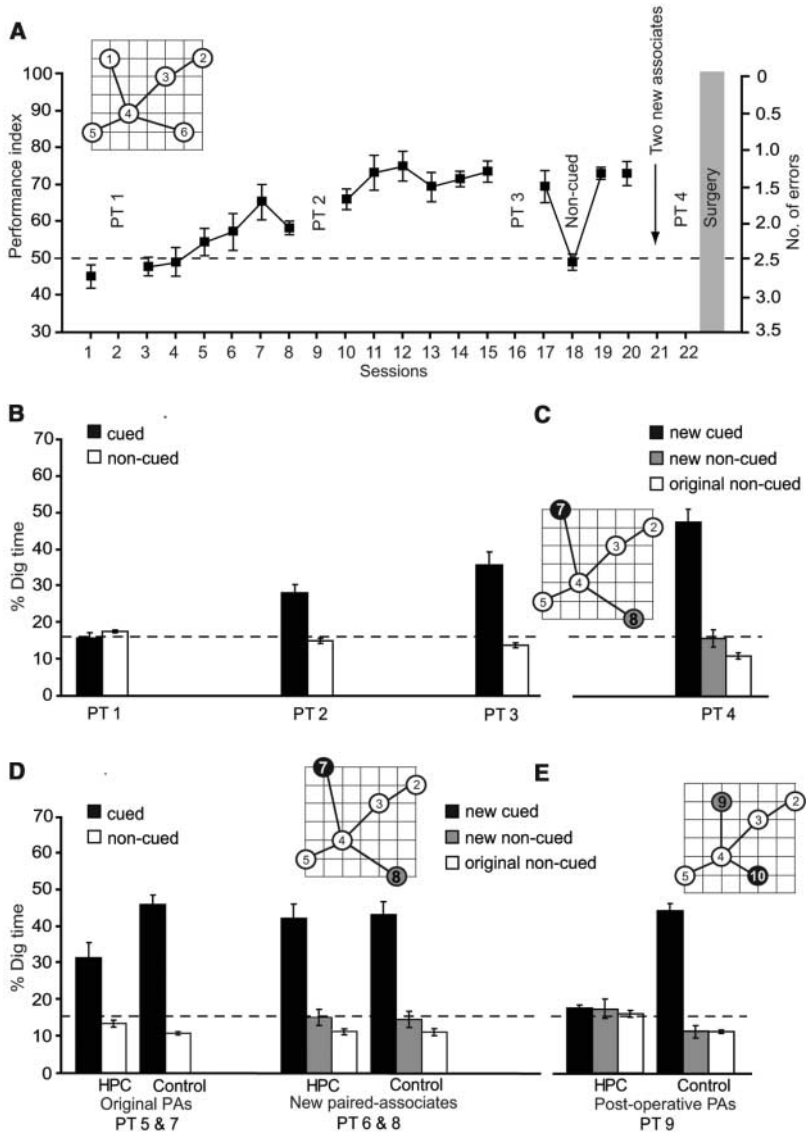

Fig. 2. Acquisition of an associative schema and its role in new learning and consolidation. (**A**) Acquisition of PAs. The animals (n = 18) made fewer choice errors over training (F = 18.24, df = 5.7/97.5, P < 0.001; Greenhouse-Geisser correction, including degrees of freedom) such that the performance index, computed as 100 − [100 × (errors/5)], was significantly above chance from session 10 onward (ts > 5.08, df = 1/17, Ps < 0.001). Removing cue flavors from the start box on session 18 resulted in performance dropping to chance and then returning to 70% correct on a succeeding normal session (session 19). (**B**) Cued-recall probe trials. Nonrewarded probe tests revealed a graded learning of the original PAs (cued flavor = solid bars) across sessions 2, 9, and 16 (F = 16.24, df = 1.54/26.22, P < 0.001; above chance in PTs 2 and 3; ts = 3.94 and 6.17, df = 17, P < 0.005 and P < 0.001, respectively). (**C**) Effective recall in PT4 of the location of the cued new PA (solid bar), coupled with avoidance of the noncued new PA (gray bar) and the remaining original associates (open bar) 24 hours after a single session of training with only one trial of each new PA (repeated-measures F = 65.28, df = 1.7/29.1, P < 0.001; cued location above chance, t = 10.29, df = 17, P < 0.001; noncued versus original, n.s.). (**D**) Postoperative retention. Both sham-lesioned (n = 8) and HPC-lesioned (n = 10) animals could effectively remember both original PAs (PTs 5 and 7) and new PAs introduced for a single trial 2 days before surgery (PTs 6 and 8). Both groups dug at the sand wells of the original associates (flavors 2 to 5) significantly more than chance (HPC t =3.60, df = 9, P < 0.01; sham t = 12.89, df = 7, P < 0.001; sham versus HPC group, t = 2.86, df = 16, P < 0.05). Both groups also dug equally at the cued locations of the new associates relative to the noncued locations (Group × Location F < 1, n.s.), and at these cued locations better than chance (ts > 8.07, df = 9 and 7, P < 0.001). (**E**) Postoperative new training. Hippocampal lesions prevented the learning of new PAs (PAs 9 and 10; Group × Location F = 60.23, df = 1.64/26.17, P < 0.001). Digging at the cued new location in PT9 was significantly above chance only in the sham group (t = 17.07, df = 7, P < 0.001) and significantly lower in the HPC group than in the sham group (t = 13.78, df = 16, P < 0.001).

place gradually, such that the learning curve of the now experienced sham-lesioned animals did not differ from the original rate of learning of the normal animals in the first event arena. The hippocampal-lesioned animals did not learn the new spatial schema despite repeated trials. Probe test performance early in training followed the same gradual pattern in the sham group, resulting in effective probe test performance only by session 67 (Fig. 3C). These findings argue against a response-based strategy, such as a learning set, because learning was no faster in the new room with new flavor-place geometry.

Completion of training in the second room offered the opportunity of returning the animals to the first arena to examine their now remote memory of the original set of PAs first learned 4 to 5 months earlier. Remarkably, the hippocampal-lesioned animals were above chance in cued spatial recall (session 68, Fig. 3D) and even showed a nonsignificant trend toward better performance than did sham-lesioned controls in a probe test as early as session 2. The sham-lesioned animals may have sustained some associative interference arising from their successful training on other sand-well arrangements in this and the other contexts, but after as few as six sessions of retraining, both groups showed effective cued recall of the original PAs (Fig. 3E). Thus, the failure to learn new PAs in a new context after a hippocampal lesion did not affect the ability to remember, after several months, information acquired before the lesion—a pattern exactly like that shown by patient E.P. in his knowledge of current and past hometown topography (*30*).

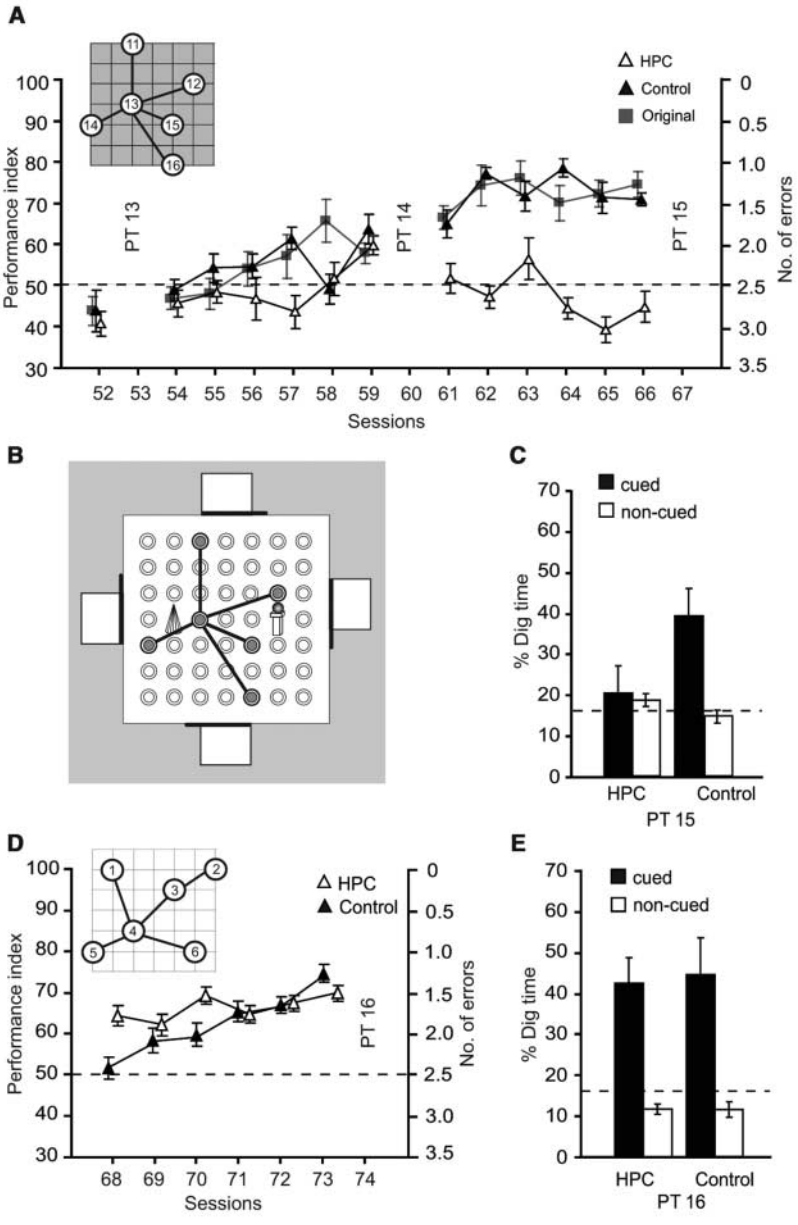

Fig. 3. Gradual acquisition of new PAs in a new context by experienced animals. (**A**) Acquisition of PAs. The now experienced sham group (n = 8) learned a new set of six PAs in the second event arena at a comparable rate to that shown by normal animals in the first event arena (Group × Session F = 1.97, df = 6.9/116.9, 0.10 > P > 0.05, treating Group as a between-subjects factor). Relative to the sham-lesioned group, the HPC-lesioned group (n = 10) failed to learn (Group F = 128.63, df = 1/15, P < 0.001; Group × Session F = 7.42, df = 5.9/89.3, P < 0.001). (**B**) Spatial arrangement of the new PAs (PAs 11 to 16) in the new event arena. (**C**) Cued-recall probe trial. Proportion of digging at the cued location relative to the noncued locations by sham- and HPC-lesioned animals (PT15, session 67). The sham group was above chance (t = 2.38, df = 7, P < 0.05); the HPC group was not (t < 1). However, the difference between groups showed only a trend toward significance (t = 1.83, df = 15, 0.10 > P > 0.05). (**D**) Return to the original event arena and flavors (flavors 1 to 6). Inset indicates transition to the original schema acquired before surgery. The HPC group is above chance at the outset (t = 3.9, P < 0.005; session 68), but neither Group nor Group × Session effects were significant for the performance index (Ps > 0.05). After six sessions of retraining, the sham group caught up, and both groups were well above chance (ts = 8.7 and 8.9, Ps < 0.001). (**E**) Performance in the probe test (PT16) indicated that both HPC and sham groups were consistently above chance in preferentially digging at the cued location (t = 4.37, df = 8, P < 0.005; t = 3.19, df = 7, P < 0.025, respectively) and did not differ from each other (t < 1, n.s.).

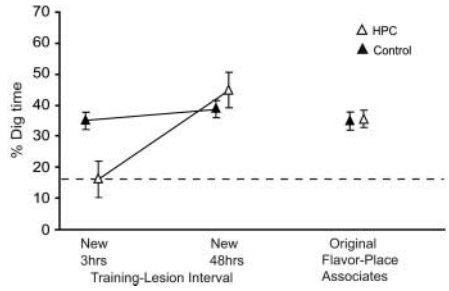

Fig. 4. Identifying the interval between training and hippocampal lesions for consolidation. A striking temporal gradient of retrograde amnesia is observed in this paradigm. HPC lesions made 3 hours after training (n = 7) on the novel flavor tested 14 days later prevented consolidation, whereas consolidation was complete when HPC lesions (n = 6) were made after 48 hours (Group × Delay F = 15.77, df = 1/13, P < 0.005). The HPC and control 48-hour groups did not differ (t < 1). The performance of the HPC 48-hour group was significantly higher than that of the HPC 3-hour group (t = 4.82, df = 11, P < 0.001), but the corresponding two control groups (ns = 9) did not differ (t < 1). The control groups were above chance at both training-lesion intervals (ts > 5.1, df = 8, P < 0.001); the HPC 3-hour group did not differ from chance (t < 1), whereas the HPC 48-hour group was above chance (t = 4.90, df = 5, P < 0.005). Separate analyses of the postsurgery memory for the original PAs learned over 14 sessions showed above-chance performance for both the HPC and sham groups (HPC t = 5.80, df = 12, P < 0.001; sham t = 9.85, df = 17, P < 0.001).

