Introduction: Beyond the synapse

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Powerful new imaging techniques can now reveal synapses changing structure and track neurotransmitter receptors shuttling into and out of the synaptic membrane. Even as synaptic plasticity is studied at a finer scale than could be imagined previously, it is important to remember that learning and memory are behaviors, not molecules, cells, or synapses. Synaptic plasticity is central, but in widening the scope of consideration, the contributors to this book reveal that there is much to learning and memory which lies beyond the synapse.

All cells in the body communicate with one another, and they all change physiologically from past experience – an elemental form of learning. Many mechanisms of intercellular communication, intercellular messenger systems, cell adhesion molecules, growth factors, interactions with the immune system cells, and gene expression, for example, are relevant to how neural circuits change their structure and function from experience. Moreover, system- and circuit-level properties of nervous system operation are fundamental to understanding the behaviors of learning and memory.

Steven Rose begins with the Hebb hypothesis, the fulcrum which has leveraged the great mass of research on learning and memory in modern times, but, as he makes clear in his chapter, the Hebb hypothesis is a fulcrum, not an endpoint. His chapter also reminds us that memory is a process, not a single event like the sudden jump in voltage of a synaptic potential in a slice of brain triggered by the flick of a switch. Memory has multiple phases, spanning from milliseconds to weeks, and it results from a sequence of very different cellular processes. Many engage machinery well outside the synapse to reach into the nucleus of the cell. Transcription factors, cell adhesion molecules, and growth factors all come into play over the course of hours or days in learning the simplest task: a chick avoiding selecting a distasteful bead after a single experience with it.

Much like the work of Rose and his colleagues on learning in the chick, the involvement of cell adhesion molecules and extracellular matrix molecules in long-term potentiation (LTP), is the focus of the chapter by Richard LeBaron and colleagues. Here, the investigators show that the specialized cell surface molecules and the associated intracellular signaling enzymes of focal adhesions in non-neuronal cells provide an important foundation for plasticity in synaptic connections between neurons in the hippocampus.

Lisa Boulanger's research concerns molecules mediating cell–cell signaling in the immune system, the major histocompatibility complex (MHC) class 1 proteins, in the context of structural and functional plasticity in the nervous system. This work is an example of how broadening thinking beyond the synaptic cleft may lead to fundamental insights into how the brain changes structure and function through experience. Molecular mechanisms of cell–cell communication used by other cells in the body are likely to be adopted by neurons in several aspects of synaptic communication and plasticity. Also, work such as this, at the intersection between the immune and nervous systems, may further illuminate how immune responses and exposure to disease may affect development and plasticity of neural circuits.

Information processing in the hippocampus underlying episodic memory is examined in the chapter by Howard Eichenbaum. "I woke, put on my pants, ran down the stairs, and ate eggs while reading the paper in the kitchen." Without this sequential coherence, fragmentary impressions of events – no matter how indelibly recorded in the altered strengths of individual synaptic connections – could not yield a useful memory any more than the frames of a filmstrip sliced into fragments could yield a meaningful sequence. Although the entire record may be preserved, without the vital episodic connections, the record cannot provide a comprehensible memory. What are the cognitive processes and circuitry

that preserves the sequence of records in an episodic memory? Eichenbaum argues that three cognitive processes supported by the hippocampus: associative representation; sequential organization; and relational networking, must be integrated to provide a coherent episodic memory.

William Greenough and colleagues approach the subject of memory from the opposite direction of most: beginning from the behavior and tracing to the roots of cellular changes induced by experience. His research shows that there are indeed changes in neurons and neuronal structure with experience, but the cellular changes in the brain sculpted by experience are hardly limited to neurons. Glia of many different types (and blood vessels) are altered by functional experience, along with changes in synapse number, synapse morphology, and neurogenesis. One particularly intriguing finding is that the myelin insulation on axons changes in animals brought up in impoverished or enriched environments, a subject that I consider briefly in a separate chapter. Operating well beyond the synapse, changes in myelin may be an underappreciated form of nervous system plasticity, affecting information-processing in the brain by regulating the speed of conduction in neural circuits, and thus the degree of temporal summation at synapses.

