Adapted Primary Literature for Teaching

Schema-dependent Gene Activation and Memory Encoding in Neocortex

Original research report: Tse, D. et al. (2011). Schema-dependent gene activation and memory encoding in neocortex. Science. 333: 891-895.

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Abstract

This study, from labs in Japan, studied an animal model of the process by which new learning contributes to development of a more general schema. The consolidation of new learning under their conditions occurred rapidly and was associated with rapid up-regulation of "early genes"in the prelimbic cortex. Drug treatments that targeted this area could prevent both new learning and the recall of previously consolidated information. The authors claim that these results shed new light on schema formation and seem to challenge the common view that hippocampal learning is rapid but that processing in the cortex is slow.

Introduction

The authors cite other papers showing that forming lasting memory ("consolidation") requires changes in signaling in junctions ("synapses") among neurons. The early processing of newly encoded information occurs in the medial temporal lobe of the cerebral cortex where it interacts in a time-limited way with widespread neocortical areas to achieve consolidation (papers cited). Other cited studies showed that early-learning encoding in the hippocampus could be followed by a variety of changes in several areas of cortex. These changes included early gene activation, glucose use, and dendritic spine formation.

Authors cite two papers suggesting that early learning produces near-simultaneous encoding or "tagging" in both the hippocampus and cortex. They cite two other papers suggesting that when new learning occurs in the presence of relevant prior knowledge, the "assimilation" of the new association occurs very rapidly in the cortex. That is, prior knowledge may increase speed of consolidation. Other papers are cited to indicate that associated new and old information may not only involve simultaneous synapse changes but also simultaneous encoding in hippocampus and cortex. The issue is whether consolidation is driven "bottom-up" from the hippocampus or whether it reflects the influence of activated prior knowledge already stored in the cortex.

Methods

The study used young adult rats housed in groups, except housed separately in Study 2 because those rats had indwelling drug-infusion cannuale. They were tested in a square-shaped open field box in which certain "events" happened in specific locations and rats were to learn which event occurred in a given location (Fig. 1).

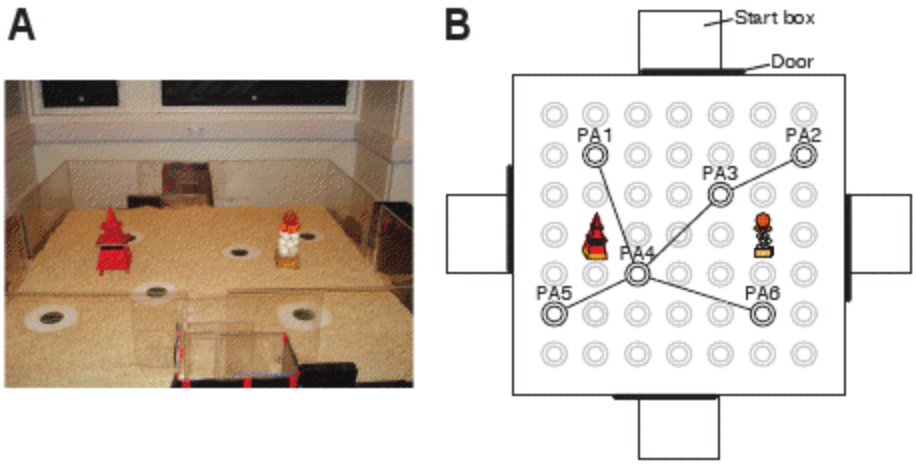

Figure 1. Photograph of the event arena (left) and diagram of the location of the six paired associations in the standard schema.

The event arena was placed in a lab room that had many prominent and distinctive cues outside of the arena that the rats could use to help them orient to locations (other studies had shown that rats in an uncovered box use objects they can see outside the box on walls and ceiling as location cues). In Study 1, 21 rats were trained in 17 paired-associates sessions and a final "critical" training session. Seven rats in a control group were handled gently for the first 3-4 days and brought from the animal house to the control room for every session along with the trained group.

