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The neurobiology of learning and memory: some reminders to remember

Larry Cahill, James L. McGaugh and Norman M. Weinberger

We have learned much about the neurobiology of learning and memory in the past 100 years. We have also learned much about how we should, and should not, investigate these complex processes. However, with the rapid recent growth in the field and the influx of investigators not familiar with this past, these crucial lessons too often fail to guide the research of today. Here we highlight some major lessons gleaned from this wealth of experience. These include the need to carefully attend to the learning/performance distinction, to rely equally on synthetic as well as reductionistic thinking, and to avoid the seduction of simplicity. Examples in which the lessons of history are, and are not, educating current research are also given.

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'Those who cannot remember the past are condemned to repeat it.' George Santayana 1863–1952

Not so long ago, all major investigators studying the neurobiology of learning and memory could easily meet in one small room. Today that would be impossible, as interest in the field has grown rapidly in recent years and is now the focus of investigators from many areas of neuroscience. However, because of this, researchers from different areas often know little about the most important issues in other closely related areas. For example, 'cognitive neuroscientists' might know nothing about LTP (long-term potentiation) or CREB (cAMP-response element binding protein), and 'molecular neuroscientists' might know nothing about the HERA (Hemispheric Encoding/Retrieval Asymmetry) model or ecphory (the interaction between a stored trace and retrieval conditions that creates the recollective experience).

All of us, however, face the same essential problems in relating brain to memory; the problems faced by everyone from Hull[1] to Hebb[2], Köhler[3] to Konorski[4]. To help ensure that our field moves forward rather than in circles, there are some key lessons that have been learned from more than a century of investigation into brain and memory that are worth remembering.

Learning and memory cannot be simply assayed

No one has ever measured learning or memory. They can be only inferred from behavior. Therefore, in order to know what an animal (including a human) has learned, we must ask it carefully. For example, Liddell[5] (a student of Pavlov) wanted to know what a sheep learned when it was trained to lift its leg off a shock pad upon hearing a tone. Had it learned a simple stimulus–response association: hear tone, lift leg? To find out, Liddell turned the sheep upside down, putting its head on the pad. When the tone was sounded, the sheep moved its head, not its leg. Understanding what the sheep had learned (a predictive relationship between two stimuli – a tone and a shock) required asking the sheep the appropriate question. Even a single Pavlovian conditioning trial creates learning that can be detected by careful behavioral testing. For example, after only a single pairing of two stimuli (e.g. a light followed by a shock) rats can learn not only that the light predicted shock, they can also learn about the length of temporal interval between the two stimuli even with an interval of over 50 seconds[6]. Because it involves simultaneous

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learning of many associations, even 'simple' Pavlovian conditioning is not simple at all[7].

David Krech[8] eloquently observed that 'memory is a big and rich and awesome phenomenon,' and it must be investigated as such. For example, all experiences involve multiple stimuli, which can enter memory not only in association with each other, but also as catalysts, either potentiating or interfering with the associativity of other stimuli. Unfortunately, the complexity of memory is often not recognized and many studies of learning and memory restrict their behavioral analyses to training and testing techniques that are currently in vogue. But memory is not easily examined by off-the-shelf assays. Placing a rat or mouse in a water maze and starting a timer might be the beginning of an assessment of memory, but it is no more than that. Finding out what the animal learns and remembers about the water maze training is not a simple matter. But it is certainly a crucial matter. For studies of brain and memory, naiveté about either the neurobiological or behavioral variables will produce flawed inferences. Krech made the point forcefully 30 years ago in discussing experiments combining behavioral and neurobiological variables: 'Anyone who is to do a good study involving these two sets of variables had damn well better be sophisticated, and careful, and disciplined, and knowledgeable and thorough in both sets of variables.'[8] The lesson is no less true today.

We ignore the learning/performance distinction at our peril

Behavior is affected by many processes other than learning and memory. These other processes must be excluded before we can draw conclusions about memory. Edward Tolman[9] first emphasized this 'learning/performance' distinction in his seminal studies of 'latent learning'. A satiated rat allowed to freely explore a maze containing food in the goal box might display no evidence of learning about the maze. However, when subsequently made hungry and returned to the maze, the rat will demonstrate that it had learned the pathway leading to the food. The satiated rat that appeared to have learned nothing had in fact learned a great deal, but this 'latent' learning was obscured by a performance factor: a lack of hunger motivation.