Several other chapters are devoted to consideration of glia, brain cells which have been ignored in the majority of studies of learning and memory. Glia remain absent from all computational neuroscience and that will remain so for some time to come. At present our knowledge of glia and their interactions with neurons is simply too limited to incorporate into a quantitative theory of brain function in plasticity, yet these cells have a powerful influence on synaptic and neuronal function. Dmitri Leonoudakis and colleagues consider the involvement of cytokines released from astrocytes in regulating α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking in central nervous system (CNS) neurons. Sarina Elmariah and colleagues examine the involvement of neurotrophin signaling among neurons and glia during synaptogenesis. Nedergaard and colleagues have pioneered studies on the involvement of astrocytes in hippocampal synaptic transmission, and the chapter by Liu and colleagues considers how inhibitory synaptic transmission is regulated through calcium-dependent release of glutamate from astrocytes. Eric Newman's pioneering studies of glia in regulating information-processing in the retina show that calcium signaling, glutamate, and purinergic signaling between neurons and glia contribute to light-evoked responses in the retina. The retina, being the most accessible part of the CNS, is a window into how circuitry in the brain operates, and through that window we see glia as a vital part of the mechanism.

Behavioral states of arousal are fundamental to learning, and Korz and Frey combine behavioral experiments with cellular electrophysiology to untangle the hormonal effects of stress and novelty on learning. Cain, Debiec, and LeDoux examine consolidation and reconsolidation of Pavlovian fear conditioning, and isolate in detail the mechanisms of emotional memory storage at a molecular level, with important implications for treating fear disorders in humans. The relationship between memory consolidation and reconsolidation emerges from studies of fear conditioning, with a wealth of information on the circuitry, receptors, ion channels, intracellular signaling cascades mobilized to consolidate short-term memories into long-term memories, and reconsolidate the memory once it is retrieved. Neurotrophin signaling, nitric oxide signaling, and neuromodulators and hormones as well as regulation of gene transcription are considered in this comprehensive chapter of fear conditioning memory.

The phases of memory consolidation extend through cycles of sleep and wakefulness, and in their chapter, Walker and Stickgold review the largely mysterious role of sleep in supporting memory consolidation and reconsolidation. Neuroimaging studies show that a task learned in the daytime re-emerges in the hippocampus during slow wave sleep. Cellular studies show that sleep can contribute as much to changes in synaptic connectivity as visual experience does to visual cortical neurons. Gene array studies show that sleep is hardly an idle period in brain function, transcription of approximately 100 genes is increased during sleep. This includes many of the same immediate early genes associated with memory formation.

Sex hormones are powerful agents driving behavior, and their effects on the brain are dramatic. Changes in synapse number and morphology are encountered during the phases of hormonal cycles, and male and female hormones have different effects on cognition and neuronal protection and plasticity. Foy, Baudry,

and Thompson consider the role of estrogen in hippocampal synaptic plasticity, and Romeo, Waters, and McEwen examine sex differences and similarities in steroid-induced hipocampal synaptic plasticity.

The role of neurotrophins in neurogenesis during development is an active area of research, and Lu and Chang consider the involvement of neurotrophins in neurogenesis associated with learning and memory in the hippocampus. Neurogenesis and neurotrophins are regulated by many extrinsic factors, including, intriguingly, voluntary exercise. Although it is now clear that thousands of new neurons are born in the adult hippocampus each day, how they are encorporated into circuits supporting learning and memory remains a difficult link to make. In their chapter Lu and Chang present evidence that the original circuitry may be changed after learning by adding new neurons to replace existing neurons in the circuit.

In addition to the classical neurotransmitters, non-traditional transmitters are being isolated which have unique properties that regulate neuronal function differently from classic neurotransmitters, and more intriguingly, encompass other non-neuronal cells in the signaling. D-serine is a glial transmitter released from astrocytes, which can act on synapses by regulating *N*-methyl-D-aspartate (NMDA) receptor function through the glycine binding site on the NMDA receptor. Mustafa, Kim, and Snyder review research showing that D-serine is an endogenous co-agonist of the NMDA receptor. The actions of D-serine at synapses in the hippocampus and cerebellum can put the regal receptor most closely associated with synaptic plasticity, the NMDA receptor, under direct control of perisynaptic glia.

