occurrence of spikelets in vivo all remain uncertain. Furthermore, the hypothesis that axonal gap junctions are crucial for high-frequency ripple oscillations in the hippocampus needs to be tested in vivo. Assuming that this will be confirmed, what behavioral and neuromodulatory conditions trigger the ripples, and what turns them off? The Schmitz et al. study will surely motivate and facilitate studies that address these questions.

Although the gap junctions studied by Schmitz et al. are located in axons, their existence will also be of interest to those who study dendrites. The spikelets observed in this study strongly resemble the "fast prepotentials" previously attributed to dendrites (Spencer and Kandel, 1961; Turner et al., 1993). Although the evidence for dendritic spikes is not in doubt, some small-amplitude spikes observed in vivo could be due to axonal gap junctions. In addition, the model proposed by Traub suggests that some of the action potentials contributing to ripples may remain restricted to the axon, while failing to trigger an action potential in the soma and dendrites. Hebbian plasticity at dendritic synapses may not occur during such events, because of the need for backpropagating action potentials to invade the dendrites (Magee and Johnston, 1997). Excitatory synapses on dendrites are often active during ripples, however, so this would appear to predict a situation where Hebb's rule might be violated: the axons of both pre- and postsynaptic cells may be active, but their coincidence could not be detected, because the hyperpolarized soma prevents postsynaptic action potential firing from being conveyed from the axon to the dendrite.

Modeling studies motivated the search for gap junctions between axons and offered clues as to how they might be found. The success of this elegant approach highlights the usefulness of models mimicking realistic neurons. The identification of gap junctions between axons, the fast electrical communication they mediate, and the implication of their importance in mediating fast neuronal oscillations should lead others to continue the search for these junctions and their molecular machinery throughout the brain. As more is learned, we may soon witness the emergence of the electrical synapse as an important element in textbook wiring diagrams of the vertebrate brain.

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Protooncogenes Subserve Memory Formation in the Adult CNS

Studies of the signal transduction mechanisms underlying learning and memory have provided many new insights into the molecular mechanisms underlying associative conditioning in mammals. In this issue of *Neuron*, Gean and colleagues report the discovery that the PI-3 kinase/AKT(PKB) pathway contributes to LTP and the consolidation of amygdala-dependent cued fear conditioning in rats.

Several decades of studies of cancer mechanisms have led to the identification of key signal transduction cascades in oncogenesis. In early studies of rous sarcoma virus-induced cancers, the *v-ras* gene was discovered and termed an "oncogene" for its cancer-inducing capacity. Shortly thereafter, normal cellular homologs of viral oncogenes were identified and termed "protooncogenes," and, not surprisingly, the normal products of these genes are now known to be critical regulators of cell division. In some instances, protooncogene products also play the complementary role of inhibiting cell death by suppressing apoptosis. The picture is emerging in the field of oncology that oncogenesis involves dysregulated cell division and aberrant suppression of apoptotic pathways, due to mutations in the protooncogenes coding for signal-transducing proteins controlling these processes (Marte and Downward, 1997).

Tracking down and identifying protooncogenes has led to a watershed of new insight into the signal transduction molecules and mechanisms operating to regulate normal cell division. Identified players include the protooncogene products ras, phosphatidylinositol-3 kinase (PI-3 K) and its target AKT (named for the transforming AKT8 retrovirus strain and also known as protein kinase B), the colony stimulating factor receptor (encoded by the c-fms protooncogene), neurofibromin (the neurofi-

bromatosis 1 gene product), several members of the mitogen-activated protein kinase (MAPK) superfamily, and numerous growth factor receptors. In broad brush strokes, these pathways can be described as transducing cell surface signals, via protein phosphorylation, to the nucleus to regulate gene expression and to the cytoskeleton to regulate morphological changes and cell division and migration. By and large, these pathways have the attribute that they do not utilize readily diffusible second messengers but, rather, rely on multiprotein signaling complexes and the translocation of activated proteins to various subcellular locales in order to achieve their effects.

This is all well and good in providing insight into the regulation of cell division in oncogenesis and normal development, but are these pathways relevant to those interested in the normal function of the adult CNS? After all, adult neurons are essentially all nondividing and terminally differentiated. Against this backdrop, it has been somewhat enigmatic that these same protooncogene products are abundantly expressed in the adult CNS (see Husi et al., 2000). The work by Lin et al. (2001), in this issue of *Neuron*, provides a key piece in an emerging solution to the puzzle of the roles played by protooncogenes in the adult CNS. Their studies show that the protooncogene product PI-3 K and its associated target AKT play a crucial role in normal synaptic plasticity and memory formation. PI-3 K is thereby added to a short but growing list which indicates that, in the adult CNS, protooncogene products are involved in the signal transduction processes subserving learning and memory. A theme is becoming more clear—neurons in the adult CNS have adapted the powerful signal transduction mechanisms used in controlling cell division during development to the purpose of controlling neuronal plasticity and information storage in the mature CNS.