If systems consolidation within the neocortex can take place in as little as 48 hours, it becomes of interest to find out the minimal time required for it to occur. Some theoretical models suppose that a memory trace stored in the hippocampus, serving as an "index" or "pointer" to cortically encoded information, must last sufficiently long to guide the slower systems-level consolidation process that is thought to take place in sleep, requires sharp-wave activity, and has previously been shown to involve hippocampal-neocortical interactions over time (*31*–*35*). The prediction is that hippocampal lesions made 3 hours after training to animals that do not sleep during this short training-surgery interval should prevent neocortical consolidation. In experiment 3 (using a new set of 18 rats that acquired the basic schema of PAs 1 to 6 over 14 sessions as before), we compared the impact of hippocampal lesions given 3 or 48 hours after the training of two new PAs in single trials (PAs 7 and 8). This experiment used a "reverse" day-night cycle (with all testing during the animal's night) to minimize, in the case of the 3-hour interval, the likelihood of sleep episodes between the end of training and the time of the lesion. A partial within-subjects design was also used (fig. S8), with some animals having hippocampal lesions at appropriate time points soon after novel PAs 7 and 8, and others that were only anesthetized in this first phase given hippocampal or sham lesions after the later introduction of PAs 9 and 10. Cued recall was examined for the new associates shortly before surgery and was found to be effective for all animals. After surgery, cued recall for the new one-trial PAs was at chance for those animals subject to hippocampal lesions 3 hours after acquisition, but—replicating the results of experiment 2—it was effective when lesions were made 48 hours after training (Fig. 4). This is a strikingly steep upward temporal gradient of remote memory.

Causal role for schemas in learning. The final issue to consider is whether an activated schema is causally necessary for rapid memory consolidation (*5*). An alternative account of these experiments could be that the animals find it increasingly easier over the course of training to encode, store, and/or consolidate individual PAs as a result of increasing familiarity with the context of learning, with the "schema" concept being superfluous. To contrast these alternatives, we trained normal animals in two event arenas concurrently (experiment 4). In one room, they were trained on a "consistent" schema in which flavors 1 to 6 were always placed consistently at locations 1 to 6, respectively (schema 1 = PAs 1 to 6; Fig. 5B). In the other room, the animals were trained on "inconsistent" schema in which a single set of six locations (locations 11 to 16) and a set of six flavors (flavors 11 to 16) were used, but the mapping of flavors to locations was changed every two sessions (Fig. 5B). The scheduled inconsistency was therefore in the relational pairing of the items rather than the identity of the flavors or the locations of the sand wells. Moreover, a change only every two sessions did not preclude the animals attempting to learn these PAs across sessions, but would have precluded the creation of a context-specific schema. Choice performance gradually improved in the consistent schema room but not in the inconsistent room (Fig. 5A); nonrewarded probe tests also established that the animals dug preferentially in the cued location in the arena of a start-box flavor in the consistent but not the inconsistent context (Fig. 5C). This difference between the two contexts is not in itself surprising and would occur even if the animals were still trying to learn individual PAs in the inconsistent room. However, this differential rate of learning sets the stage for a last and crucial test of the schema concept.

This test involved the learning of new PAs. If animals learn PAs as isolated "facts," and if they do so ever more quickly because of context familiarity as training in this protocol proceeds, the rate of learning in the two contexts should be the same. However, if the animals bring something like "activated schema" to bear on the process of learning, a difference between the two contexts might be observed. The "consistent schema" would only be activated in its appropriate context. Procedurally, the comparison in the rate of

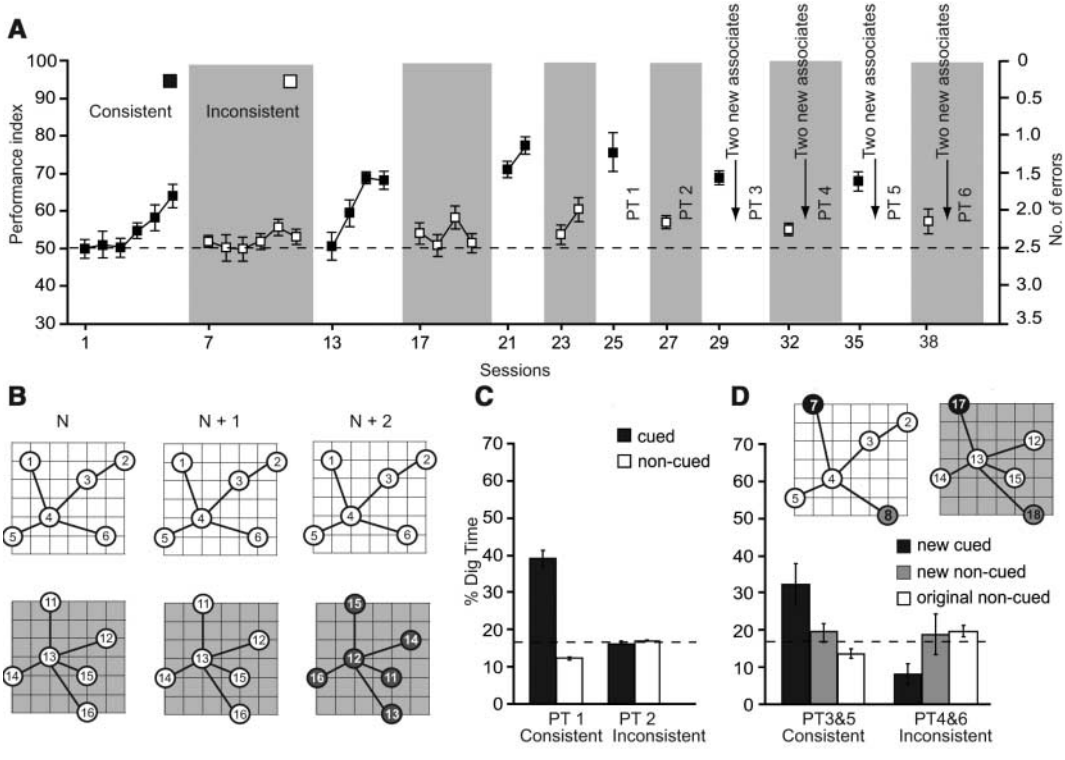

Fig. 5. A consistent activated schema promotes effective memory. (**A**) Differential acquisition of consistent and inconsistent schemata. Effective acquisition by normal rats (n = 9) occurred when mapping of flavors to places remained consistent, with six, four, two, and then single sessions (sessions 1 to 40; white background). Above-chance performance was consistent from session 15 onward ($P < 0.025$ for each comparison with chance). The same animals failed to learn a series of inconsistent schemas in the second event arena (selected days are above chance, e.g., session 27, but performance never rose above 60% correct; gray background). (**B**) With the consistent schema, the mapping of flavors to places is consistent across sessions; inconsistent schema used a common set of six flavors and locations that were associated for two sessions but then changed every third session (see N + 2, shaded gray). (**C**) Preferential digging in the probe trials at the cued locations for the consistent schema (PT1: $t = 10.9$, df = 8, $P < 0.001$) but not for the inconsistent schema (PT2: $t < 1$). (**D**) New PA probes. Performance 24 hours after exposure to the two new cue flavors and their locations when the animals would be encoding information using a consistent activated schema (PTs 3 and 5) was consistently good to the cued new location, whereas performance after use of an inconsistent schema was not (PTs 4 and 6; Group × Location $F = 13.92$, df = 1.64/26.30, $P < 0.001$). Approach latencies from the start box to the correct sand well during these probe trials were equivalent in the consistent (20.9 ± 1.9 s) and inconsistent (20.0 ± 2.5 s) contexts, indicating comparable motivation to perform each task.

learning new information had to be done in a manner that ensured an identical behavioral protocol in the consistent and inconsistent rooms. In this phase, beginning at session 29, the animals were therefore trained on four successive sequences of three training sessions beginning as follows: session 29, further consistent-context training of flavors 1 to 6; session 30, two new PAs trained in a session consisting of only two trials (PAs 7 and 8); session 31, a nonrewarded probe test for these novel associates. This three-session sequence was then repeated in the inconsistent context (sessions 32 to 34) using flavors 11 to 16, then PAs 17 and 18 followed by a nonrewarded probe test; and again in the consistent and then the inconsistent context with PAs 9 and 10 and PAs 19 and 20, respectively. The sequence ended with PT6 on session 40 (Fig. 5). The use of only two rewarded trials instead of the usual six trials per day on session 2 of this three-session sequence ensured that both the behavioral procedure and the memory-encoding demands on the animals were identical in the two training contexts on session 2. Figure 5D shows successful acquisition and 24-hour retention of these new PAs only when encoding occurred in the consistent-schema context. The apparent motivation of the animals to perform these two learning tasks was equivalent, as indexed by equivalent approach latencies to the target sand well in both the consistent and inconsistent contexts (Fig. 5D).

These findings indicate that animals—no less than people—can bring activated mental schemas to bear in a PA learning task and thereby encode, assimilate, and rapidly consolidate relevant new information after a single trial. The capacity of animals to make deductive inferences on the basis of their "mental models" of the world is, of course, far more limited than that of humans (*4*), but the principle that associative schemas can be useful in memory is not unique to humans.

In experiment 1, animals used hippocampal-dependent learning to acquire several PAs concurrently, of which one member of each pair was a spatial location in a familiar environment. This enabled the animals to treat these several associates as a connected spatial set, rather than as individual "facts," and so build up a framework in which similar new information could be stored. The construction of this "schema" took about a month—approximately the same period that several studies of retrograde amnesia have suggested is always required after learning for effective systems consolidation to occur. We observed, however, that if the several weeks of schema building was completed before new learning, the assimilation and consolidation of novel information within these neocortical schemata could be very rapid (experiments 2 and 3). We also established that the possession of an activated schema is causally important in the acquisition of new information (experiment 4). The use of rigorous control protocols (e.g., the noncued memory test, arena rotation) established that performance is mediated by PA memory rather than by cryptic uncontrolled olfactory cues. Similarly, the use of two new PAs exploring associative assimilation into a schema, rather than a single PA, ensured that the effective recall in probe trials was not an artifact of stimulus novelty.

Discussion. These findings have implications for a number of key issues in the neurobiology of learning and memory. First, they indicate that the rate at which systems consolidation occurs in the neocortex can be influenced by what is already known. In contrast, in the complementary learning systems approach (*36*, *37*), the hippocampus is said to be "specialized for rapidly memorizing specific events" (*37*) and the neocortex for "slowly learning the statistical regularities of the environment." Consolidation of memory traces in the neocortex is held to be a largely time-dependent process determined by the specific patterns of information representation, anatomical connectivity, and synaptic plasticity expression rules that it can support. Broadly speaking, this is a fair characterization of a large body of data (*27*), but it does not quite capture the potential that the neocortex has for rapid consolidation when newly acquired information is compatible with previously acquired knowledge. Given our observation that the neocortex can sometimes consolidate very rapidly, it follows that it must also be able to encode associative memory traces very rapidly—perhaps even "on-line" within sensory-perceptual systems. The widely held supposition that the neocortex is a slow learner therefore needs to be reappraised. The distinct temporal dynamics of these memory processes may contribute to the usual finding that the cortex does learn more slowly than subcortical structures—a generality that extends to conditional-associative motor learning (*38*)—but that this may not always occur.