There is now wide recognition that diffusible gases, such as nitric oxide and carbon monoxide, are important intercellular messengers, regulating synaptic function, and communicating between neurons and non-neuronal cells. The chapter by Avshulumov *et al.* considers a less well-known intercellular messenger, hydrogen peroxide, in regulating dopamine release in the striatum. Hydrogen peroxide works together with the traditional neurotransmitters, glutamate, and gamma-aminobutyric acid (GABA), to regulate dopamine release from dorsal striatum. Their chapter also considers reactive oxygen species in neuron survival and the important role of glia in releasing antioxidants for neuroprotection. These non-traditional signaling molecules expand beyond the synapse and interact with non-neuronal cells to modulate neurons in normal and pathological conditions.

In their chapter, Du *et al.* link bipolar disorder to impaired intracellular signaling and AMPA receptor trafficking at synapses. Calcium-signaling and several protein kinase pathways involved in learning are also implicated in bipolar disorder through effects on AMPA receptor trafficking. Many mood stabilizers and antidepressants modulate synaptic plasticity in association with hormonal effects and complex intracellular signaling networks.

Learning does not require gene transcription or translation into protein, but long-term memory does. Without activating transcription of new genes in the nucleus, short-term memories will quickly fade. But how do signals reach the nucleus to activate transcription of the necessary genes to make memories permanent? Bukalo and I consider this question, where our research indicates that the widely assumed requirement for a synapse-to-nucleus signaling molecule to activate gene transcription for late-phase LTP is not necessary. Our work showing that action potentials are the critical factor, again, shows the importance of thinking beyond the synapse in understanding the mechanisms of memory.

Part I
Spanning scales of neural plasticity

1 · Memory beyond the synapse

Steven P. R. Rose

> Let us assume then that the persistence or repetition of a reverberatory activity (or "trace") tends to induce lasting cellular changes that add to its stability. The assumption can be precisely stated as follows: When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.
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> The most obvious and I believe much the most probable suggestion concerning the way in which one cell could become more capable of firing another is that synaptic knobs develop and increase the area of contact between the afferent axon and efferent [cell body]. There is certainly no direct evidence that this is so . . . There are several considerations, however, that make the growth of synaptic knobs a plausible perception.
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> (Hebb, 1949, pp. 62–63)

HEBB SYNAPSES

When Donald Hebb (1949) formulated this now famous proposition in his classic book *The Organization of Behavior* it was no more than a challenging hypothesis. For sure, it was not entirely original. Earlier versions may be traced back to Tanzi (1893) and even pre-date the discovery of synapses themselves, as hints can be found in Sechenov and even Descartes. However, it is Hebb's formulation that now appears in all the standard textbooks, and what was when he advanced it a mere speculative idea has become, at least for neuroscientists working at the molecular and cellular level, an item of faith, the paradigm within which for the most part our experimental questions are set and our results interpreted.

Put at its simplest, the Hebbian paradigm states that when learning occurs there is a change in neural connectivity, brought about by the modulation of particular synaptic strengths. This modulation may be transient, in which case memory for the learned experience is not retained (short-term memory), or it may be converted into some more permanent modification (consolidation; long-term memory). Implicit in this model is the corollary that recalling a memory of the learned experience requires a retracing of the same neural pathways, via the restructured synapses. But are all forms of memory similar? Hebb did not concern himself with the varied taxonomies of memory that are now common in the literature. Thus there is a key distinction between procedural memory (knowing *how*) and declarative (knowing *that*; itself divisible between semantic and episodic or autobiographical memory). It is easy to distinguish between these forms in humans, much harder in animals, although Clayton (2004), working with scrub jays, has made valiant attempts. As an animal can only tell us it has learned by performing some task, are any observed synaptic changes related to the performance, or the memory on which that performance is based? Are synaptic changes associated with procedural memory similar to those for declarative memory, differing only in which synapses are involved, or are different biochemistries and mechanisms involved?