In picking a behavioral model system with which to study learning and memory, Lin et al. chose a robust learning paradigm that capitalizes on the capacity of mammals, including rodents, to associate an environmental cue with a mild aversive stimulus. This type of learning, generally called "fear conditioning," is an example of classical associative conditioning similar to Pavlovian conditioning. Many aspects of the neuronal circuitry underlying this behavior have been worked out, and it is clear that the amygdala is involved in memory formation in this system. In addition, the fear conditioning paradigm has been quite fruitful of late as a model in which to study molecular mechanisms underlying learning and memory. Using this behavioral paradigm, recent studies have implicated the PKA, PKC, CaMKII, and ERK kinase families, as well as their target CREB, in conditioned fear (see Schafe et al., 2000, for just one recent example). One reason for the high level of enthusiasm for pursuing this behavioral paradigm is its potential relevance as a model system for human anxiety disorders.

Lin et al. trained animals by pairing a light cue with mild footshock and confirmed learning by measuring a light cue-evoked startle potentiation 24 hr later. Potentiation of the animal's normal startle response by re-presentation of the conditioned stimulus (light in this case) is one of the standard indices of cue-evoked fear and, hence, an indication of the animal having formed a lasting association between the light cue and the fear-evoking stimulus. In investigating the molecular mechanisms underlying the associative learning, Lin et al. observed amygdalar activation of a target of PI-3 K, AKT, shortly after training—an observation in nice congruence with the known dependence of fear conditioning on the amygdala. More importantly, this finding provided direct evidence for a possible role for the PI-3 K/AKT pathway in classical fear conditioning.

AKT activation was measured using an antibody selective for phosphorylated, activated AKT. It is important to note that PI-3 K itself phosphorylates inositol-containing phospholipids, which subsequently activate AKT by binding to its pleckstrin homology (PH) domain (see Figure). The phosphorylation event measured by Lin et al. is actually an accessory phosphorylation that occurs concomitant with activation. Thus, the increased phosphorylation of AKT observed by Lin et al. is an indirect but reliable indication of PI-3 K activation in the amygdala following fear conditioning training.

The PI-3 K/AKT activation occurred $\sim$1 hr after training, in good agreement with other studies of CNS protein kinase activation after fear conditioning. Particularly compelling is their observation that amygdala PI-3 K activation occurs selectively with paired light/footshock and not in unpaired controls that experience identical environmental stimulation but do not learn to associate the stimuli. They also extended their studies using pharmacology in vivo, by showing that amygdalar infusion of a PI-3 K inhibitor, wortmannin, selectively blocked long-term (24 hr) but not short-term fear conditioning. They bolstered these findings by demonstrating the efficacy of their PI-3 K inhibitor for blocking amygdala PI-3 K activation in response to behavioral training.

In investigating the cellular basis for the effects of PI-3 K inhibitors on learning, Lin et al. capitalized on recent seminal findings strongly linking amygdala LTP with amygdala-dependent fear conditioning (reviewed in Maren, 1999). Lin et al. found that the PI-3 K inhibitors wortmannin and LY 294002 blocked amygdala LTP. Further dissecting the biochemical cascades involved, Lin et al. showed that the ERK MAP kinase cascade is activated by LTP-inducing tetanic stimulation and that ERK activation is blocked by PI-3 K inhibitors (see Figure). Similarly, elevation of cAMP levels caused ERK activation, and this was blocked by PI-3 K inhibitors and a selective PKA inhibitor. They also rounded out this phase of their studies by showing that PI-3 K is activated in response to elevated cAMP. Finally, they identified a target of the PI-3 K/AKT/ERK cascade, the transcription factor CREB, using an appealing combination of physiologic, pharmacologic, and behavioral approaches. Overall, these studies nicely dissect essential components of the PI-3 K/AKT/ERK cascade in the amygdala. While previous studies by the LeDoux and Kandel groups had shown a necessity for PKA and ERK activation in various forms of amygdala LTP, the mechanisms of ERK regulation in the amygdala were quite mysterious. Lin et al. have nicely fleshed out several steps in the cascade linking amygdala neuronal activity with intracellular ERK activation and synaptic plasticity.