On the day before the study began, rats were trained to dig for food in sand-wells in their home cages.[1] The next day, "habituation day 1," the rats were put into the event arena which had no sand wells for 10 minutes to explore the arena and cues within (and outside) the arena. They entered the arena via a start box where they first ate a control 0.5 gram food pellet. On habituation days 2-6, a similar test was done, with a rat being placed in a different start box each day and a food-bearing sand well in the center of the arena. On habituation day 2, a food pellet was placed on top of the sand well. On day 3, the sand well had a pellet on top and another buried half way. Day 4 was similar, except that two pellets were buried. On day 5, one pellet was buried mid-way in the well and two buried at the bottom. On day 6, three pellets were buried at the well bottom. At the end of habituation day 6, all rats had learned to run quickly to the sand well and dig up and eat the food pellets.

Prior to the main study, rats were trained to associate a flavor of pellet with its location. Six such pairings (Paired Associates, PA) were taught (for example, strawberry and place 1, banana at place 2, …. and bacon at place 6). On any given trial,[2] all six sand-wells were accessible, but only one contained the appropriately flavored pellet reward. The other five wells had only sand and a masking mixture of flavors. The location of the rewarded sand-well changed from trial to trial. A training trial began with presentation of a flavored pellet in the start box, which served as the cue. After eating this pellet, the door opened and the rat entered the arena and searched among the sand-wells until finding the location of the one that contained the three pellets of the same flavor. Rats took the pellets, one at a time, back to the start box to eat. After retrieving the third pellet, the door was closed and the rat taken back to its home cage after eating the pellet.

[1] In studies involving food reward, animals are intentionally given less of their regular diet so they will be motivated to seek food rewards in the testing environment.

[2] A "session" consisted of multiple "trials," each with a different PA.

Each rat was trained on the different PAs across six trails within a session, and the order of pairings was counterbalanced across rats and sessions. Start boxes were pseudo-randomly assigned.

Measurement during each trials involved recording the number of sand-well errors made before the rat located the correct one. To count as an error, a rat had to place one or more paws on or into an incorrect well. During each trial, errors were counted and the time noted from when the rat left the start box and when it reached the correct sand-well. . Rats were monitored remotely by video camera.

To further test for memory development, three non-rewarded probe trials were conducted on Sessions 2, 9, and 16. During these tests rats could dig in any of the six sand-wells though all lacked food. Rats were cued with a flavor as usual and then allowed to roam in the arena for 120 seconds. So as not to extinguish any learning, rats were given the correct-flavor pellets at the correct location at the end of each probe test.

The "critical training" session occurred at the end when rats had learned the six PAs. The rats were divided into three groups, one group (OPA) was based on the original PA learning, a second based on two new (NPA) and four original PAs, and a new map group (NM) which had 6 new PAs. This critical training consisted of six trials, each with a different PA, followed by a cued-recall test after a 80-minute delay. For the first 4 trials, both Groups OPA and NPA were presented with original PAs 2-5 and the Group NM was presented with new PAs 9-12. The inter-trial interval was 30 min. To map the early gene expression induced on Trials 5 and 6 of the training without major influence from Trials 1-4, all rats were placed in the home cage for 180 min after Trial 4, and they were sacrificed 90 min after Trial 5. This schedule was used because published research by others showed that the early-gene mediated expression of proteins examined in this study becomes elevated in cortex between 30 min and 2 hours after a novel learning event.

On Trials 5 and 6, Group OPA was presented with original PAs 1 and 6. The Groups NPA and NM were presented with new PAs 7 and 8. The inter-trial interval was 5 min. The animals were then placed in their home cages for 80 min until the following cued-recall test. The performance index and latency to dig at the correct sand-wells on Trials 1-4 and Trials 5-6 in the critical session are shown in Fig. 1B and Fig. S3 respectively. Eighty minutes after Trial 6, the cued-recall probe test was performed to see if the rats remembered the PAs with which they had been presented on Trial 5.

Immunohistochemistry. Ninety minutes after the training of Trial 5 in the critical training session, rats were deeply anesthetized with sodium pentobarbital and their brains perfused with buffered saline, followed by formaldehyde. Brains were removed from the skull and set overnight in formaldehyde, then transferred to 50-ml tubes containing 30% sucrose in buffered saline and kept at 4^{o}C until they sank to the bottom. Coronal brain sections (40 μm) were serially cut using a freezing sliding microtome and divided into 6 interleaved sets. Each of these sets included sections at 240 μm (40 μm × 6) intervals and collected into an antifreeze solution (50% ethylene glycol and 50 % glycerol) and maintained at –20°C for later processing.