A second finding is that the storage and recall of allocentric spatial memory can occur outside the hippocampus in the rat, even for information that has been acquired in a single trial as a consequence of hippocampal-dependent processing. This conflicts with both the cognitive-map theory and the multiple-trace theory of memory consolidation (*7*, *39*, *40*). Spatial memory has been shown previously in rats with hippocampal lesions, but the information was either acquired postoperatively and inflexibly over very extended training (*41*, *42*) or "semanticized" over many months before the lesion (*43*). The long-sought upward gradient of remote spatial memory in rats when varying intervals of time are systematically scheduled before making hippocampal lesions (*44*–*47*) is now definitively shown using a cued-recall protocol for information acquired in one trial. The temporal gradient is much steeper than might have been expected on the basis of prior work using a within-subjects design for contextual fear conditioning (*26*). Moreover, the effective remote spatial memory in hippocampal-lesioned animals upon their return to the first event arena, learned as young animals, is strikingly similar to that displayed by patient E.P. (*30*). It is unclear why effective remote spatial memory is found here but not in the water maze (*48*). One possibility is that the water maze is more "recall-like" in character (*10*), requiring an animal to generate its own reminder cues. The PA paradigm used here could allow apparent cued recall to be mediated in part by cued recognition based on proximal intramaze cues.

Third, the failure of animals with near-complete hippocampal lesions to acquire PAs over many trials of training (experiments 1 and 2) calls into question the capacity for effective "semantic-like" learning in the absence of functional hippocampal tissue. This idea emerged particularly in studies of developmental amnesia (*49*), but it has proved difficult to distinguish whether the intriguing dissociations between impaired episodic and intact semantic memory in such patients are due to intact neocortical learning of semantic information (*50*), to functional reorganization in the developing brain, or to islands of residual hippocampal function in these amnesic patients. When the medial temporal lesions are large, as in patient E.P., essentially no declarative fact learning occurs (*51*). Our findings suggest that, in animals in which it is possible to make selective 90% lesions of the hippocampus as adults, the acquisition of new flavor-place PAs is also consistently blocked and not rescued by multiple training trials. The generality of this observation beyond the spatial domain should be followed up in young animals, including primates, in order to model the situation in developmental amnesia more closely.

That the acquisition of a schema took about a month points to the possibility of it involving some kind of neuroanatomical growth process in the neocortex that creates an associative "space" in which new PAs can be rapidly stored without interference—analogous to "phase sequences" (*52*). Intercortical synaptic connections may be created or unmasked within a functional network that has only silent or baseline synaptic strengths. These could then be rapidly potentiated by relevant information when the network is in an "active" state (an activated schema). The initial growth process would necessarily take a period of days or weeks—the very time period that has hitherto been thought to mediate systems consolidation and to occur only after learning (*20*). Thus, an intriguing speculation to emerge from the present data, with conceptual similarities to the principles of synaptic tagging and capture (*53*, *54*), is that an associative space into which new information can be assimilated can be constructed before the exposure to that information. However, this construction of associative interconnections can be noncommittal or "experience-expectant" in character (*55*).

The findings bring to neuroscience a set of ideas hitherto largely discussed in the context of psychological studies of human memory. The concept of "activated schemas" has been discussed only in relation to humans (*3*), as it implies a conscious awareness that rats are unlikely

to possess. However, even if they are implicit, schemas are an economical way to characterize the gradual acquisition of an organized framework of associative "semantic-like" information from "episodic-like" events that, once acquired, allows relevant new information to be assimilated and stored rapidly. Given that animals have daily activities such as finding food and water, it is important for them to retain an organized body of knowledge about where these may be found and to be able to update such a framework rapidly, within one trial. This inferential flexibility of rodent cognition is now established in several domains (*9*).

References and Notes

1. F. C. Bartlett, *Remembering* (Cambridge Univ. Press, Cambridge, 1932).
2. K. Craik, *The Nature of Explanation* (Cambridge Univ. Press, Cambridge, 1943).
3. J. D. Bransford, *Human Cognition: Learning, Understanding and Remembering* (Wadsworth, Belmont, CA, 1979).
4. P. N. Johnson-Laird, *Mental Models: Towards a Cognitive Science of Language, Inference, and Consciousness* (Cambridge Univ. Press, Cambridge, 1983).
5. J. D. Bransford, M. K. Johnson, *J. Verb. Learn. Verb. Behav.* **11**, 717 (1972).
6. E. A. Maguire, C. D. Frith, R. G. M. Morris, *Brain* **122**, 1839 (1999).
7. J. O'Keefe, L. Nadel, *The Hippocampus as a Cognitive Map* (Clarendon, Oxford, 1978).
8. B. O. McGonigle, M. Chalmers, *Nature* **267**, 694 (1977).
9. H. Eichenbaum, *Neuron* **44**, 109 (2004).
10. L. R. Squire, *Psychol. Rev.* **99**, 195 (1992).
11. Y. Dudai, R. G. M. Morris, in *Brain, Perception and Memory: Advances in Cognitive Sciences*, J. Bolhuis, Ed. (Oxford Univ. Press, Oxford, 2001), pp. 147–162.
12. J. L. McClelland, B. L. McNaughton, R. C. O'Reilly, *Psychol. Rev.* **102**, 419 (1995).
13. M. Day, R. F. Langston, R. G. M. Morris, *Nature* **424**, 205 (2003).
14. M. Bunsey, H. Eichenbaum, *Nature* **379**, 255 (1996).
15. R. P. Kesner, M. R. Hunsaker, P. E. Gilbert, *Behav. Neurosci.* **119**, 781 (2005).
16. S. Wirth *et al.*, *Science* **300**, 1578 (2003).
17. K. Sakai, Y. Miyashita, *Nature* **354**, 152 (1991).
18. Y. Miyashita, *Science* **306**, 435 (2004).
19. B. Bontempi, C. Laurent-Demir, C. Destrade, R. Jaffard, *Nature* **400**, 671 (1999).
20. P. W. Frankland, B. Bontempi, *Nat. Rev. Neurosci.* **6**, 119 (2005).
21. H. Eichenbaum, P. Dudchenko, E. Wood, M. Shapiro, H. Tanila, *Neuron* **23**, 209 (1999).
22. The food used as one member of each PA (diet pellets manufactured in a range of different flavors) was hidden in a sand mixture that had been adulterated by ground-up pellets consisting of 1% of each of the six flavors used on any daily session (6% total). This and other procedures masked any olfactory cue that might have guided the animals to the correct sand well. The animals were shown to use recall of spatial location exclusively in making their sand-well choices. No food pellets were present in the sand mixture during nonrewarded probe tests.
23. See supporting material on *Science* Online.
24. S. M. Zola-Morgan, L. R. Squire, *Science* **250**, 288 (1990).
25. J. J. Kim, M. S. Fanselow, *Science* **256**, 675 (1992).
26. S. G. Anagnostaras, S. Maren, M. S. Fanselow, *J. Neurosci.* **19**, 1106 (1999).
27. P. J. Bayley, J. J. Gold, R. O. Hopkins, L. R. Squire, *Neuron* **46**, 799 (2005).
28. T. Maviel, T. P. Durkin, F. Menzaghi, B. Bontempi, *Science* **305**, 96 (2004).
29. H. F. Harlow, *Psychol. Rev.* **56**, 51 (1949).
30. E. Teng, L. R. Squire, *Nature* **400**, 675 (1999).
31. T. J. Teyler, P. DiScenna, *Behav. Neurosci.* **100**, 147 (1986).
32. G. Buzsaki, *Neuroscience* **31**, 551 (1989).
33. A. G. Siapas, M. A. Wilson, *Neuron* **21**, 1123 (1998).
34. B. L. McNaughton *et al.*, in *Sleep and Synaptic Plasticity*, C. Smith, P. Maquet, Eds. (Oxford Univ. Press, New York, 2003), pp. 225–246.
35. R. G. Morris, *Eur. J. Neurosci.* **23**, 2829 (2006).
36. R. C. O'Reilly, J. W. Rudy, *Psychol. Rev.* **108**, 311 (2001).
37. K. A. Norman, R. C. O'Reilly, *Psychol. Rev.* **110**, 611 (2003).
38. A. Pasupathy, E. K. Miller, *Nature* **433**, 873 (2005).
39. R. S. Rosenbaum, G. Winocur, M. Moscovitch, *Behav. Brain Res.* **127**, 183 (2001).
40. M. Moscovitch, L. Nadel, G. Winocur, A. Gilboa, R. S. Rosenbaum, *Curr. Opin. Neurobiol.* **16**, 179 (2006).
41. R. G. M. Morris, F. Schenk, F. Tweedie, L. E. Jarrard, *Eur. J. Neurosci.* **2**, 1016 (1990).
42. H. Eichenbaum, C. Stewart, R. G. M. Morris, *J. Neurosci.* **10**, 3531 (1990).
43. G. Winocur, M. Moscovitch, S. Fogel, R. S. Rosenbaum, M. Sekeres, *Nat. Neurosci.* **8**, 273 (2005).
44. J. J. Bolhuis, C. A. Stewart, E. M. Forrest, *Q. J. Exp. Psychol. B* **47**, 129 (1994).
45. R. J. Sutherland *et al.*, *Hippocampus* **11**, 27 (2001).
46. R. E. Clark, N. J. Broadbent, L. R. Squire, *Hippocampus* **15**, 260 (2005).
47. S. J. Martin, L. de Hoz, R. G. M. Morris, *Neuropsychologia* **43**, 609 (2005).
48. R. G. M. Morris, P. Garrud, J. N. Rawlins, J. O'Keefe, *Nature* **297**, 681 (1982).
49. F. Vargha-Khadem *et al.*, *Science* **277**, 376 (1997).
50. M. Mishkin, W. A. Suzuki, D. G. Gadian, F. Vargha-Khadem, *Philos. Trans. R. Soc. London Ser. B* **352**, 1461 (1997).
51. P. J. Bayley, L. R. Squire, *J. Neurosci.* **22**, 5741 (2002).
52. D. O. Hebb, *The Organization of Behaviour* (Wiley, New York, 1949).
53. U. Frey, R. G. M. Morris, *Nature* **385**, 533 (1997).
54. A. Govindarajan, R. J. Kelleher, S. Tonegawa, *Nat. Rev. Neurosci.* **7**, 575 (2006).
55. W. T. Greenough, J. E. Black, in *Developmental Behavioral Neuroscience*, M. R. Gunnar, C. A. Nelson, Eds., vol. 24 of *Minnesota Symposia on Child Psychology* Erlbaum, Hillsdale, NJ, 1992); pp. 155–200.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5821/76/DC1
Materials and Methods
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REPORTS

Nonstoichiometric Dislocation Cores in α-Alumina

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Little is known about dislocation core structures in oxides, despite their central importance in controlling electrical, optical, and mechanical properties. It has often been assumed, on the basis of charge considerations, that a nonstoichiometric core structure could not exist. We report atomic-resolution images that directly resolve the cation and anion sublattices in alumina (α-Al_2O_3). A dissociated basal edge dislocation is seen to consist of two cores; an aluminum column terminates one partial, and an oxygen column terminates the second partial. Each partial core is locally nonstoichiometric due to the excess of aluminum or oxygen at the core. The implication for mechanical properties is that the mobile high-temperature dislocation core structure consists of two closely spaced partial dislocations. For basal slip to occur, synchronized motion of the partials on adjacent planes is required.

The core structures of dislocations are critical to the electronic, optical, and mechanical properties of a wide range of materials. For most simple monometallic crystals, dislocation core termination can be determined; however, in complex crystals such as oxides, either cation or anion columns (or both) can be the terminating atomic columns even with the same dislocation character (i.e., characteristic displacement vectors called Burgers vectors, **b**). The possibility of nonstoichiometric cores also arises but has usually been rejected because it suggests the possibility of charged dislocations (*1*, *2*) and the presence of long-range Coulomb fields with a high associated electrostatic energy. This has been suggested to be the reason why the close-packed {111} crystal plane in alkali halides cannot be an easy slip system (*2*, *3*). Detailed knowledge of dislocation core structures and compositions is critical to understand dislocations in ionic crystals.