However these questions are answered, the Hebbian view of memory makes it a special case of synaptic plasticity – though it is important to recognize that plasticity is a term more often adduced than inspected, as it has several meanings. Any release of neurotransmitter from a synapse involves a temporary alteration in its composition, morphology, and physiology: millisecond plastic changes after which the synapse may revert to its prior form. Longer-term and more stable changes occur during development (including apoptosis) as a result of experience and injury. All these are forms of plasticity. So too, are the continual making and breaking of synaptic connections which may be

observed in time-lapse studies of living brains even in adults (Purves, 1988). Training an animal to acquire some new skill – learning – may involve any or all of these forms of plasticity, and there is a conceptual distinction to be drawn between identifying a synaptic change that occurs as a consequence or correlate of such training and a change which in some sense is part of the representation of that memory within the brain's encoding systems.

So, an important question within the Hebbian paradigm is whether the synaptic modulation that occurs during memory consolidation is of the same type, and involves the same molecular and physiological processes, as occur during the formation of more or less stable synaptic connections during development. It is how we answer this question that determines, for instance, whether we regard the well-documented changes in hippocampal synaptic physiology that occur after injection of a train of high-frequency pulses, long-term potentiation, as a mechanism of memory, or merely a model system for the study of synaptic plasticity (Bliss, Collingridge & Morris, 2004).

THE CHICK AS A MODEL SYSTEM

These questions have been studied in a variety of animal models ranging from molluscs to mammals. In this chapter, however, the focus is on my own studies in a simple learning model in the young chick. First, I briefly review past work which, seemingly, leads to a straightforward endorsement of the Hebbian paradigm. Training chicks on a one-trial passive avoidance task results in a molecular cascade occurring in a defined region of the chick forebrain, which culminates in seemingly lasting changes in synaptic morphology, biochemistry, and physiology. (A fuller review of the earlier results may be found in Rose, 2000). I will also discuss recent data concerning the role of the amyloid precursor protein (APP) in this cascade, and its potential relevance therefore to Alzheimer's disease. However, as is often the case in neuroscience, more detailed analysis, especially of the processes involved in recall of already learned memory, produces paradoxical results, which suggest a much more labile and dynamic model of memory beyond the synaptic level.

The one-trial passive avoidance task, introduced by Cherkin (1969), is based on the young chick's tendency to peck at small, bright objects such as beads. It has the merit of being rapid and sharply timed (chicks peck a bead within 10 seconds), enabling us to distinguish between the molecular correlates of the immediate training experience (the taste and sight of the bead, the motor act of pecking) and the downstream events associated with memory consolidation. In the standard version of the task in our laboratory, day-old chicks are held in pairs in small pens, pre-trained by being offered a small, dry white bead, and those which peck are trained with a larger (4 mm diameter) chrome or coloured bead coated with the distasteful methylanthranilate (MeA) (Lossner and Rose, 1983). Chicks that peck such a bead show a disgust reaction (backing away, shaking their heads, and wiping their bills) and will avoid a similar but dry bead for at least 48 hours subsequently. However, they continue to discriminate, as shown by pecking at control beads of other colours. Chicks trained on the bitter bead are matched with controls which have pecked at a water-coated or dry bead, and which peck the dry bead on test. Generally some 80% of chicks in any hatch group may be trained successfully and tested on this protocol. Each chick is usually trained and tested only once.

We have used two approaches to identify the cellular sequelae of pecking the bitter bead. In the correlative approach, appropriate brain regions may be dissected from trained and control birds at specific post-training times, and tissue is processed for biochemical, immunocytochemical, autoradiographic, or microscopic analysis. In the interventive approach all the birds are trained on MeA and injected either before or after training with drug, antibody or antisense, or vehicle to explore the possible enhancing or amnestic effect of the agent. Chicks which peck the previously distasteful bead on test are considered to be amnesic for the training. As this pecking response requires a positive and accurate act by the bird, it also controls for effects of the agent on attentional, visual, and motor processes.