Two cohorts of rats were used for immunoperoxidase staining. A single experimental cohort of four rats was processed together, sections from each trained rat in the same container as those of its caged control to minimize the impact of any immunohistochemical variation. All immunohistochemical incubations were done at room temperature in a free-floating state. Sections were first rinsed in buffered saline to remove the antifreeze solution and then treated with 0.3% hydrogen peroxide in saline for 30 minutes to reduce endogenous peroxidase activity. Then after rinsing, sections were incubated with a 1:3000 dilution of rabbit antibody to the early-gene produced protein "Zif268." They were then later incubated with a secondary antibody to complete the staining. (Many staining details omitted here in the interests of simplicity and brevity). A similar staining process was used to detect presence of a second early-gene produced protein, Arc.

Regions of interest were selected to be the hippocampus and specific parts of the cortex, based on cited studies of others reporting them to be involved in memory storage processes. Sensory/motor cortex area was examined as a control area because it is not known to be primarily involved memory consolidation. Histological drawings and slides were presented but not shown here for brevity and simplicity. Likewise, staining patterns were shown but not presented here.

Stained protein analysis. The Zif268 protein is exclusively expressed in neuron nuclei, and stained cells could be counted under low (10x) magnification. The Arc protein is expressed throughout neurons, and therefore high magnification was needed (100X) to distinguish cytoplasmic (nuclear?) staining from stains distributed throughout the many small neuronal dendrites. All counting was done blind (i.e., investigators doing the counting did not know which experimental group the brain slices came from). (Other counting details are omitted here in the interests of brevity.)

For each brain area, the counts of stained neurons for each type of protein for Groups OPA, NPA, and NM were normalized to the average raw count of the members of the control group. For Zif268 protein staining, separate counts were made for superficial versus deep layers of the cortex.

An additional measure of staining of the Zif268 protein used optical density of the hippocampus slices. These values were normalized to the average density of the control slices.

Study 2. Drug Intervention. In these experiments, rats were anesthetized and placed in a "stereotaxic frame," a head holder that allows accurate placement in three dimensions into a specified part of the brain. Skull was surgically exposed, holes were drilled over desired locations, and six small anchoring screws were placed into skull bone. Guide cannulae were implanted through the holes into target brain areas, and dental cement was placed around all screws and cannulae to hold them fast to the skull. Cannulae were temporarily plugged with wires to keep the cannulae open. Local anesthetic was injected around the incision sites after surgery. Rats were given seven days to recuperate and recover their pre-surgery weight. Food was then restricted so the rats would have incentive to learn foot locations in the subsequent training and memory testing.

Drugs selected for testing included blockers for the NMDA receptor and the AMPA/kainite receptor. When activated, these receptors are known to excite the neurons (Details omitted here for brevity). During the experiments, when infusions were performed, the dummy cannula were removed and replace by infusion cannulae that protruded 0.5 mm from the ends of the implanted guide cannulae. Drugs were delivered by a microinfusion pump via flexible polyvinyl chloride tube and a microsyringe at a rate of 0.4 microliter/min over 1 to 2 min, after which the infusion cannulae remained in place another minute and were then replaced with the dummy cannulae.

After experiments, histological staining was used to verify the location of the implanted cannulae tips (details omitted here for brevity).

PA Training and Testing. Each PA was presented for one trial/session in its designated location in the arena. To exclude the possibility that an olfactory cue led rats to the correct associated location, authors included a single non-cued session of six trials where the usual protocol was unchanged but there was no cue flavor in the start box. Once rats had learned the PAs in Study 1, they were then tested for the effect of the two receptor-blocking drugs. Drug tests occurred either 1) on days after asny new learning and shortly before memory retrieval tests, or 2) before new encoding sessions with retrieval tests conducted 24 hours later. Standard training sessions were interpolated between these tests. The experiment used repeated measures on the same subjects.

Results

Study 1. Errors were scored with an index using 100 – [100 x (#of errors/5)]. During training on the six PAs, the performance index was around 50 (chance level) at first but reached a stable level of around 70 with minimal variation from sessions 5-17 (data not shown here). In terms of number of errors, the initial number was about 2.5 errors, while the stable trained level was around1 error.