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Supporting Online Material for

Schemas and Memory Consolidation

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This PDF file includes:

1. Materials and Methods

1.1 Subjects

The subjects were adult male Lister-hooded rats (Charles River, UK), aged 8-10 weeks at the start of experimentation and weighing 250-270 gm. They were housed in groups of 3/4 rats per cage (Experiments 1, 3 and 4) or single cages (Experiment 2). They had free access to water at all times and were maintained at 85% of their free-feeding weight. Experiments 1, 2 and 4 were conducted on a 12 hr (on)/12 hr (off) light cycle, with training during the light phase (8am-8pm). Experiment 3 was conducted using a reverse day/night cycle of the same length, with training during the night phase. A total of 59 rats were used (Experiment 1: n = 12; Experiment 2: n = 18; Experiment 3: n = 20; Experiment 4: n = 9). All procedures were compliant with national (Animals [Scientific Procedures] Act, 1986) and international (European Communities Council Directive of 24 November 1986 [86/609/EEC]) legislation governing the maintenance of laboratory animals and their use in scientific experiments. We used the minimal number of rats for the necessary statistical power and there was minimal suffering associated with any of the experimental procedures.

1.2 Apparatus

The two 'event arenas' (*See* ***Reference S1***) in which rats were trained to find flavored food were made of plexiglass and placed in adjacent laboratory rooms containing a number of prominent and distinctive cues (*see detailed apparatus description in* ***Reference S2***). They measured 1.6m x 1.6m, with floors containing a 7 x 7 grid of 49 circular holes (6cm diameter) at 20 cm spacing. Six of these holes contained a sand-well with the remainder covered by plastic lids. The sand-wells were in different locations in different parts of each of the experiments. The entire floor was covered by 3 cm of sawdust. Two distinctive landmarks (*Arena 1:* a glued stack of golf balls and a pyramid; *Arena 2:* a different pyramid shape, and a rectangular shape with a ball on top) were placed in 2 locations: row 4, column 2; and row 4, column 6. Access to the arenas by the rats was from any of four plexiglass start boxes (25 cm x 25 cm x 25 cm), covered with black paper to make them dark inside, that were centrally placed in each wall with remotely controlled sliding doors for arena access. The illumination of the laboratory room was maintained at a moderate level by wall mounted halogen lamps. The rats were observed by means of CCD cameras connected to video recorders and their movements tracked by means of custom LabView computer software

which could also record the time taken to locate the correct sand-well, the time spent digging at each sand-well and other parameters of performance.

A key design principle was to support paired-associate learning and recall in the absence of artifactual cues. This was achieved as follows. The plastic sand-wells that could be inserted into the holes in the arenas were 4cm deep with a removable metal mesh grid fixed half way down. The area below the grid was filled with a mixture of food pellets that also included all the flavors being used during the experiment; the area above was filled with a mixture of 90g sand plus ground-up food (25g per 2.5kg sand) which included all the flavors used in the experiments. To make food reward available in a sand-well, 3 pellets (0.5g each; manufactured in various flavors all with equal nutritional value) were hidden on the upper surface of the metal grid under the sand layer. The rats could then dig through the sand mixture to search for and retrieve each food pellet. The sand and food mixture was renewed every 2/3 days. These olfactory control precautions were designed to ensure that when the rats entered the arena, their search was guided only by memory for the flavor-location association and not by odor emanating from the sand-well containing the food. The cue to determine which sand-well to approach was flavored food made available to the rats in the start-boxes. As the rats preferred to return to the start box to eat each of the 3 pellets dug up from a sand-well, water was placed in the start-boxes for drinking.

1.3 Hippocampus lesions

All surgical procedures were carried out by RFL. Complete hippocampus lesions (HPC lesions, Experiments 1-3) were made bilaterally by stereotaxic injection of ibotenic acid throughout the entire hippocampus, including dentate gyrus and CA fields. Sham operated control rats (sham controls, Experiments 1-3) received anaesthetic, surgery including removal of a large section of the skull and piercing of the dura, but no injections. Anaesthetised control rats (Experiment 3) were anaesthetised for the same time period as rats receiving complete hippocampus lesions, but did not undergo any invasive procedures. The number of surgeries was: Experiment 1: HPC n = 6, Sham n = 6; Experiment 2, HPC n = 10, Sham n = 8; Experiment 3, HPC n = 12, Sham n = 8.

Ibotenic acid hydrate was dissolved in phosphate-buffered saline (pH 7.4) at 10mg/ml, following the protocol of Jarrard (***Reference S3***). Anaesthesia was induced and maintained using halothane and rats were positioned in a stereotaxic frame. Bilateral craniotomy was carried out at the target site to expose the dura above the hippocampus. Thirteen (Experiment 1) or fifteen (Experiments 2 and 3) injections of ibotenic acid were

made into each hippocampus at different rostrocaudal and dorsoventral levels via a 1µl syringe securely attached to the frame by a stereotaxic arm. The needle tip was bevelled, with the hole facing posterior. Ibotenic acid was injected at a rate of 0.1 µl/min; beginning 30s after the needle was lowered. The needle was removed very slowly 60s after the injection. The co-ordinates **(*Table S1*)** were modified from de Hoz, Knox and Morris (***Reference S4***), given the different size of the rats at the time of surgery. Sham lesions were carried out in the same way, but no injections were made. Instead, the dura was pierced repeatedly with a needle to simulate the mechanical damage caused by needle entry in lesioned rats. After completion of the ibotenic acid injections (or piercing of dura for sham rats), gelatine sponge was placed over areas where bone had been removed and a subcuticular suture technique used to close the skin over the top of the skull. Each rat received 0.03ml carprofen analgesia in 5ml saline subcutaneously at the end of the procedure. Analgesia was also administered in oral form in the rats' water supply. This analgesic solution was freely available to all rats from 24 hours pre-surgery until 96 hours post-surgery. The rats were given access to unrestricted food 24 hours before surgery and thereafter for 11 days. Food restriction began 12 days into the post-operative recovery period, and behavioral testing recommenced after 14 days.

1.4 Perfusion, histology and lesion analysis

All rats were terminally anaesthetised with 1.4 ml/kg sodium pentobarbital then perfused intracardially with 0.9% saline followed by 4% formalin. The brains were removed and stored in 4% formalin for a minimum of 24 hours, then placed into cuboid moulds filled with fresh egg yolk and incubated at 37°C in a shallow 4% formalin bath for a further 24 hours during which the egg-yolk became solid. The brains with the egg-yolk coating were then removed from the plastic moulds and placed back into jars of 4% formalin for a further 48 hours. Coronal 30µM sections were cut using a cryostat with one in every five sections recovered for histological analysis. Theses sections were mounted on slides, stained with cresyl violet and cover slipped using DPX.

Fig. S1 shows representative sections of a sham operated brain and a hippocampus lesion at 3 different rostral to caudal locations. The extent of the lesions was assessed by calculating the volume of hippocampus spared in each of the brains (DG and CA fields). Each coronal section was placed under a Makroscope and the image transferred to a computer using a camera. The images could then be opened in Leica QWin software and the calibrated area measurement tool in the program allowed calculation of the amount of

hippocampus tissue remaining in each brain section, with the program recording the size of the area in mm^2. The brain sections from control rats were measured in this way to give a standardised value (i.e. an intact hippocampus with no lesion, 100%). The sum of all the areas of hippocampal sparing in each of the lesioned rats (i.e. the overall volume of sparing) was then compared with the average value for all control rats to calculate a percentage sparing of hippocampus tissue. The lesion extent varied slightly across experiments ranging from 9.67% spared tissue through to 19.09% **(*Table S2*)**. These are large lesions by established standards.

Qualitative analysis showed that the hippocampus lesions were specific to the target area, producing maximal damage to the hippocampus whilst avoiding damage to entorhinal, perirhinal, postrhinal and retrosplenial cortices. There was some minor damage to the overlying parietal cortex in the lesioned rats which is represented in the 3D reconstruction image in Fig. 1 (Main Paper). Presubiculum and parasubiculum were generally intact. However, in the larger hippocampus lesions there was some damage to a small portion of the subiculum both dorsally and ventrally. To assess any effects of this damage, Pearson correlations were carried out where relevant to see if there was any correlation between the subicular damage (lesion size) and behavioral performance (% correct dig time). These correlations were carried out for postoperative probe tests PT5-PT8 (Experiments 2 and 3, ***Fig. S4*** and ***Fig. S8***) and probe tests PT10-PT13 (Experiment 3, ***Fig. S8***). No significant correlations were found between lesion size and behavioral performance (all *ps*>0.05, 2-tailed), but this is unsurprising given the considerable consistency in lesion volume across rats.

1.5 Behavioral training

The experimenter conducting the behavioral training (IB/RFL/DT) was blind to which rats had hippocampus lesions and which were sham controls. The protocols and additional results beyond those reported in the main paper are described below for each experiment.

1.6 Statistical Analysis

Several measures of performance were assessed, including number of errors in main training and time spent digging in each sand-well during probe trials. Statistical significance (SPSS) was determined by t-test and ANOVA analysis where appropriate. The level of significance was set at $p < 0.05$ with the more important findings at a substantially greater level of significance (z scores > 5).

2. Experiment 1

2.1 Aims

- **to establish whether the learning of flavor location paired-associates requires the integrity of the hippocampus**.

2.2 Habituation

Habituation (Sessions -8 to -3): 12 days after recovery from surgery, rats (HPC = 6, Sham = 6) were habituated to the event arena and trained to search and dig for control food-pellets (non-flavored) in the sand-wells. They carried these 0.5 gm pellets to the start box, and ate them there. Habituation consisted of a series of stages across days, allowing exploration of the arena and its cues, experience of each of the 4 start-boxes, digging for food, and carrying pellets to the start boxes. By the end of habituation, all rats were running quickly into the arena, collecting food and returning to the start boxes to eat each pellet. Habituation lasted 6 sessions with the full protocol shown in **Fig. S2**.

2.3 Training of paired-associates

Training (Sessions 1-13): The key feature of the protocol was the concurrent training of 6 flavor-place paired-associates (PAs) each day. After 2 sessions in which only 3 of the 6 flavors were used (pre-training), all PAs were trained, each flavor/place pair for 1 trial per session throughout the 13 sessions of acquisition with PA1 (Rum, in L1), PA2 (Strawberry in L2), PA3 (Ginger in L3), PA4 (Banana in L4), PA5 (Very Berry in L5) and PA6 (Bacon in L6). On any trial, all 6 sand-wells were accessible, but only one contained its appropriate flavor reward. A trial began with the rats receiving a 0.5g 'cue' flavor in one of the start boxes. After a period of 30 s in the start box during which the rats ate this cue, the door was automatically opened. Initially, the rats would run into and explore the arena, and then dig at one or more sand-well until finding the correct location containing 3 food pellets of the same flavor as the cue (0.5g each). These were carried back in the start box, pellet by pellet, and eaten in turn. After a rat returned to the start box with the 3rd pellet, the door was closed. The experimenter allowed a short period of eating time and then entered the testing room to return the rat to its home cage and prepare the arena for the next rat. As training progressed, trials became quicker (reaching an asymptote of 3-4 min per trial). Choice performance also improved for the sham rats as they learned to associate a specific flavor with a specific

location (Fig. 1C). The rats were trained on alternate days in 2 groups of 6 rats each (3 lesioned and 3 control rats in each group) with each group receiving 3 training sessions per week. As all 6 rats were trained consecutively, the intertrial interval for an individual rat between successive flavor-place pairings was circa 1 h, resulting a total daily session time of up to 6 h. The rats were returned to their home cage between trials. The various possible sequences of different flavored pellets across 6 trials within a session were carefully counterbalanced across rats and sessions. On a given session, half the rats were cued from one start box (e.g. South) while the other half were cued from the opposite start box (e.g. North). The start locations were then pseudo-randomly assigned (N, S, E or W) across training sessions.

Non-rewarded probe test PT (Session 14): To examine cued-recall memory, a non-rewarded probe test (PT) was scheduled. During this test, all six sand-wells were open as usual, and the rats could dig in any of them, but none contained food reward. The rats were cued with a single flavor as usual, and then allowed into the arena for a total of 120 s, with good memory revealed as preferential digging at the cued location. The digging time at each of the sand-wells was measured using the computerized tracking system. This probe test also served as a further control for artifacts and uncontrolled factors. The lack of reward at the target sand-well precluded any olfactory guidance to the correct location. Additionally, to prevent the rats using olfactory traces from a previous rat to locate the reward, the sand used in the wells in different locations was mixed between each trial and the sawdust surrounding each sand-well distributed around the arena between trials. None of these manipulations affected performance.