Other performance factors include those affecting motor function, attention, sensory receptor sensitivity, motivation and general arousal level. All must be controlled as fully as possible when studying learning and memory as it is easy to draw false conclusions when influences of performance factors are not taken into account. For example, investigators initially thought that a 'knockout' genetic lesion impaired water maze learning in mice[10], until careful behavioral testing showed that the mice could not swim properly[11]. Similarly, rats with amygdala lesions might appear to have little memory of Pavlovian 'fear conditioning', as indexed by their freezing behavior. But, because amygdala lesions also impair unconditioned freezing behavior, such findings do not provide evidence that the amygdala lesions impair memory of the conditioning. Rats with amygdala lesions will show that they can acquire and remember 'fear conditioning' if they are allowed to avoid the place where the conditioning occurred[12,13], or to learn to escape from the conditioned stimulus[14]. Clearly, careful attention to the learning/performance distinction is absolutely essential for studies of brain and memory.

We can not get to the top of the mountain by only working our way down

The great learning theorist Jerzy Konorski[4] distinguished two complementary approaches to understanding brain and memory: the analytical and the synthetic. The analytical (or reductionistic) approach seeks to explain phenomena by breaking them down into component pieces. The synthetic approach seeks to integrate pieces into a cohesive and comprehensive whole. Neither approach is 'the correct one' as each has limitations as well as advantages. Rather, they are complementary, and both are essential for understanding how the brain creates learning and memory.

The problem occurs when neuroscientists assume the superiority of one approach over the other. The field of brain and memory, traditionally strongly tilted towards reductionism, appears increasingly so in recent years as a result of the excitement generated by cellular and molecular neuroscience. Not surprisingly, the reductionistic bias in our field produces large numbers of investigators who equate, perhaps implicitly, the word 'mechanism' with 'cellular and/or molecular mechanism'. The power of reductionistic approaches to answer many questions can create the false impression for many that they will answer all the questions. For others, however, the limitations of purely reductionistic approaches are becoming clearer. For example, Marc Vidal[15] recently noted that 'forced into a reductionistic approach to molecular biology, mostly by technical limitations, we have trained ourselves to think and design experiments on a one-gene-one-protein-at-a-time basis' and that 'more integrative approaches will be needed.'

The influential geneticist Seymour Benzer, no doubt speaking for many with similarly reductionistic predispositions, observed that 'it takes a long time of going down before you start looking to go up again. Down is a much easier way to go.'[16] And up we must go if we want to explain brain and memory, because memory is revealed only at the behavioral level. Synthetic thinking is not, of course, limited to the behavioral level (the double helix and the periodic table of the elements are two masterpieces of synthetic thinking), but it does demand thoughtful integration of other levels with the behavioral level in order to create insights into the neurobiology of memory. From a purely reductionistic, molecular-level perspective, a gene acting as a universal 'consolidation switch' for memory might seem plausible, perhaps even probable.

However, from a synthetic, behavioral-level perspective, consolidation appears highly multidimensional, involving many brain systems and time-courses. Thus, from this perspective a genetic 'consolidation switch' seems no more probable than a gene for perception or emotion. The complex processes of learning and memory are not likely to be accounted for by any single gene or molecule. Because it is easier to go down, we must constantly remind ourselves that we must also climb up.

Simplicity is seductive, and often wrong

Unfortunately there is a long history in our field of overly simplistic ideas that influence research primarily because of their simplistic appeal. The idea that all animal behavior could be explained by stimulus–response connections formed by reinforcements (e.g. rewards) (Hull) was one such highly influential idea during the first half of the last century. It was abandoned and is now rarely cited because the experimental evidence did not support it. Rewards are not necessary for learning (e.g. latent learning). Learning does not typically consist of specific responses made to specific stimuli. We and the other animals learn and remember events and places and can express our memories, even of responses we have learned, in many ways. We can, if we wish, write our names (a highly practised response) with the toe of our left foot in a sandy beach. Such 'response transfer' evidence makes any simple stimulus–response theory doomed at the outset.

Occasionally the seduction of simplicity embarrasses the field, as happened with the now discredited 'memory transfer' experiments conducted several decades ago (e.g. see Setlow[17]), in which investigators injected extracts from trained animals into untrained animals to determine whether hypothesized 'memory molecules' extracted from the trained animals would transfer the memory to the untrained animals. We should be careful not to laugh in retrospect at such ideas if we remain attracted to other more contemporary simple explanations of the complex phenomena of learning and memory.

Sir Charles Sherrington, awarded the Nobel Prize for his work on spinal reflexes, described the 'reflex arc' as a 'convenient fiction.'[18] Indeed, even 'simple' spinal cord reflexes are highly intricate and multidimensional (see Carew[19]). If something as 'simple' as a rat limb flexion reflex is not simple then the neural processes underlying learning and memory are most certainly not simple.

Santayana was right, so we must study the field's history

The likelihood of oversimplification seems inversely related to the degree of historical perspective. Consider our field's love affair with the 'Hebb synapse.' As important as it might be, Donald Hebb knew that the concept was a minor player in his theories. (Regarding his famous 1949 book he once wryly noted, 'I know what a classic is – it's something cited and not read!'[20].) Yet today many view the 'Hebb synapse' and memory as isomorphic. Hebb surely did not. For Hebb, the synapse enabled memory processes based on the interactions of large assemblies of cells. Oversimplification of Hebb's theories certainly has not promoted progress.