Cued recall testing showed effective memory for the new Pas in the NPA group but no learning in the NM group (Fig. 2).

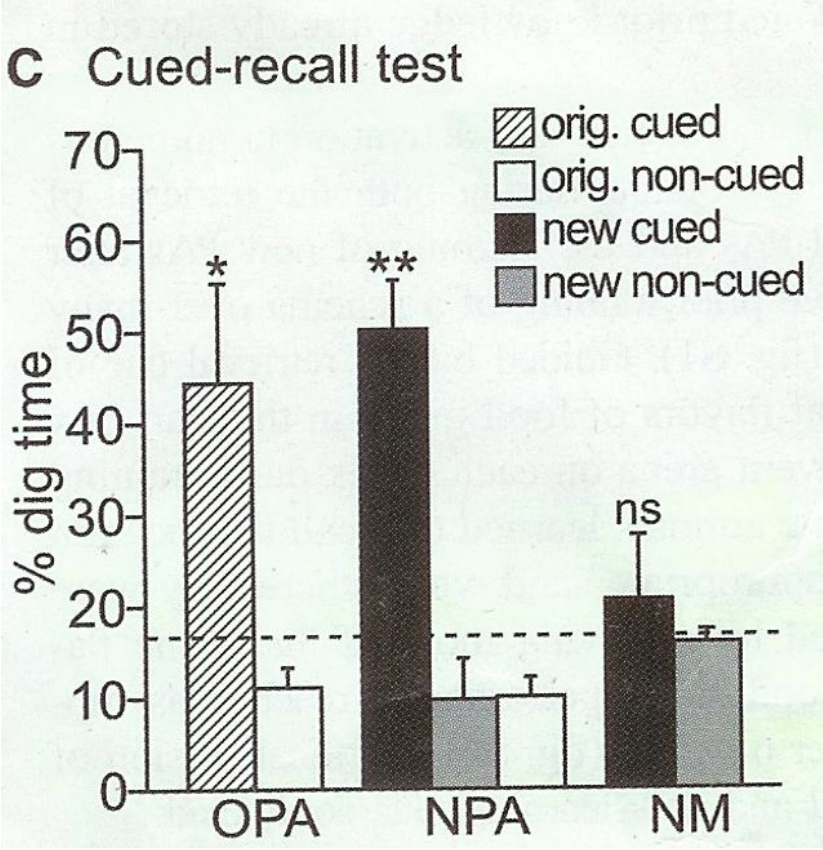

Fig. 2. Rats in the NPA (two new and four old Pas) group learned in one trial newly cued PAs, but group NM (all new Pas) did not. Stars over the error bars indicate statistical significance at the 1% level.

The protein staining results (Fig. 3) indicated a learning-induced increase in the prelimbic region (PrL) of the medial prefrontal cortex (mPFC). Gene expression was highest in the NPA rats for whom activated prior knowledge was relevant to the new information.