Performance measures: During each trial of the experiment (training and probe tests), several parameters were measured:

- **Performance Index:** the number of incorrect sand-wells rats dug in before choosing the correct sand-well (primary error measure: chance = 2.5 errors; conversion to a score computed as 100-100*(errors/5));
- **First choice:** whether rats dug in the correct sand-well first before any other (chance = 16.7%);
- **Latency:** Time before digging commenced at the correct well (sec).

and, in probe tests:

- **Cued-recall:** the time spent digging at each of 6 sand-wells over 120s (primary performance measure calculated as the proportion of time spent at cued and non-cued locations). Although not rewarded *during* the 120s probe test time, the rats were given

3 half pellets (correct flavor) in the correct location at the end of each probe test to limit extinction.

The experimenters recorded a 'choice' only when a rat placed its front paw on or into a sand-well. Rats running past or merely sniffing quickly around a sand-well were not considered as making a choice. In rare cases, it was difficult to tell from the video monitors whether or not the rats had made a choice as defined here. In this case, when the experimenters entered the room at the end of a trial, they checked carefully if there were any traces of digging, i.e. whether the sand had been displaced around the sand-well(s).

3. Experiment 2

3.1 Aims

The full experimental design of this complex study is shown in **Fig S3**. The Aims were:

- **To monitor the acquisition of multiple paired-associates and the guidance of performance by cued-recall.**
- **To examine whether the prior learning of multiple paired-associates enables new paired-associates to be learned in 1 trial and retained over 24 hr.**
- **To examine the impact of HPC lesions (given within 48 hr of the learning of novel paired-associates) on the retention of the newly acquired paired-associates and the original set of paired-associates learned over 6 weeks.**
- **To examine the impact of HPC lesions on the post-operative acquisition of further new paired-associates in the same room.**
- **To examine the impact of HPC lesions on the post-operative acquisition of further new paired-associates in a new room.**
- **To examine whether the rats with HPC lesions could remember the original set of paired-associates after 4 months.**

3.2 Pre-operative training of multiple paired-associates

Initial training, probe tests and non-cued test (Sessions 1-19): 18 normal rats were trained on the same set of 6 flavors in the same geometric arrangement as used in Experiment

1: PA1 (Rum in L1), PA2 (Strawberry in L2), PA3 (Ginger in L3), PA4 (Banana in L4), PA5 (Chocolate in L5) and PA6 (Bacon in L6). The choice performance is shown in Fig. 2A and the proportion of trials in which a correct first-choice was made in ***Fig. S4A1*** (note that in this and subsequent supplementary figures, the letters used for panels correspond, where relevant, to identically lettered panels in the main figures). The measure of correct first choices shows greater variability than the standard Performance Index, but the overall pattern over the course of training is identical. The rats perform around chance level for the first 6 sessions and then choose correctly much more often during the second block of 6 sessions.

As the rats collected 3 pellets during each trial, it was also possible to calculate the Performance Index for choices of the 1st, 2nd and 3rd pellets independently. As shown in **Fig. S4A2**, the rats were extremely good at returning directly to the correct sand-well for their 2nd and 3rd food-pellets with levels of performance reaching circa 90% from near the beginning of training. This relatively direct return was probably mediated by spatial working memory rather than cued-recall, but even so, it would have likely aided the learning of the paired-association between a specific flavor and its location in the arena.

To demonstrate that it really was the cue flavor given in the start box that guided the rats' search strategy, a standard session of 6 daily rewarded trials was scheduled in which the successive set of 6 separate cues across trials were absent. This occurred on Session 18. The Performance Index measure fell to chance for retrieval of the first food pellet, but was very good on the subsequent retrievals for pellets 2 and 3 (**Fig. S4A2**). A standard training day occurred on Session 19 to restore normal performance.

Rapid acquisition of novel flavor location paired-associates (Sessions 20-21): Having learned 6 paired-associates in a single schema, we retrained the rats normally for 1 day (session 20) and then investigated whether the rats could acquire two new paired-associates in a single trial (Fig. 2A, session 21). The sand-wells for PA1 and PA6 were closed, and replaced by two new paired associates: PA7 (Marshmallow in L7) and PA8 (Apple in L8) at neighboring but not identical locations. The rats were trained for 6 trials in a single session with the 2 new PAs and the previous 4 PAs with the trial sequence positions of the new PAs counterbalanced across the rats. The reason for introducing 2 new PAs rather than just a single PA was to ensure that any preferential digging seen at the location of a new associate was not merely a novelty effect. If due to novelty, the rats would dig preferentially at both of the two new locations rather than the remaining original locations in a later probe test. In contrast, if the rats rapidly learned each of the 2 new PAs, they would preferentially visit only the new location cued by the appropriate new flavor was given in the start box.

Thus, in the probe test scheduled 24 hr later (Session 22), the performance measures now contrasted digging at the cued new PA, with that for both the new non-cued PA and the non-cued PAs.

3.3 Surgery

Surgery (Session 23): The surgery to create hippocampal and sham lesions was scheduled 24 hr after the new paired-associate probe test (HPC n = 10; Sham n = 8).

3.4 Postoperative testing

Retest on the old and new PAs (Sessions 24-27): After a 14 day post-surgery recovery period, a probe test was conducted to test memory for the old PAs (PT5), and followed immediately by 3 training trials only to the 3 other 'original' paired associates (PAs 2-5). PT6 was performed 48 hr on one of the new PAs (PAs7,8: rats tested on PA7 before surgery were now tested on PA8 and vice versa). These 2 probe tests were then repeated (PTs 7,8) so that each rat was tested on both of the newly acquired PAs and memory for the original PAs was examined more thoroughly. Cued recall of all flavor locations during this series of probe tests is shown Fig. 2D.

Post-operative learning of new paired-associates and a probe test (Sessions 28-35): Following a further 6 sessions of training with the original PAs2-5 and the new PAs7,8 two further new PAs were introduced. Two new novel flavors (Anise at L9 and Butter at L10), were introduced to replace PAs 7 and 8. The sand-wells for these new PAs were located at adjacent but distinct locations. The rats were trained for 6 trials in one session with PAs2-5 and PAs9,10 making up the 6 trials and then PT9 was given 24 hr later (session 35). There was no evidence that the HPC lesioned rats could learn these new paired-associates (Fig. 2E).

Additional performance measures: In addition to the standardized measures used throughout the main paper, the absolute dig time is also shown for various probe tests in **Fig. S5**. This measure enabled us to examine differences in total dig time between the HPC lesioned and sham control rats during the 120 s probe tests. It can be seen that the total dig time in a probe test tended to decline gradually over the course of training, but this decline did not differ between groups. A full statistical analysis was conducted and this revealed the same statistical pattern of group differences as in the standardized data.

3.5 Post-operative training of a new set of paired associates in the original room

Initial training and probe tests (Sessions 36-51): The same rats were then trained on a new set of 6 PAs placed in a different geometric arrangement to that used previously in this Experiment, but in the same room: PA11b (Pineapple in L11), PA12b (Brandy in L12), PA13b (Coconut in L13), PA14b (Very Berry in L14), PA15b (Paprika in L15) and PA16b (Butter in L16). The Performance Index data is shown in **Fig. S6A** and the layout of the new flavor paired-associates in **Fig. S6B**. The training sessions again took place over 28 days. The HPC lesioned rats did not learn this new geometric arrangement. Though the learning curve of the sham control rats was accelerated relative to their original rate of learning of the first schema, the probe test performance on sessions 37 and 44 (data not shown) and 51 followed the same gradual pattern (**Fig. S6C**), as that displayed the original schema. The rapid learning of the sham lesion rats probably reflects their familiarity with the intramaze and extramaze cues in the room. The impact of introducing a new geometry of the sand-wells in a familiar room with familiar cues is therefore an ambiguous experiment that is difficult to interpret - with some cues new and some old. To contrast schema learning with the savings expected if the rats had merely acquired a learning-set, it was necessary to examine learning by the now experienced rats in a new context with entirely new location cues.

3.6 Post-operative training of a new set of paired associates in the new room

Initial training and probe tests (Sessions 52-67): The rats were taken to a second event arena located in a different room and trained on a further set of 6 PAs placed in a novel geometric arrangement: PA11 (Pineapple in L11), PA12 (Brandy in L12), PA13 (Coconut in L13), PA14 (Very Berry in L14), PA15 (Paprika in L15) and PA16 (Butter in L16). Choice performance is shown in Fig. 3A. The layout of the new paired-associates is shown in Fig. 3B. Acquisition again took place over 28 days (sessions being scheduled once every 2 days). Rats did not show accelerated learning or improved asymptotic performance of the new flavor location paired-associates in this new context relative to their initial rate of learning. This key finding is discussed in the main paper. The Performance Index for choices of the 1st, 2nd and 3rd pellets independently was again calculated, but this time for the HPC lesion and sham control groups separately (**Fig. S7**). The HPC lesioned rats (**Fig. S7A**) did not differ from the sham control rats (**Fig. S7B**), nor from their own presurgery performance (**Fig.**

S4A2) with respect to their accuracy of returning to the correct location to retrieve the 2nd and 3rd pellets. Although they showed no learning across trials for the location of the 1st pellet, their spatial working memory within a trial appeared intact.

3.7 Return to the original paired-associates after 4 months

Training and probe test (Sessions 68-74): After training on the new sets of paired-associates in both the original and new rooms (circa 4 months), the rats were returned to the original room and re-tested on the originally trained PAs1-6. After 6 sessions of normal training as a reminder, a final probe test was given with different rats tested for memory of each of the original PAs (Fig. 3E). Both the HPC lesioned and sham control groups showed effective cued recall of the original flavors first learned 4 months earlier.

At the end of the entire experiment (Sessions 75-76), a final check was made of possible olfactory guidance cues on the arena. Rotation of the arena after the 3rd choice trial of a regular training session, and back again after the 3rd choice trial of a second session had no effect on performance (**Fig. S4A3**).

4. Experiment 3

4.1 Aims

- **To examine whether there is a temporal gradient for neocortical consolidation of paired-associate memory over 3-48 h.**

See **Fig. S8** for the successive stages and time course of this (partially) within-subjects experimental design.

4.2 Pre-operative training of multiple paired-associates

Initial training, probe tests and non-cued test (Sessions 1-19): 20 normal rats were trained on the same set of 6 paired-associates in the same geometric arrangement as used in Experiment 2. Acquisition again took place gradually over 28 days such that the learning curve of the rats in this Experiment (not shown) did not differ from that of Experiment 2. However, this third Experiment used a reverse day-night cycle with all testing during the night phase in order to minimize the likelihood of sleep episodes between the end of training and the time of lesion for the rats in the 3 h training-surgery interval group.

4.3 Novel flavor training and first surgery

Rapid acquisition of 2 novel flavor place paired-associates (Session 21): After the last standard training session (S20), the sand-wells for PA1 and PA6 were replaced by two new novel flavors, PA7 (Marshmallow in L7) and PA8 (Apple in L8). The rats were trained, as usual, for 6 trials in a single session, but with the 2 novel PAs introduced in the 4th (either PA7 or PA8) and 5th (vice versa) trials of the session. A probe test (PT4) was given 1 h after the last training trial (trial 6), examining the novel flavor-place associate of the 4th trial. All rats performed well above chance as expected during this test (data not shown).

First surgery (Session 22): Immediately after the probe test, half of the rats (n = 10) underwent surgery for a bilateral hippocampus lesion (n = 4; note that additional HPC lesions are made later) or served as anaesthetised controls (n = 6). These rats therefore received surgery (or anaesthetic only) 3 h after they had experienced their first novel flavor location pair on trial 4. The other half of the rats (n = 10) received hippocampus lesions (n = 4) or anaesthetic only (n = 6) 48 h after trial 4.

Post-operative testing on both the original and new PAs (Sessions 23-26): After a 14 day recovery period, a series of probe tests was scheduled (PTs 5-8) to test memory for the original and newly trained paired-associates. For the subset of rats with hippocampus lesions (n = 8), this was the end of the experiment.

Re-training of multiple paired-associates prior to second surgery (Sessions 27-32): The 12 rats that had served as anaesthetized controls were given a further 6 sessions of training with the original flavors Fs1-6 before experiencing 2 new flavor location paired-associates.