The sharply timed nature of the learning experience, together with a combination of these experimental strategies, has enabled us to identify a biochemical cascade associated with memory consolidation in the minutes to hours following training. Thus, a change in some biochemical marker at a specific post-training time, occurring in trained compared with control chicks, might imply its direct engagement in memory expression at that time. Alternatively, it might indicate the mobilization of that marker as part of a sequence

leading to the synthesis of a molecule, or cellular reorganization, required for expression of memory. A similar argument applies to the timing of the onset of amnesia after intracerebral drug injection.

Two regions of the chick brain are specifically involved in the biochemical responses to the learning experience. These are the intermediate medial mesopallium (IMMP; previously called the intermediate medial hyperstriatum ventrale), an association "cortical" area, and the medial striatum (MS; previously called lobus parolfactorius, a basal ganglia homologue) (Avian Brain Nomenclature Consortium, 2005). The chick brain is strongly lateralized (Andrew, 1999; Rogers & Deng, 1999), and many, though not all of the molecular events we have observed are confined to the left IMMP.

THE TEMPORAL CASCADE: THE FIRST HOUR

During training, and in the five minutes which follow, there is enhanced release of glutamate in the IMMP (Daisley & Rose, 2001). Over the same time period there is also an increase in potassium-stimulated calcium concentration in synaptoneurosomes isolated from the IMMP (Salinska *et al.*, 1999). Within the succeeding 40 minutes, although we cannot assign them a precise temporal dependency, we have found: increases in NMDA-stimulated calcium flux in synaptoneurosomes (Salinska *et al.*, 1999); in ligand binding to the NMDA-glutamate receptor (Steele, Stewart & Rose, 1995) and of phosphorylation of the presynaptic membrane protein B50/GAP43 (Ali, Bullock & Rose, 1988) coupled with a translocation of cytosolic protein kinase C (PKC) to the membrane (Burchuladze, Potter & Rose, 1990). There is increased release of the putative retrograde messenger arachidonic acid, in tissue prisms prepared 30–75 minutes post-training, though the onset time for amnesia if the arachidonic acid synthesis is blocked with phospholipase A_2 inhibitors is delayed until 75 minutes (Holscher & Rose, 1994; Clements & Rose, 1996). Intervention studies with MK801 (Burchuladze & Rose, 1992), the N-type calcium channel blocker ω-Ω conotoxin GVIA (Clements, Rose & Tiunova, 1995) and PKC inhibitors (Burchuladze, Potter & Rose, 1990), injected into the IMMP either just before or just after training, all produce amnesia with an onset time of 30 minutes to one hour. $GABA_A$ agonists are also amnestic at this time. So, too, is nitroarginine, which blocks synthesis of the putative retrograde messenger nitric oxide (NO) (Holscher & Rose, 1993; Rickard, Ng & Gibbs, 1998). Other laboratories have found an involvement of a variety of protein kinases, notably protein kinase A (PKA), over this period (Serrano, Rodriguez, Bennett & Rosenzweig, 1995).

Thus it would appear that the training experience generates a sequence of rapid synaptic transients which provide a temporary "hold" for the memory – the phases categorized as short- and intermediate-term memory by Gibbs and Ng (1977; *see also* Patterson *et al.*, 1988). As well as forming the brain substrate of the remembered avoidance over this period, these transients must serve two other functions. They must initiate the sequence of pre- and post-synaptic intracellular processes which will in due course result in the lasting synaptic changes presumed to underlie long-term memory, and they must also serve to "tag" relevant active synapses, perhaps via membrane phosphorylations, so as to indicate those synapses later to be more lastingly modified.

THE TEMPORAL CASCADE: ONE TO EIGHT HOURS

A key step in the intracellular cascade must be the link between synapse and nucleus. Calcium is clearly a major player here, and that intracellular calcium signaling may be important is indicated by our recent observation that within 10 minutes post-training there is also a mobilization of synaptoneurosomal ryanodine-sensitive calcium stores (Salinska, Bourne & Rose, 2001), whilst dantrolene, which blocks calcium release from these stores, injected 30 minutes before or 30 minutes after training, produces amnesia by three hours post-training. Synaptoneurosomes are largely pre-synaptic, though they contain resealed post-synaptic (dendritic) elements as well, so it is not possible to distinguish whether the mobilized calcium stores are located at one, the other, or both sides of the cleft.