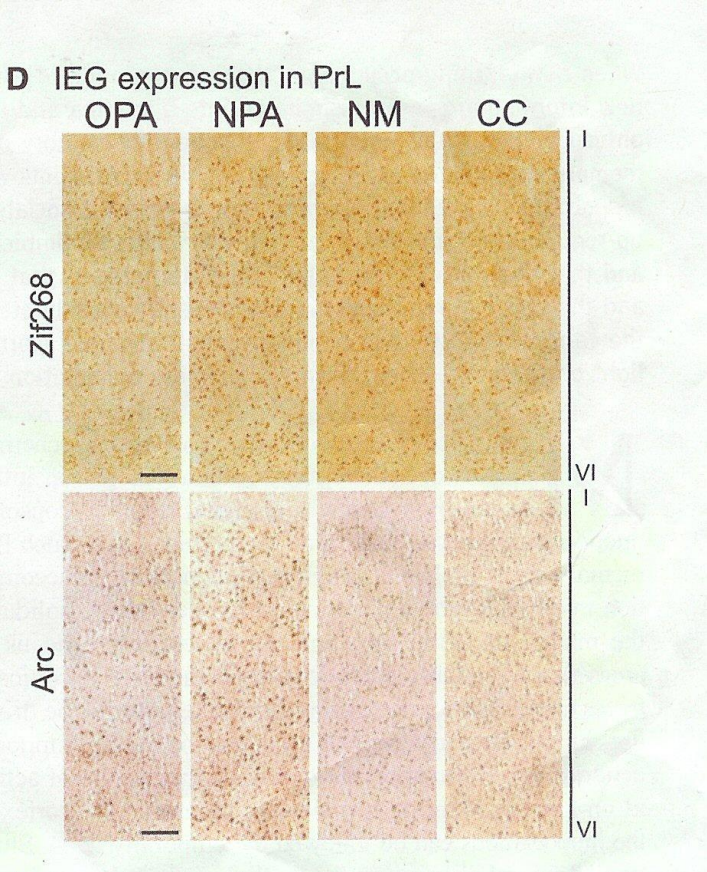

Fig. 3. Histological staining maps of early-gene activated proteins. Early gene expression in prelimbic cortex, as indicated by staining of two relevant proteins, in the various test groups. For both proteins, the greatest expression occurred in NPA rats. This impression was confirmed by actual counts which were statistically significant at the 1% level (data not shown here).

For other brain areas, three patterns of response were evident. One was that seen with prelimbic cortex [and retrosplenial (RSc) and anterior cingulate cortex (ACC)} in which the greatest expression occurred in NPA rats even though NM rats had more new learning exposure (Fig. 2A). Non-memory influences, such as motivation, were ruled out by lack of difference in latency to approach sand-wells (data not shown here).

A second category of response occurred in the hippocampus, where a large increase in Arc protein expression occurred in both NPA and NM groups.

A third category of cortical areas revealed no difference in staining of either protein in any group (data not shown here).

Study 2. New animals with brain injection cannulae were trained in the same protocol over six weeks and then tested for the impact of the receptor-blocking drugs. PA performance was stable over six months of training and testing, leading to the conclusion that the memory schema was fully consolidated in the cortex.