4.4 Novel paired-associate training and second surgery

Rapid acquisition of 2 more novel flavor location paired-associates (Session 33): Two further flavors and locations were introduced as in Experiment 2: PA9 (Anise in L9) and PA10 (Butter in L10), at adjacent but distinct locations to the sand-wells for PAs 7 and 8 (which were removed). The rats were trained for 6 trials in one session with PAs 2-5 and PAs 9,10 each given a single training trial, and a probe test (PT9) scheduled 1 h after the last training trial, as in Session 21.

Second surgery (Session 34): The remaining 12 rats were then divided again into 2 further groups with a 3 h or a 48 h interval between the first experience of the latest novel flavor location paired-associates and the surgery. In the 3 h interval group, 3 rats received hippocampus lesions (1 rat died 2 hours after surgery, leaving 2 rats from which we could

obtain data) and 3 rats received sham lesions. In the 48 h interval group, 3 rats received hippocampus lesions and 3 served as sham operated controls.

This within-subjects design enabled data to be secured from 13 rats with hippocampal lesions (6 at a 3hr consolidation interval and 7 at a 48 hr interval) and 18 control rats (9 at each consolidation interval), using only a total of 20 rats.

Post-operative testing on both the original and new flavors (Sessions 35-38): After a 14-day recovery period, a series of probe tests was scheduled (PTs 10-13) to test the original and new PAs. Pooling the data from PTs 5-8 and PTs 10-13 enabled us to examine the temporal gradient of remote memory over a 3 to 48 h consolidation period. The key result is is shown in Fig. 4.

5. Experiment 4

5.1 Aims

- **To establish whether an activated schema at the time of training plays a *causal* role in the rapid encoding and consolidation of flavor location paired-associates.**

5.2 Training of consistent and inconsistent paired-associate schema

Initial training (Sessions 1-24): Intact rats (n = 9) were trained in the two event arenas concurrently. In one arena with one set of contextual cues ('consistent'), the rats were trained on the same set of 6 flavors in the same geometric arrangement as used in Experiment 2. This constituted what we refer to as a 'consistent' schema (Fig. 5A,B). The rats were trained on these paired-associates for 6 sessions. Beginning on sessions 7-12, these same rats were then introduced to another event arena ('inconsistent', in another room but with the room allocation counterbalanced) in which they were confronted by another set of 6 flavors in a different geometric arrangement of 6 locations. In this room however, the mapping of flavors to locations was changed every 2 days (Fig. 5B). Flavors were each assigned to one location for 2 sessions, and then randomly swapped between the 6 locations for successive sets of 2 sessions. Briefly, rats were trained in sessions 7-8 with PA11 (Pineapple in L11), PA12 (Brandy in L12), PA13 (Coconut in L13), PA14 (Very Berry in L14), PA15 (Paprika in L15) and PA16 (Butter in L16); in sessions 9-10, the mapping was changed such that Paprika was now in L11, Very Berry in L12, Brandy in L13, Butter in L14, Pineapple in L15 and

Coconut in L16; in sessions 11-12, the mapping of flavors to locations was switched again. This constant re-mapping using a common set of 6 flavors and 6 locations constituted an 'inconsistent' schema of PAs. The initial 12 sessions of training (6 on the consistent schema followed by 6 on the inconsistent schema) were followed by another 4 sessions on each type of schema, then 2 sessions on each type of schema and then a single session of consistent paired-associate schema training followed by a single inconsistent session.

Probe tests for the original flavor-place associates (Sessions 25-28): Probe tests were given on Sessions 26 and 28 to test whether the rats could recall the paired-associates in both schema. As expected, the rats showed effective cued recall of the appropriate location when given a flavor cue in the consistent schema, but performed at chance level in the inconsistent schema (Fig. 5C). The critical part of the experiment came next.

5.3 Acquisition of novel PAs in consistent and inconsistent contexts.

Training and probe tests for novel paired-associates (Sessions 29-40): The next step was to establish whether the rate of learning new paired-associates differed when it occurred in the consistent and inconsistent schema. In the consistent schema, the sand-wells for PA1 (Rum in L1) and PA6 (Bacon in L6) were closed, and replaced by two novel flavors: PA7 (Marshmallow in L7) and PA8 (Apple in L8) at neighboring but not identical locations. Unlike all previous experiments in this series, the rats were then trained for a session consisting of only 2 trials - just the two novel paired-associates. The order of presentation of the 2 new flavor trials was counterbalanced across rats. In the inconsistent schema, the sand-wells occupying L11 and L16 were closed, and two novel PAs were created: Anise at L17 and Orange and L18, these being neighboring but not identical locations. The row and column locations of L7 and L8 in the consistent event arena and of L17 and L18 in the inconsistent arena were identical. The rats were also then trained for only 2 trials, which consisted of just the two new PAs. The order of presentation of these 2 new flavor trials was also counterbalanced. These test sessions were repeated twice for each type of schema as laid out in Fig. 5A. The main result, learning restricted to training in the consistent context, is shown in Fig. 5D.

Table S1: Injection sites for complete hippocampus lesions.

A Complete hippocampus lesion co-ordinates for Experiment 1
Mean weight of subjects = 289g

AP	ML	DV	uL ibotenic acid (10mg/ml)
-2.4	+/- 1.0	-3.0	0.05
-3.0	+/- 3.0	-2.7	0.10
	+/- 1.4	-2.1	0.05
		-2.9	0.05
-4.0	+/- 3.7	-2.7	0.10
	+/- 2.6	-1.8	0.05
		-2.8	0.05
-4.3	+/- 4.0	-7.0	0.05
-4.9	+/- 3.9	-3.5	0.05
		-7.0	0.10
-5.9	+/- 5.1	-4.5	0.08
		-5.3	0.08
	+/- 4.3	-3.9	0.10

B Complete hippocampus lesion co-ordinates for Experiments 2 & 3
Mean weight of subjects = 459g

AP	ML	DV	uL ibotenic acid (10mg/ml)
-2.4	+/- 1.0	-3.0	0.05
-3.0	+/- 3.0	-2.7	0.10
	+/- 1.4	-2.1	0.05
		-2.9	0.05
-4.0	+/- 3.7	-2.7	0.10
	+/- 2.6	-1.8	0.05
		-2.8	0.05
-4.3	+/- 4.0	-7.0	0.05
-5.0	+/- 3.0	-3.0	0.10
	+/- 3.9	-7.0	0.10
	+/- 5.4	-5.1	0.10
-6.1	+/- 3.9	-3.6	0.05
		-7.0	0.05
	+/- 5.0	-4.5	0.05
-6.6	+/- 4.6	-4.6	0.05

Table S2: Mean % intact hippocampus relative to sham controls (% ±S.E.M.).

Region	Exp 1	Exp 2	Exp 3 3hr delay	Exp 3 48hr delay
Dorsal	13.14 +/- 6.94	6.71 +/- 3.24	9.24 +/- 3.52	4.20 +/- 1.84
Ventral	6.23 +/- 2.73	16.46 +/- 2.99	28.88 +/- 4.53	20.70 +/- 3.81
Total	9.67 +/- 4.41	11.60 +/- 2.24	19.09 +/- 2.80	12.48 +/- 1.36

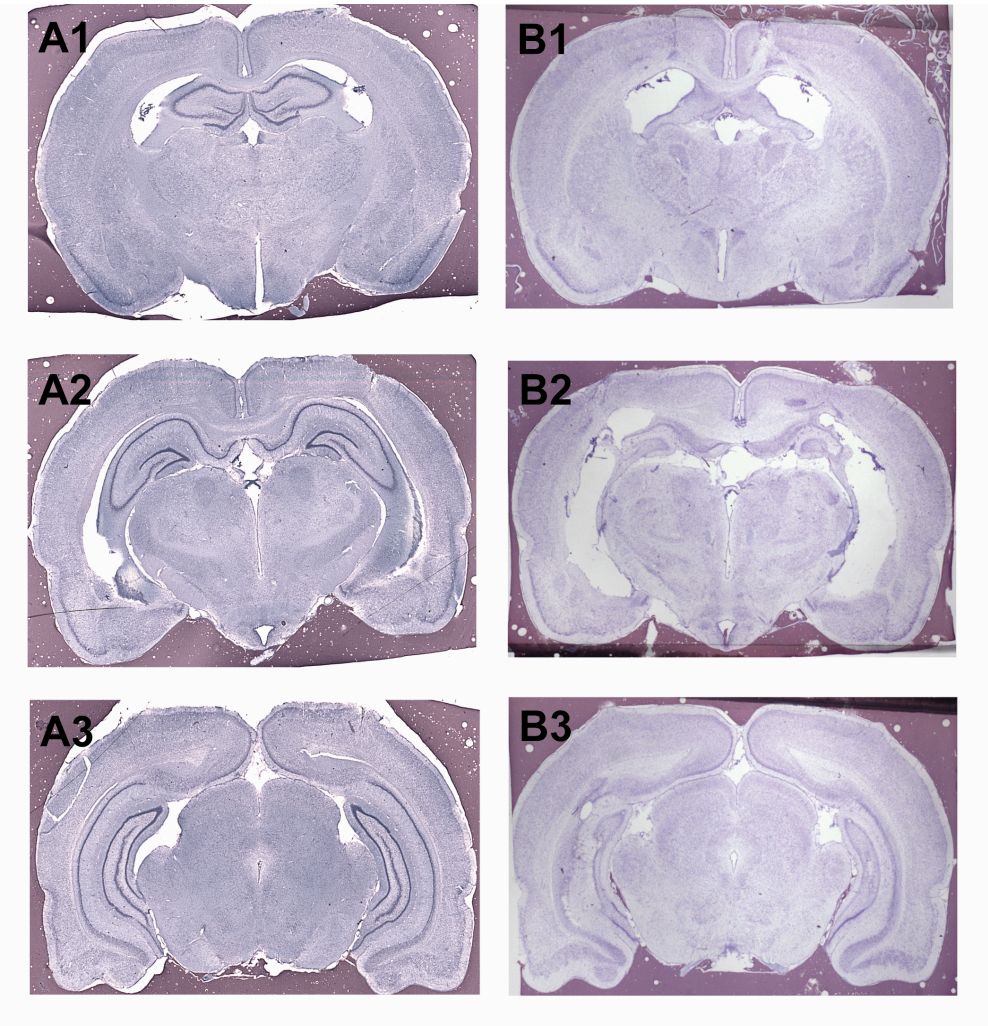

Figure S1: (A) Sections from a typical sham operated brain, and (B) a large complete hippocampus lesion at 3 sites posterior to Bregma: (1) -3.0mm; (2) -4.8mm; (3) -6.0mm. Both brains are from rats that underwent surgery alongside the experimental rats of Experiment 2, but were sacrificed after 14 days. This interval was chosen as the same critical time point as the experimental rats were undergoing postoperative probe tests.

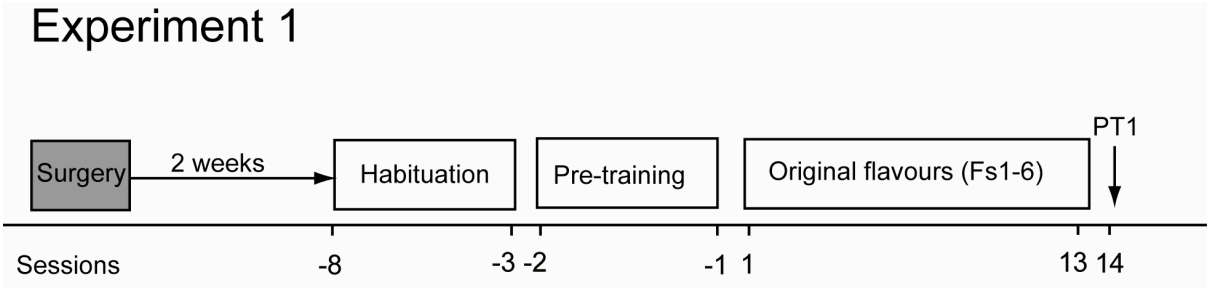

Figure S2: Timeline showing the design of Experiment 1.

