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Work conducted during AFOSR-89-0383 indicates that long-term potentiation is induced and stabilized by variants of the chemistries that regulate adhesive relationships. Expression of the potentiation effect involves modification of a subgroup of post-synaptic receptors; this modification includes a change in the kinetics of the receptor's ion channel. New evidence linking long-term potentiation to memory was obtained during the tenure of the grant and pharmacological agents that promote its occurrence were identified. Based on this information, attempts to design and synthesize memory enhancing drugs have been initiated. The following paragraphs briefly describe these developments and cite relevant publications; a fuller description is found in the application for continuation of AFOSR support.

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Final Technical Report for AFOSR grant 89-0383
"Synaptic Plasticity and Memory Formation"
Principal Investigator: Gary Lynch

Summary: Work conducted during AFOSR 89-0383 indicates that long-term potentiation is induced and stabilized by variants of the chemistries that regulate adhesive relationships. Expression of the potentiation effect involves modification of a subgroup of post-synaptic receptors; this modification includes a change in the kinetics of the receptor's ion channel. New evidence linking long-term potentiation to memory was obtained during the tenure of the grant and pharmacological agents that promote its occurrence were identified. Based on this information, attempts to design and synthesize memory enhancing drugs have been initiated. The following paragraphs briefly describe these developments and cite relevant publications; a fuller description is found in the application for continuation of AFOSR support.

The signal transmitted at synapses in the brain can be adjusted within seconds to a higher intensity by applying a brief sequence of high-frequency pulses in a specific pattern which mimics endogenous brain activity as it typically occurs during learning. The duration of this "long-term potentiation" (LTP) depends on the particular circuitry but can in some brain areas last for months or longer. There is considerable evidence that LTP encodes some forms of memory in forebrain networks and a description of its cellular bases should therefore be of considerable theoretical and practical significance.

Significant progress has been made during the last grant period (AFOSR 89-0383) in defining the processes responsible for induction, expression, and stabilization of LTP. Most importantly, several independent lines of research have provided converging evidence that the principle locus of the stable change resides on the postsynaptic side, more specifically in the AMPA type glutamate receptor which mediates fast synaptic transmission (4, 7, 8, 28, 29). LTP was found to alter a waveform parameter (the decay time constant or decay tau) directly related to the mean open time of the AMPA receptor (34). Changing the size of individual synaptic EPSPs did not affect the waveform of the responses and the LTP associated change in decay tau was obtained in slices from immature brains which have primitive spines and dendrites. These results confirm that the distortion in waveform produced by LTP is due to a modification of the kinetics of the voltage independent AMPA receptor channel. Further insight into the nature of the change was obtained with the drug aniracetam which, as shown in an AFOSR sponsored experiment, prolongs the mean open time of the AMPA receptor channel (33). The effects of aniracetam on the amplitude and shape of synaptic responses were substantially modified by LTP (but not by enhanced release) in a manner expected if potentiation alters the kinetics of the AMPA receptor channel (23, 30, 38). It should be noted that the waveform and pharmacological data accord well with the earlier discovery that LTP differentially affects response components mediated by AMPA receptors vs. the co-localized NMDA receptors (see previous Progress Reports). By themselves, the observed modifications would not enhance post-synaptic currents and so must occur as part of a receptor configuration that *in toto* involves channel kinetics and a shift in the conductance or affinity. Tests for a change in receptor affinity proved negative (31), pointing to a conductance increase as the agent of LTP expression. However, another group using a different autoradiographic technique has

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reported that LTP increased binding to the AMPA receptor (43). The discrepancies between their results and ours need to be examined but it is likely that LTP expression is due to a change in receptor conformation that affects ligand affinity, channel conductance, and channel kinetics.