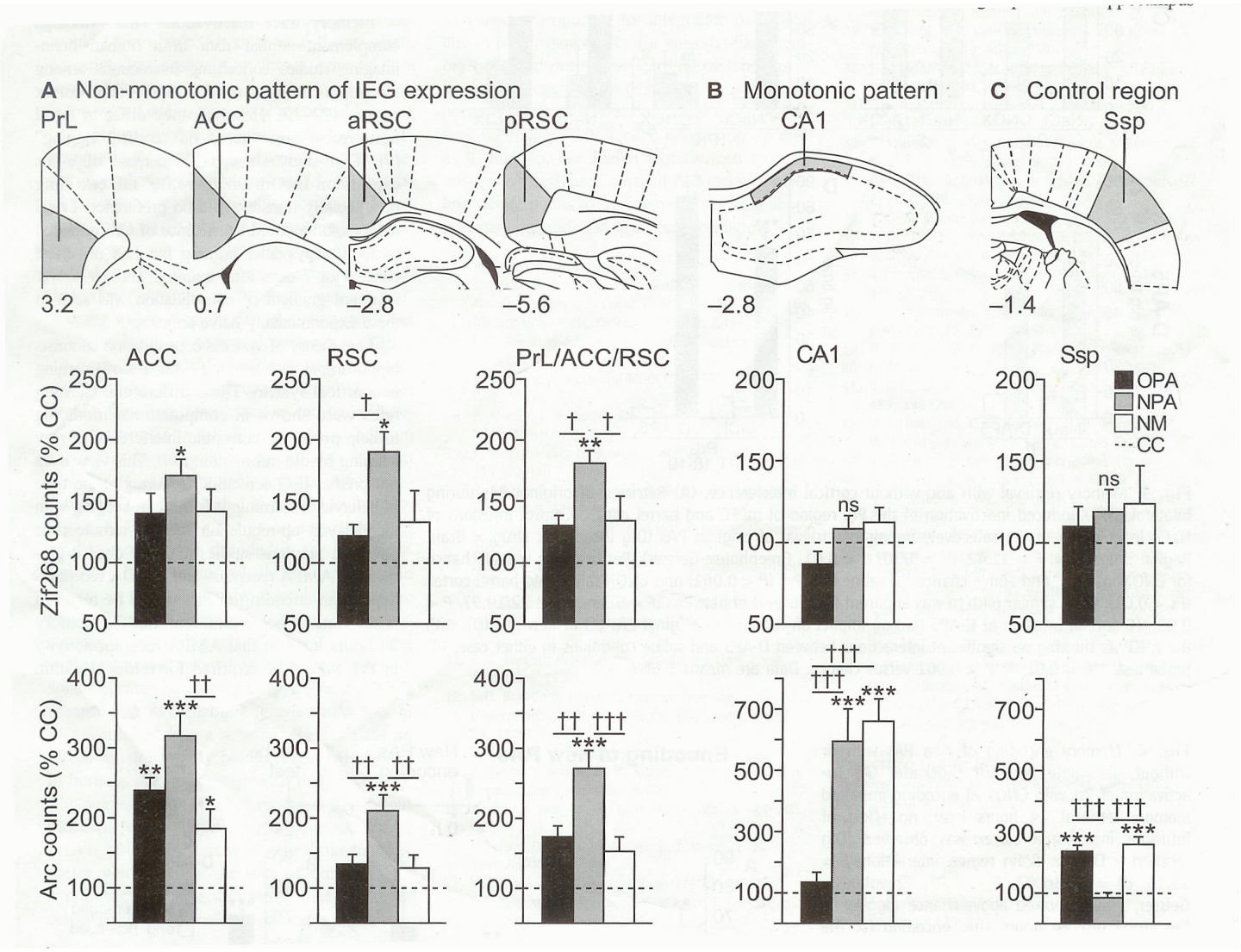

Fig. 4. A Staining of early-gene expressed proteins in various areas of cortex. Greatest expression occurred in the NPA rats. *=5% versus the control group, **=1%, and ***=0.1%. † = 5%, 1%, and 0.1% versus trained group.

B - Arc protein staining, but not Zif268, was much greater in the CA1 region of hippocampus in both NPA and NM (***= 0.1% significance level).

Blocking the receptors in prelimbic cortical areas interfered with memory consolidation of new PA learning (data not shown here).

Discussion

The authors interpreted the results to support the notion that pre-existing knowledge about PAs promoted rapid encoding and assimilation into neocortical brain areas. Since this kind of learning task is known to require the hippocampus for consolidation, the two brain regions operate together as a simultaneously acting system. In the NPA group, which had four old and two new PAs), early gene activation was immediate in the prelimbic cortical areas (ACC and RSC). This result, coupled with lack of early gene activation in the NM group, suggested that a gene activation response to new

learning was enhanced by pre-existing memory of prior learning. The NM group by definition had no pre-existing learning "schema." Thus these rats were unable to incorporate their all new PAs under these conditions. Yet, the NM group did show higher Arc expression in the hippocampus, indicating this structure's attempt at processing the new learning.

Temporary disruption of the excitatory neurotransmitter receptors during new PA learning did prevent memory formation, as expected. Thus, Arc expression is assumed to be necessary for memory consolidation, even as the authors now claim that both hippocampal and cortical areas participate as a cooperative consolidation system.

These results call into question the old idea that fast learning occurs via the hippocampus and that the cortex achieves learning slowly. This view would not predict early gene activation in the cortex at the time of learning. Nor does it predict that the receptor systems in the cortex be active at the time of learning.

A second idea, called multiple-trace theory, supposes that a new learning event encodes in parallel as separate traces in both hippocampus and cortex. This idea is consistent with present results, except that the authors cite their earlier work showing that the hippocampus is needed for future learning of PAs. The point is made that both regions of brain work together simultaneously in memory consolidation. Whether or not early genes are activated depends on the relevance of new information to stored information (that is, the pre-existing schema).

Thus the data suggest both bottom-up and top-down processes operating simultaneously to consolidate new learning into existing schema. The idea is extended to make the point that recall of stored information would not occur if the cortex receptors were blocked. The hippocampus alone could not support recall.

The authors conclude that this new view of consolidation should generate new experiments in which brain electrical activity is monitored in the hippocampus and cortical areas. Of special interest would be to monitor oscillatory activity in activated cortical networks of prior knowledge.