That activation of a number of transcription factors must be among the next steps in the process is clear from the elucidation of a role for cAMP response element-binding protein (CREB) in several mammalian learning paradigms. We, however, have focussed on the role of immediate early genes, c-fos and c-jun, both of which show increased expression in the hour after training

(Anokhin *et al.*, 1991). Further evidence as to the necessity of fos expression for longer-term memory is provided by the observation that antisense to c-fos, given six or more hours before training, blocks its synthesis (Mileusnic, Anokhin & Rose, 1996) and chicks become amnesic within three hours after training.

One of the few universal findings in studies of biochemical processes in memory formation is that long(er)-term memory is protein synthesis-dependent (Davies & Squire, 1984). Passive avoidance training is no exception, and anisomycin injected into the IMMP either before or up to some 60 minutes post-training results in amnesia for the avoidance. If the anisomycin is injected before training, amnesia sets in by the end of the first post-training hour, leading to the suggestion that beyond this period memory is protein-synthesis independent (Gibbs & Ng, 1977). However, the earlier view that beyond this time a protein synthesis-independent, long-term memory has been established is no longer tenable. Whilst anisomycin injections two and three hours after training are without effect on memory, injections given four or five hours post-training are amnestic in animals tested at 24 hours (Freeman & Rose, 1995). Thus there is a second, downstream, wave of training-related protein synthesis that we interpret as being the period during which late genes are activated and structural proteins are synthesized.

Although much attention within the learning and memory community is directed toward the roles of the many transcription factors involved in the early phases of memory formation, we have focussed on identifying the later gene products, and in particular the cell adhesion molecules (CAMs), transmembrane molecules, whose glycosylated extracellular domains may bind either homophilically or heterophilically, providing a mechanism for associating pre- and post-synaptic membranes. Their potential role in synaptic plasticity has long been emphasized by Edelman (1985). As well as their adherent properties, they have a second role, in transmembrane signaling. Two, in particular, are required for longer-term memory: NCAM and NgCAM/ L1 (Scholey *et al.*, 1993; Scholey *et al.*, 1995).

Specific blocking of neural cell adhesion molecule (NCAM) synthesis with antisense, injected over the 24-hour post-hatching period before the birds are trained, does not prevent the chicks learning the avoidance, but amnesia sets in within three hours (Mileusnic, Lancashire & Rose, 1999). However, interference with the functioning of already-synthesized CAM molecules is also amnestic. Thus, if antibodies which bind to the extracellular domains of either NCAM or L1 are injected into the intermediate medial mesopallium (IMMP) at five to six hours post-training, chicks show amnesia when tested at 24 hours (Scholey *et al.*, 1993, 1995), a time at which the antibodies themselves are no longer detectable in the brain. Antibodies to NCAM are not amnestic if injected at other times, but antibodies to L1, injected 30 minutes before training, are also amnestic when the chicks are tested at 24 hours. The extracellular domains of L1 include fibronectin and immunoglobulin regions, and using recombinant fragments to these regions we found that blocking the immunoglobulin domain at –30 minutes, but not at +5.5 hours, resulted in amnesia, whereas by contrast blocking the fibronectin domain at +5.5 hours but not at –30 minutes resulted in amnesia (Scholey *et al.*, 1995). This biochemical version of a double dissociation experiment led us to postulate that it was the cell-signaling function of L1, mediated via the immunoglobulin domain, which was engaged in the early phases of memory formation, whilst the fibronectin domains of NCAM and L1 were required in the de-adherence or re-adherence processes at the later time-point. It is presumably at this time, five to eight hours downstream of the training event, while their epitopes on the external domains are open to attack, that antibody binding can occur and hence amnesia results.