One unanswered question from their studies is how PI-3 K couples up to ERK activation. The prevailing model from studies of nonneuronal systems is that these

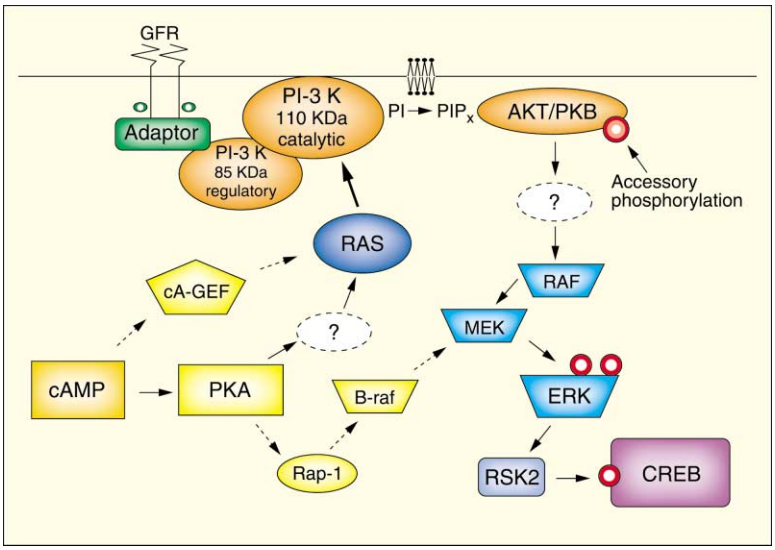

Figure 1. Potential Signal Transduction Routes under Study by Gean and Coworkers

Mechanisms coupling cAMP to PI-3 K are speculative but based on the available literature. Red circles represent the specific phosphorylation events measured. Abbreviations: PI-3 K, phosphatidylinositol-3-kinase; PIP_x, (poly) phosphorylated derivatives of phosphatidylinositol; RAS, the low molecular weight G protein ras; GEF, guanine nucleotide exchange factor; PKA, cAMP-dependent protein kinase; GFR, growth factor receptor; RSK, ribosomal S6 kinase; CREB, cAMP response element binding protein; AKT/PKB, the kinase AKT, also known as protein kinase B; Rap-1, RAF, MEK, and ERK are all components of the extracellular signal-regulated kinase (ERK) cascade, a subfamily of the mitogen-activated protein kinases.

two cascades are antagonistic (or in some cases parallel). Serial linkage as suggested by the work of Lin et al. is somewhat unusual, although serial linkage of this sort has been found for G protein $\beta\gamma$ subunit activation of PI-3 K/AKT/ERK and for integrin stimulation of MAPK in cultured cells (King et al., 1997). An alternate scenario is suggested by recent work from Perkinton et al. (1999) using cultured striatal neurons. In this system, calcium-permeable AMPA receptors activate ERK and CREB phosphorylation through activating PI-3 K. This is an intriguing possibility, as calcium-permeable AMPA receptors have already been proposed as potentially playing a role in amygdala LTP and fear conditioning (Mahanty and Sah, 1998). Overall, across a wide variety of tissue types, the serial linkage of PI-3 K and ERK as observed by Lin et al. is atypical, but perhaps neurons will specifically utilize the pathways in this serial fashion. A similar disconnect between neuronal and nonneuronal cells has already been observed for the PKA and ERK pathways, because, whereas in most cells PKA inhibits ERK activation, in neurons the generalization is that cAMP and PKA are upstream activators of ERK.

This is quite an impressive series of studies that provides a compelling case for a role for PI-3 K in synaptic plasticity and memory formation. These studies also strengthen the link between amygdala LTP and classical associative fear conditioning, as inhibiting PI-3 K blocks both phenomena. Moreover, PI-3 K is activated with both LTP and fear conditioning—in a sense, one can consider PI-3 K activation as a "marker" for LTP having been induced, and the observation that PI-3 K is activated with fear conditioning is consistent with the hypothesis that LTP has occurred in the amygdala when animals are conditioned.

The study by Lin et al. greatly expands our appreciation of normal physiologic roles for the PI-3 K pathway in the adult CNS. To date, most work on PI-3 K/AKT in neurons has focused on its role as an antiapoptotic signaling system that regulates neuronal cell death during development and which promotes survival in adult neurons (reviewed in Brunet et al., 2001). In addition, in one recent study, Bai Lu's group found that the PI-3 K cascade contributes to neurotrophin-induced synaptic plasticity using a neuromuscular junction preparation in vitro (Yang et al., 2001), and Kelly and Lynch (2000) have presented evidence that long-term potentiation in the dentate gyrus involves PI-3 K. The study by Lin et al. takes our understanding of roles for PI-3 K to a new level, by demonstrating that the cascade subserves cognitive function in the behaving animal.