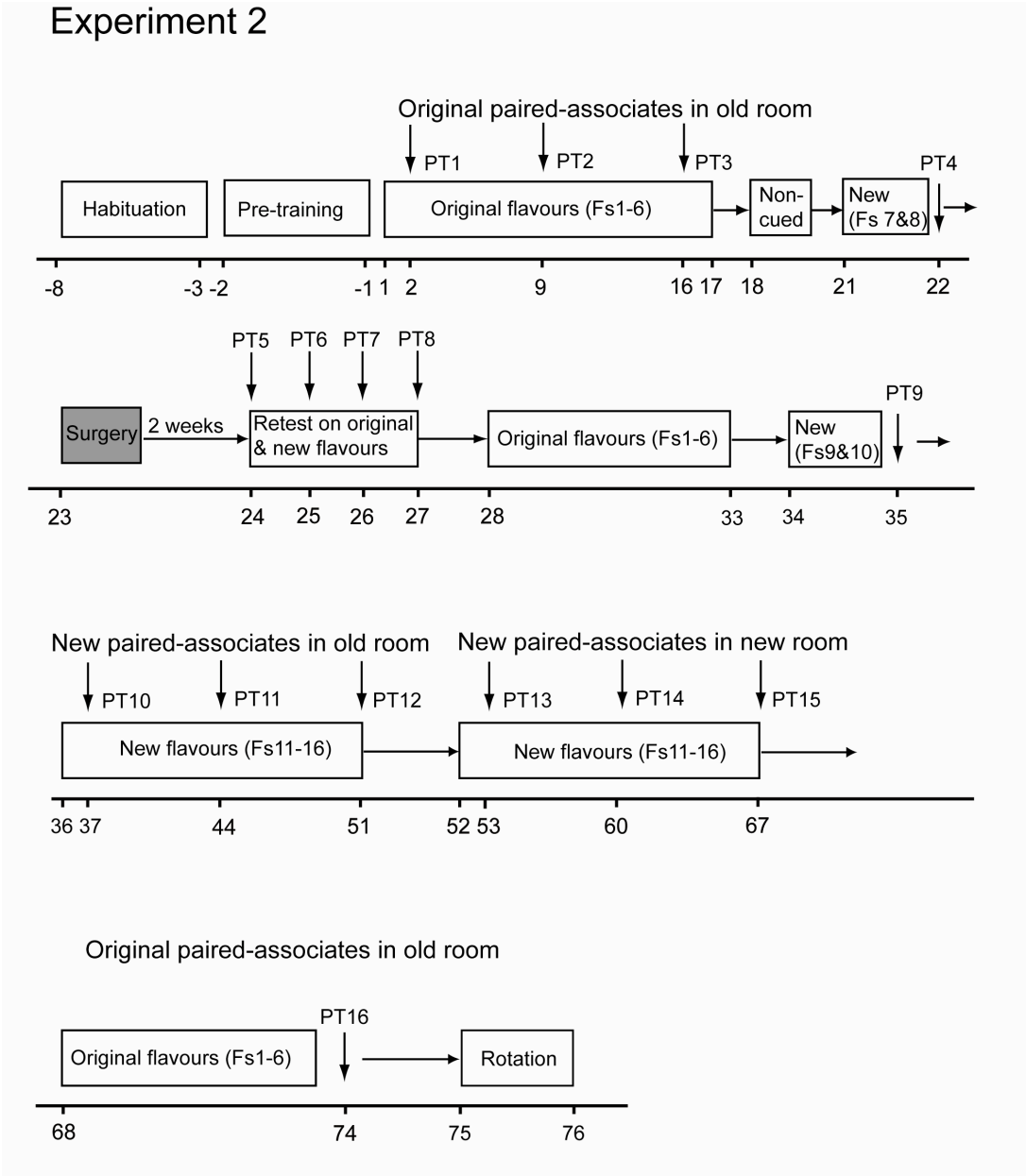

Figure S3: Timeline showing the design of Experiment 2.

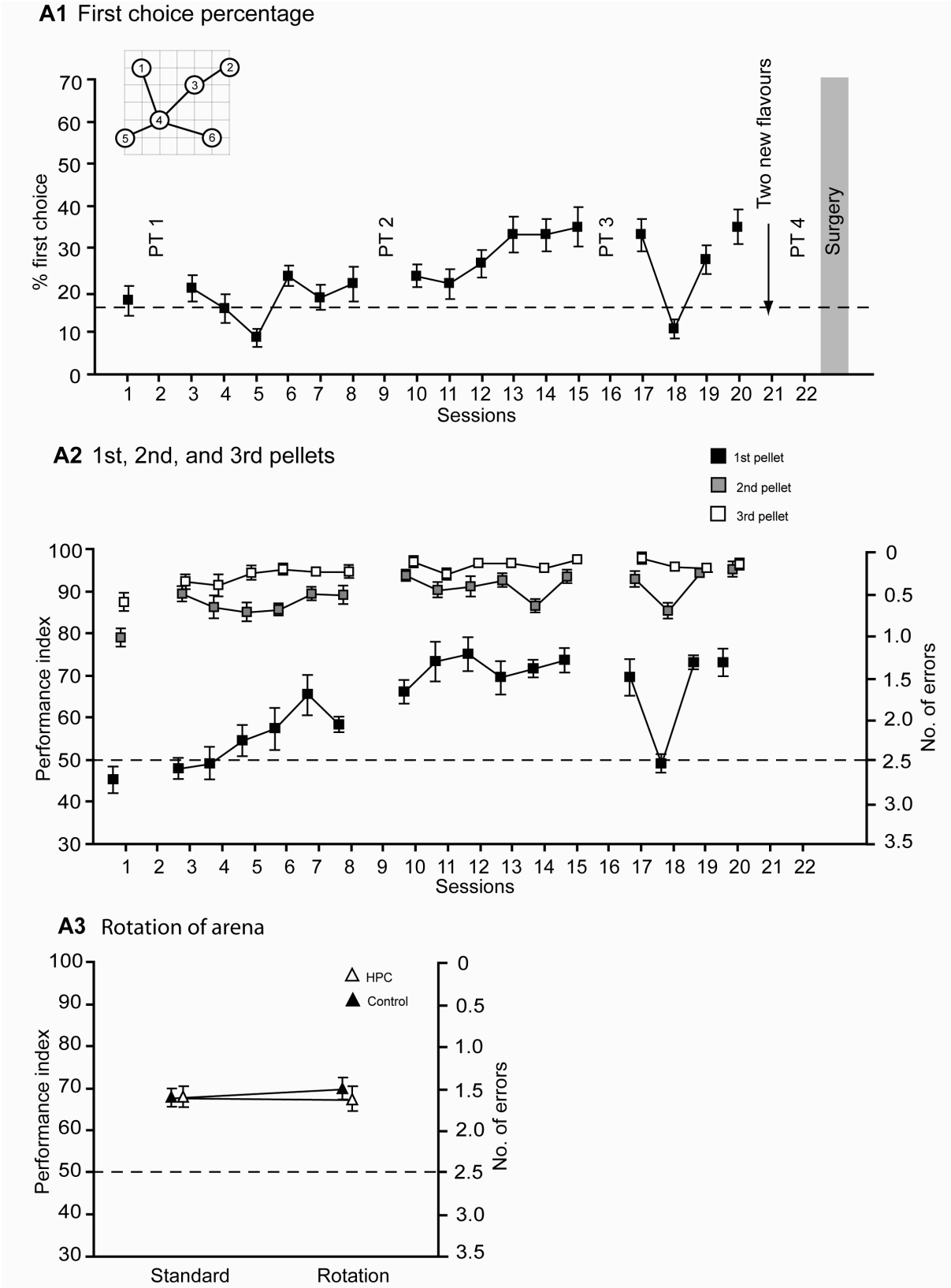

Figure S4: Additional behavioral measures analysed in Experiment 2. (---- = chance level). A1 & A2: n = 18 intact rats, A3: HPC n = 10, Control n = 8.

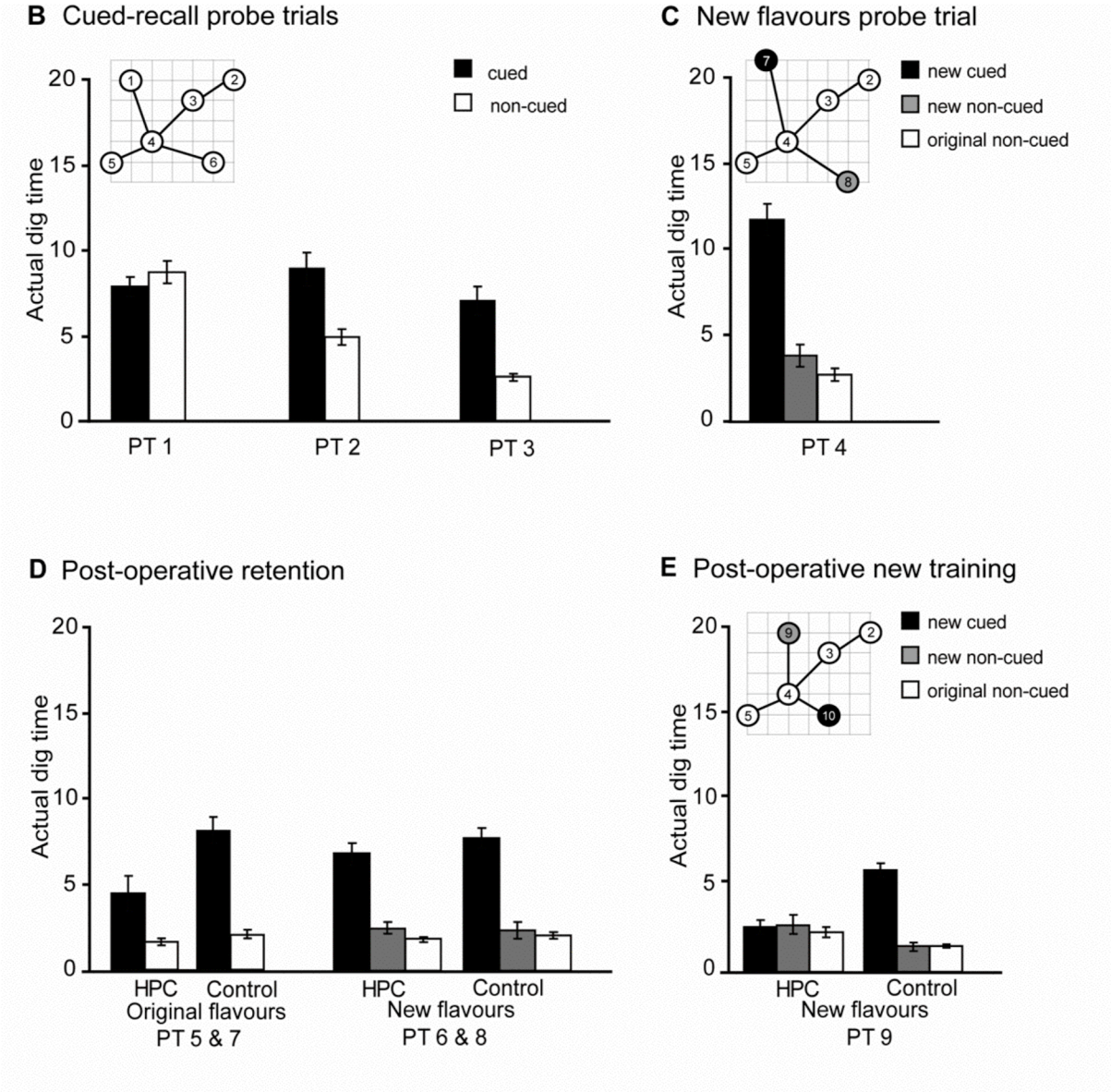

Figure S5: Absolute dig time data. B & C show pre-operative performance (n = 18 intact rats). D & E show absolute dig time data for comparison between groups (HPC: n = 10; Sham: n = 8).