Demonstrating a role of the CAMs in memory formation led us to think about the possible involvement of another adhesion molecule, APP. APP is a rapidly turned-over protein, whose extracellular domains have been implicated in a variety of functions, including neurite outgrowth and synaptic plasticity. Improper processing of APP results in the cleavage of the 42-amino acid Abeta fragment, which accumulates in the plaques characteristic of Alzheimer's disease, and as is well-known, memory loss is a characteristic early feature of the disease. A monoclonal antibody to the C-terminal of APP, injected prior to training, results in the rapid onset of amnesia. So, too, does down-regulating APP levels by injection of antisense (Mileusnic, Lancashire & Rose, 2000). More significantly, we have shown that it is possible to rescue the memory lost by either antibody or antisense injection by administering a small peptide, the palindromic sequence tripeptide Arg-Glu-Arg (RER), homologous

to part of the growth-promoting domain of APP. The peptide also acts as a cognitive enhancer in weak versions of the training task. RER binds displaceably to two membrane proteins, of molecular weights 66–69 kD and 110 kD, present in both chick and human neuronal membranes (Mileusnic, Lancashire & Rose, 2000, 2004), and our working hypothesis is that it substitutes for APP in the transmembrane signaling required to activate the internal cascade leading to synaptic modulation. The potential therapeutic role of this peptide is currently under intense study.

STRUCTURAL ENDPOINTS?

The longer-term consequence of this cascade is thus the modification of synaptic connectivity, detectable biochemically in terms of changes in the configuration and distribution of NCAM, among other synaptic markers. The presumed endpoint for memory storage is modulation of synaptic connectivity, by altering synaptic number or relocating or structurally modifying existing synapses and dendritic spines, or both. Stewart and colleagues have been able to show changes in both pre- and post-synaptic elements. Thus, 24 hours after training there is increased dendritic spine density in projection neurons of the IMMP (Patel, Rose & Stewart, 1988) and, at the same time, changes in the numbers and dimensions of synaptic junctions, presynaptic boutons, and synaptic vesicle number in both IMMP and MS.

SIGNALING FACTORS BEYOND THE SYNAPSE

Having pecked a bead coated in MeA, chicks avoid a similar but dry bead for at least 24 hours subsequently. However, if the aversant is made less strong by, for instance, using a 10% solution of MeA in alcohol, the birds peck and display a disgust reaction, but will avoid similar beads for only six to nine hours subsequently (Sandi & Rose, 1994a; *see also* Burne & Rose, 1997). Although in so far as we have compared them, weak training initiates a similar set of synaptic transients to those produced in the strong version of the task, these are apparently not sufficient to result in gene expression, as CAM synthesis does not occur. Our assumption is that the temporal relationship between the fading of the memory trace for the weak training beyond six hours and the wave of glycoprotein synthesis that occurs at this time with strong training is not fortuitous (Rose, 2000). However, there are many factors which may affect the salience of this "weak-learning" experience, and which result in memory being retained as for the strong learning.

Chicks are normally held in their pens in pairs, as this diminishes stress. If they are trained on 10% MeA, and then separated, stress levels increase, and retention persists for 24 hours. The normal training procedure is indeed stressful, as is shown by the fact that for five to ten minutes after training chicks on the strong, but not the weak, version of the task there is an increase in plasma corticosterone levels (Sandi & Rose, 1997). Further, if corticosterone is injected into the IMMP just before or just after weak training, retention is also enhanced (Sandi & Rose, 1994a). The enhancing effects of stress may be blocked by injection of antagonists of glucocorticoid receptors into the IMMP, which is rich in such receptors. Blockade of these receptors is also amnestic for strong training (Sandi & Rose, 1994b), as is inhibition of peripheral corticosterone synthesis with metyrapone or aminoglutethimide (Loscertales, Rose & Sandi, 1997). As might be anticipated, the effects of corticosterone are dose-dependent in the classic inverted-U form: 1 μg injected prior to weak training enhances retention, whereas higher doses do not; 1–5 μg injected prior to strong training diminishes retention (Sandi & Rose, 1997). Similar enhancing effects are also apparent with neurosteroids such as dehydroepiandosterone (DHEA) (Migues, Johnson & Rose, 2001). Neurotrophins also affect the salience of weak training. Recombinant brain-derived neurotrophic factor (BDNF), but not nerve growth factor (NGF) or neurotrophin 3 (NT-3), injected just before or just after weak training, will enhance 24-hour retention. Reciprocally, antibodies to BDNF are amnestic for strong training, amnesia setting in within three hours (Johnston & Rose, 2001).