Overall, these studies are a beautiful combination of biochemistry, physiology, pharmacology, and behavior—a trend that is becoming more widespread as neuroscience advances toward trying to understand the molecular basis of cognition. These studies are also a further example (if one were even necessary) of the prominent role played by protein (and lipid?) kinases in learning and memory. Importantly, these studies point out the continuing recognition of the wide diversity of signal transduction mechanisms necessary for complex neuronal information processing.

Finally, these studies fit into a rapidly developing picture and provide an important complement to earlier studies. Prior studies have shown roles in memory for other signal transduction molecules first identified as regulators of cell division and differentiation, in particular, the ERK MAPK cascade (reviewed in Sweatt, 2001), ras-GRF (Brambilla et al., 1997), and neurofibromin 1 (Silva et al., 1997). Evidence suggests that, like Lin et al. have shown for PI-3 K/AKT, these molecules play roles in memory formation in the adult animal that are independent of their roles as protooncogenes in development. It is interesting that neuroscientists for many decades have believed that important and fruitful analogies could be drawn between development and memory. These recent studies suggest that a more direct comparison may be drawn—development and memory may in several ways be not only analogous but homologous processes at the molecular level, sharing identical molecular mechanisms to achieve their ends.

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Attention! V1 Neurons Lining Up for Inspection

In this issue of *Neuron*, Roelfsema and Spekreijse report that macaque V1 neuron responses are correlated with target choice in a task requiring monkeys to attentively trace a line to plan a saccade. These results provide evidence that V1 is actively involved in the interpretation of visual stimuli.

One of the organizing principles that has driven much of vision research over the last 40 years is that visual areas are organized hierarchically, with retinal information flowing through successive stages of processing, each of which serves as the input for the next stage. According to this view, primary visual cortex autonomously performs elementary analysis of inputs from the lateral geniculate nucleus and passes the results forward to higher-order areas where task-dependent processes like attentional selection and decision making take place. In this issue of *Neuron*, Roelfsema and Spekreijse challenge this view by providing evidence that V1 responses accord with the monkey's interpretation of a stimulus rather than with the stimulus itself (Roelfsema and Spekreijse, 2001). This gives impetus to an emerging view of V1 as a participant in a functionally interdependent visual system whose elements selectively influence one another during the execution of a given visual task.

They trained monkeys to trace a curved line without moving their eyes (see Figure). At the beginning of each trial, monkeys fixated a small spot at the center of a computer monitor. Once fixation was attained, two curved lines appeared, one of which, designated the "target curve," originated at the fixation point. After a brief delay, the fixation point disappeared, and the monkey was rewarded for making a saccade to the other end of the target curve. The second curve, termed the "distractor curve," also began near the fixation point, and then it either passed near the target curve or else intersected with it within a so-called "critical zone." In order to perform the task correctly, monkeys had to determine whether or not the target and distractor curves crossed within the critical zone, prior to making the required eye movement.

Psychophysical experiments have found that human observers solve this sort of task by moving visual attention along the target line from one end to the other. When subjects perform a line-tracing task, they are better at judging the color of the target line than that of the distractor, evidence that the traced line is attended. The time required to determine whether two ends of a line are connected to one another scales linearly with line length (e.g., Joliceur et al., 1991). In previously published experiments using the same task, Roelfsema and colleagues have provided physiological evidence that monkeys adopt a similar strategy. As in the present experiment, they recorded responses of primary visual cortical neurons whose receptive fields fell along one of the lines. Firing rates were higher when the line that passed through the receptive fields was the target line. Elevated responses appeared first at the starting end of the target line, near fixation, and only later reached the far end of the line (Roelfsema et al., 2000).

The key advance of the present study is that the authors have related neuronal responses to behavioral performance on a trial-by-trial basis, using a logic that has been applied successfully in the analysis of visual motion processing (e.g., Britten et al., 1996). They found that the monkey chose eye movements in accordance with the responses of neurons in primary visual cortex. On trials in which the monkeys erroneously made a saccade to the distractor curve, the firing rate enhancement switched over to the distractor curve after the critical zone. Responses were elevated on the initial target curve segment, prior to the critical zone, regardless of whether the ultimate eye movement was right or wrong. This is exactly what one would expect if monkeys failed to correctly trace the target curve through the critical zone.

This study raises a number of important issues. The authors propose a model in which rate enhancement spreads along horizontal connections among V1 neurons with collinear orientation preferences, thereby labeling the V1 representation of the line (Roelfsema et al., 2000; Roelfsema and Spekreijse, 2001). This model is motivated, in part, by the finding that the time to complete line tracing in human subjects scales linearly with line length. Similar path length-dependent reaction time patterns have also been observed when human subjects mentally traverse a drawn maze (Crowe et al.,