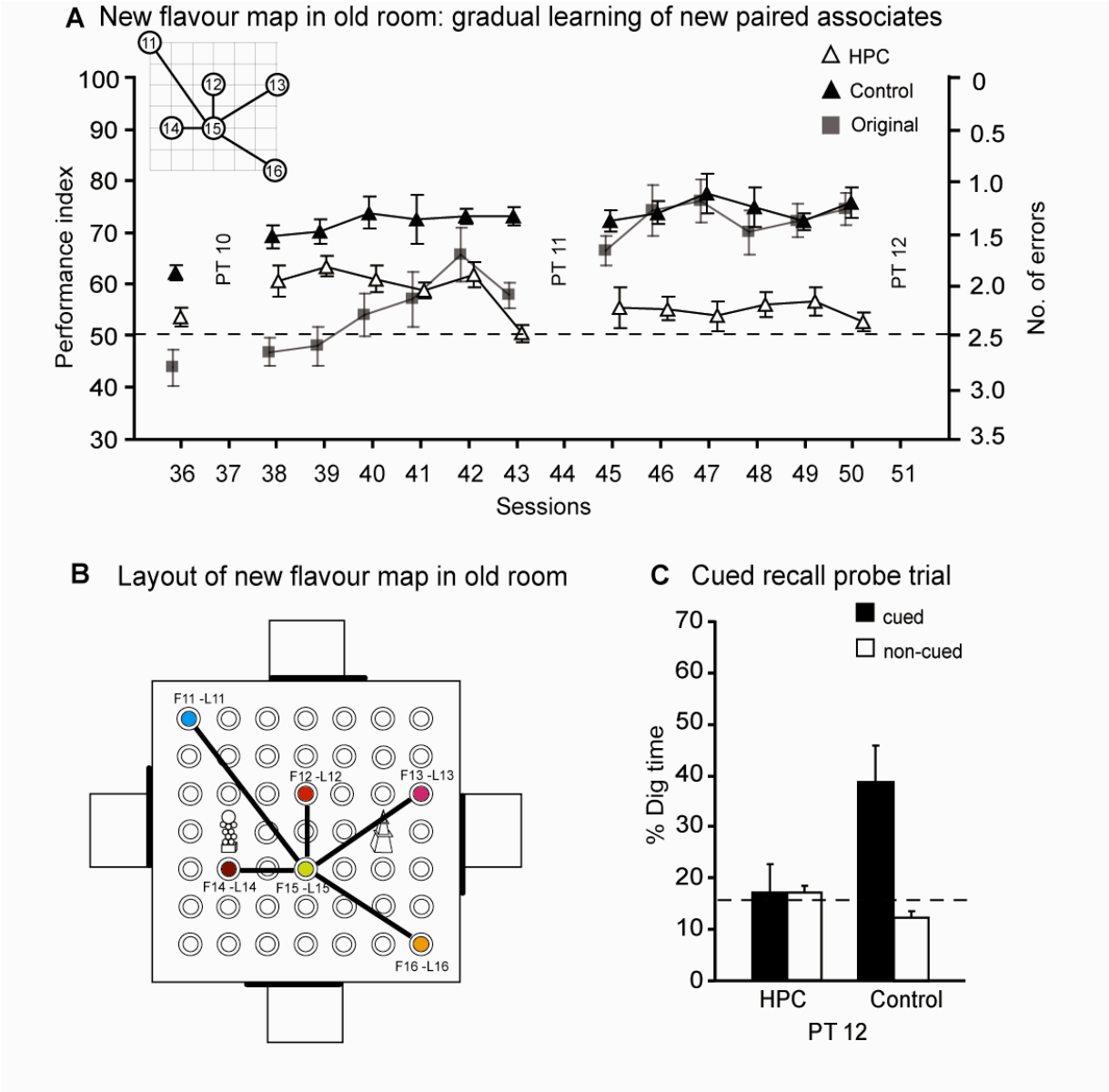

Figure S6: A new geometric arrangement of 6 new flavor location pairs was introduced in the same arena. In Panel A, Original n = 18 (intact rats' pre-operative performance); HPC n = 10; Control n = 8 (post-operative performance of the same rats).

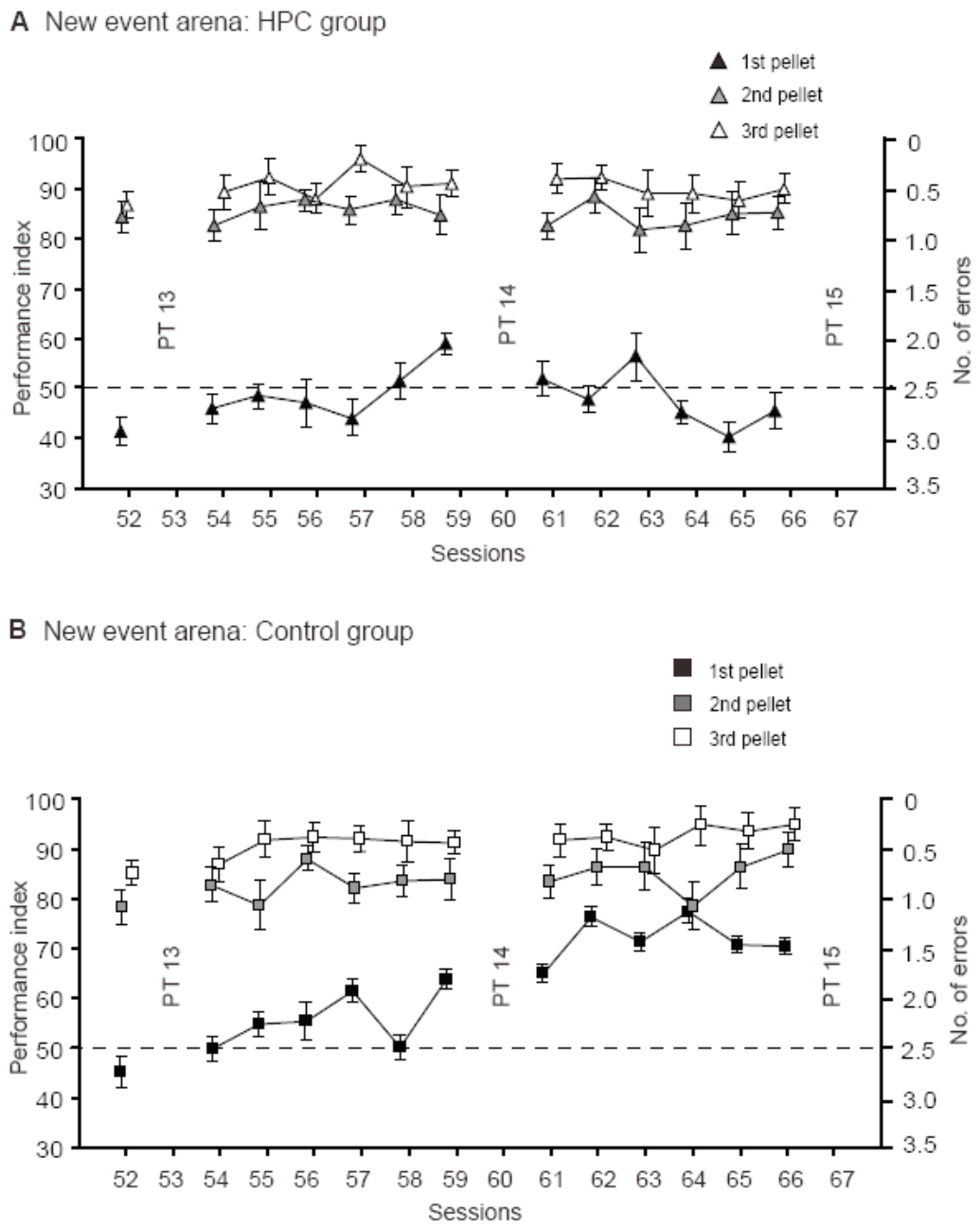

Figure S7: Accuracy of retrieval of the 1st, 2nd and 3rd pellets in Experiment 2 during postoperative learning of a new set of paired associates in a new room. (---- = chance level). A: HPC n = 10, B: Control n = 8.

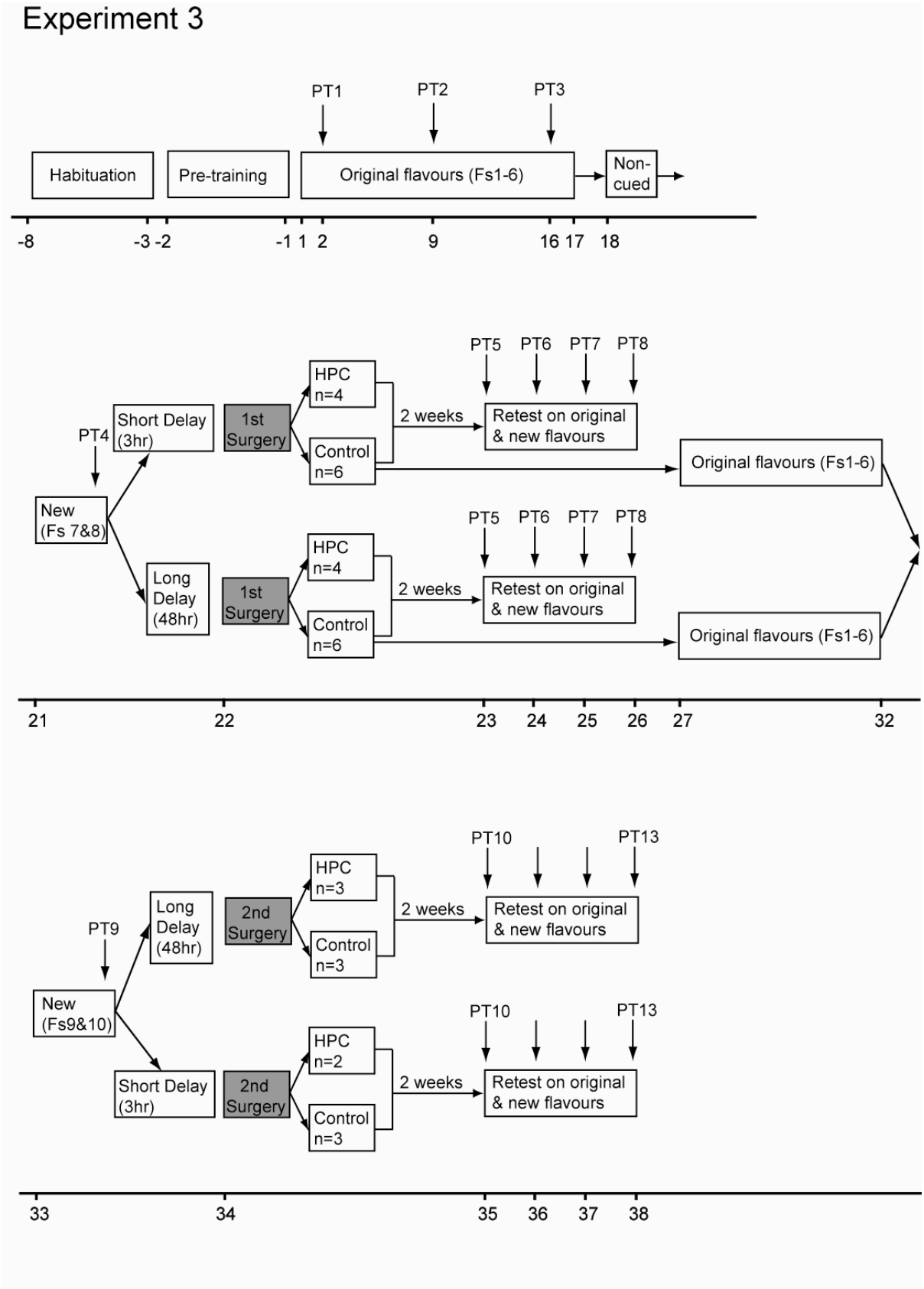

Figure S8: Within-subjects design for Experiment 3.

6. References

S1. Day, M., Langston, R. & Morris, R.G.M. (2003). Glutamate-receptor-mediated encoding and retrieval of paired-associate learning. *Nature* 424: 205-209.

S2. Bast, T., da Silva, B.M. & Morris, R.G.M. (2005). Distinct contributions of hippocampal NMDA and AMPA receptors to encoding and retrieval of one-trial place memory. *J. Neurosci.* 25:5845-5856.

S3. Jarrard, L.E. (1989). On the use of ibotenic acid to lesion selectively different components of the hippocampal formation. *J. Neurosci. Meth.*, 29:251–259.

S4. de Hoz, L., Knox, J. & Morris, R.G.M. (2003). Longitudinal axis of the hippocampus: both septal and temporal poles of the hippocampus support watermaze spatial learning depending on the training protocol. *Hippocampus*, 13:587–603.