Consider also the current revival of interest in hypothetical memory 'reconsolidation' processes – the hypothesis that reactivation of an old memory initiates reconsolidation of the memory, thus making it susceptible to amnestic treatments. Earlier studies of 'reconsolidation' clearly showed that memory disruption produced with treatments given during 'reconsolidation' periods is not only unreliable across laboratories and experimental paradigms[21–25], but is unlikely to be true amnesia as the supposedly lost memories show spontaneous recovery[26–28]. A crucial test of 'reconsolidation' by Squire and colleagues[22] in which human subjects recalled previously learned information just before they received an electroconvulsive therapy (ECT) treatment provided no support for the 'reconsolidation' hypothesis. The information recalled just prior to the ECT treatment was not forgotten. Each of these facts remains crucial to current debate on 'reconsolidation,' yet none was discussed in a series of recent commentaries on the topic in an influential review journal[29]. Is history primed to repeat itself with the 'reconsolidation' issue?

Fortunately, the lessons of history are guiding many other investigations today. For example, post-training, reversible manipulations, first introduced over four decades ago[30] to dissociate learning and performance mechanisms, as Tolman taught us to do, remain a potent tool today, and in fact are enjoying renewed interest (see for example Riedel *et al.*[31]). Careful attention to both the neurobiological and behavioral levels, as Krech admonished us to do, continues to yield new insights. For example, Sutton and colleagues[32] recently uncovered a surprising 'dip' in retention of sensitization in *Aplysia* several hours after training, at precisely the time predicted by their neurobiological model of the learning. Finally, the active synthesis of research involving experimental animals and humans, facilitated by brain imaging methods, is yielding powerfully convergent evidence and greater cross-fertilization of research in each domain[33].

Progress is being made in understanding how the brain makes and preserves memory. But progress comes only when we draw proper inferences from behavior, when we carefully integrate different levels of analysis, and when we view our findings in proper historical perspective. After all, a field that forgets the lessons of its past is a field with a serious memory disorder.

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Ischemic injury and faulty gene transcripts in the brain

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The brain has the highest metabolic rate of all organs and depends predominantly on oxidative metabolism as a source of energy. Oxidative metabolism generates reactive oxygen species, which can damage all cellular components, including protein, lipids and nucleic acids. The processes of DNA repair normally remove spontaneous gene damage with few errors. However, cerebral ischemia followed by reperfusion leads to elevated oxidative stress and damage to genes in brain tissue despite a functional mechanism of DNA repair. These critical events occur at the same time as the expression of immediate early genes, the products of which *trans*-activate late effector genes that are important for sustaining neuronal viability. These findings open the possibility of applying genetic tools to identify molecular mechanisms of gene repair and to derive new therapies for stroke and brain injury.

Brain injury of the ischemia–reperfusion type, which occurs in stroke and cardiac arrest, induces neuronal damage. The mechanisms of neuronal injury include decreased intracellular pH and ATP concentration and increased levels of extracellular glutamate, intracellular Ca^{2+} and reactive oxygen species (ROS). ROS are generated as byproducts of oxygen metabolism. Under physiological conditions, most ROS are generated in mitochondria, but pathological conditions can cause ROS to form in the cytoplasm as well (Box 1). Mitochondrial DNA is prone to damage[1] and mitochondria have functional repair processes[2]. Because of a short half-life, the hydroxyl radicals generated in the mitochondria under normal conditions might not react with nuclear DNA to a significant extent. In experimental models of ischemia, ROS are produced within 30 minutes of the ischemic episode, predominantly in the penumbral region[3]. Recent studies show that the radical form of nitric oxide and superoxide anion might participate in nuclear gene damage in the brain (Box 1). The content of nitric oxide increases in the CNS after cerebral ischemia–reperfusion, traumatic head injury and spinal cord injury[4–7]. The nitric oxide radical has dual effects: first, it combines with the superoxide anion to form peroxynitrite; second, it interferes with superoxide dismutase, whose antioxidant effect is reduced[8,9]. The mechanisms by which peroxynitrite damages nuclear DNA are unclear, but could involve diffusion from the mitochondria and cytoplasm and cleavage in the nucleus to form hydroxyl radicals or singlet oxygen[10,11]. Brain injury also results in an elevated extracellular concentration of glutamate, which activates neuronal nitric oxide synthase via Ca^{2+} influx[12,13]. Other routes that induce the formation of ROS have been presented in recent reviews[14–16]. There is evidence that the cellular accumulation of nitric oxide can damage nucleic acids

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