These findings are of both theoretical and practical relevance. First, they remind us that although, especially under the influence of the neurophysiological observations of synaptic interactions during LTP, cellular theories of memory formation are heavily based on Hebbian models, memory is not just a pre- or post-synaptic event. Rather, whether any particular experience is learned or not depends on a much wider array of

neural and peripheral factors, humoral and perhaps also immunological (*see* McGaugh, 1989; Damasio, 1994). The entire animal is thus involved in any learning experience. Second, together with the observations on APP described above, they may point the way toward developing effective agents for therapeutic intervention in conditions of memory deficit.

MEMORY BEYOND THE IMMP

I have so far focussed on the sequence of biochemical events occurring in the chick IMMP consequent on passive avoidance training, and I have argued that the cascade we have identified, leading as it does to measurable morphologic changes in synaptic connectivity, is a necessary part of memory consolidation. Does this, however, mean that the IMMP contains some lasting representation of the association between bead and bitter taste, the elusive engram? A combination of electrophysiologic and lesioning experiments that have been conducted in parallel with those described here makes clear that this is not simply the case (Rose, 2000). Within the hours after training biochemical changes occur in brain regions other than the left IMMP, including the right IMMP and MS, and the memory trace, if such it is, becomes both fragmented and redistributed. The IMMP seems to retain some aspects of the memory including colour discrimination, whereas others, related perhaps to the size and shape of the bead, may be located to the MS (Patterson & Rose 1992; Barber et al., 1999). Again this points to the conclusion that learning and memory formation and retention engage not simply a discrete neuronal ensemble in a small brain region, but a much wider set of spatially and temporally dynamic processes, linked and given coherence by some form of binding mechanism (Rose, 2004).

REACTIVATING MEMORY

The evidence adduced so far suggests that although synapses are modified as a result of training, in accord with the Hebbian hypothesis, this modulation involves more than just intersynaptic signals, but engages wider systemic properties, growth factors, systemic and neuro-hormones, and neurotrophins. Furthermore, over time the memory trace becomes distributed, rather than localized to a simple neural network. There is a yet further complexity to be added. Within the simple Hebbian paradigm, the memory trace, once established, is permanent. However, there have been persistent reports in the literature suggesting that even well-established memories may be rendered labile and susceptible to amnestic agents if they are reactivated by giving an animal a reminder (Sara, 2001). In particular, it has been shown in a number of species and learning paradigms that if anisomycin is administered around the time of the reminder, the animal is rendered amnesic for the task. However, a debate has ensued as to whether this is a lasting or merely transient amnesia (Nader, 2003); that is, does "reconsolidation" recapitulate consolidation, or does the amnestic agent merely transiently block access to the memory? In our hands, both the temporal dynamics and kinetics of the amnesia after administration of anisomycin coupled with a reminder are different. Notably, the effects are transient, and lower doses of the inhibitor are required to produce it (Anokhin, Tiunova & Rose, 2003). Furthermore, whilst a reminder resulted in increases in 2-doexyglucose uptake into both IMMP and MS, as is the case following initial training, immediate early gene expression is enhanced only in the MS (Salinska, Bourne & Rose, 2004). One suggestion is that reactivating a memory might not require the full biochemical cascade triggered by training, but involves only local, synaptic, protein synthesis. It is relevant in this context that blocking axonal flow around the time of training with colchicine results in transient amnesia, there is no such effect following a reminder.

CONCLUSIONS

What are the lessons from these experiments? A biochemical cascade leading to modulation of synaptic connectivity is a necessary consequence of exposing an animal to a learning situation, and that this cascade is required for memory consolidation is clear. Our experiments have mapped this cascade in a specific and simple learning task in the young chick. We cannot say from this that we have identified a universal mechanism, even for all forms of learning in a single species. However, the similarities both in molecular processes and temporal dynamics between this cascade and those observed in other tasks and vertebrates (Izquierdo & Medina, 1997) are encouraging. It would be nice to be able to say that what is true for *Gallus gallus domesticus* is also true for *Homo sapiens sapiens*, in which case it may well be that our