As indicated above, one can distinguish several phases in the formation of stable LTP. The process is set in motion by activation of NMDA receptors. Antagonist of this receptor were found to cause a substantial impairment of olfactory discrimination learning in rats (1); this finding has contributed to the evidence that LTP is a process subserving learning. In *in vitro* tests, the activity of the NMDA receptor can be modulated by a number of agents acting through several receptor sites. We have focused in our studies on one of these sites which binds glycine with high affinity (2, 5) and have shown that its activation is essential for receptor function inasmuch as its occupation by a selective antagonist prevented long-term potentiation (10, 11, 17). In a further approach, activation of NMDA receptors with exogenously added agonists was shown to produce a long-lasting enhancement of synaptic responses (6); whether the characteristics of this form of potentiation share similarities with those of LTP remains to be determined.

Various forms of evidence suggest that potentiation develops within 30 seconds of the induction. Once established, however, it appears to remain vulnerable to disruption for a period of minutes. This was shown in experiments in which a transient application of adenosine or a brief exposure to oxygen-free atmosphere followed the induction of LTP (12, 21). In both cases, the responses, after being transiently suppressed, returned to the level at which they had been before LTP induction. These findings indicate that the changes in the synaptic domain which account for LTP have to be consolidated over a period of about five minutes. Several biochemical mechanisms have now been demonstrated to be essential components of this process. One of them involves the activation of a receptor for platelet-activating factor (PAF)(22, 35); LTP induced in the presence of inhibitors of this receptor slowly decays as the responses return back to their pre-induction level. The PAF system in many peripheral cells aids in mobilizing calcium to orchestrate cellular changes and it is likely that it serves a similar function in the remodeling of the synapse during LTP. The effects of calcium are at least in part mediated by calpain, a calcium-sensitive protease, which is widely distributed in nerve cells including their dendrites (13) and has as its preferred targets a variety of structural proteins whose function it is to stabilize cell morphology; LTP elicited in presence of a calpain inhibitors consistently decayed back to baseline (9, 19). These observations have led to the idea that LTP involves a structural remodeling of the synapse in which calpain presumably would first degrade some of the existing structural components (e.g. spectrin) which then would lead the synaptic zone to reassemble in a new configuration. The processes involved in re-organization of the synaptic domain do not by themselves explain how the emergent configuration becomes stabilized. During the tenure of AFOSR 89-0383, we obtained evidence that transmembrane adhesion receptors belonging to the integrin family play a critical role in anchoring LTP. Specifically, small peptides that compete for the extracellular attachment site of a subgroup of integrins were found to prevent stabilization of potentiation (15, 32). Biochemical work then led to the purification of a protein greatly enriched in synaptic membranes that possesses the essential characteristics of an integrin including recognition by appropriate antibodies (24, 26, 39). Some integrins outside the brain are known to be latent in that they become adhesive only after an activation event; it is possible that the newly discovered synaptic integrin-like receptors

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belong to this group. Thus, the re-forming of adhesion between adjacent cells, and presumably between pre- and postsynaptic elements, may be an essential part of the synaptic remodeling. We also obtained evidence that at least some of the proteins mediating cell adhesion are substrates of calpain (27).

As indicated above, the AMPA receptor is now thought to be the critical element in the expression of long-term potentiation. For this reason, efforts have been made to identify mechanisms which may control its functional properties. One conclusion from these studies has been that receptor affinity can be selectively altered after treating the membranes in which the receptors are embedded with certain phospholipases or supplementing them with phospholipids (14, 25). It is not unlikely that this is relevant for receptor function in intact cells as phospholipase inhibitors disrupted the stabilization of LTP (20). In another study we have found evidence that the AMPA receptor preferentially exists in two distinct affinity states and that certain manipulations such as the dissolution of the synaptic environment promote a conversion from the low into the high affinity state (41). The successful reconstitution of partially purified AMPA receptors (42) should allow us now to correlate these affinity states with functional properties of the receptor associated ion channel.

The above results provide a reasonably detailed, though in some aspects still tentative, picture of the events that induce, express, and stabilize a form of synaptic potentiation that is linked to the encoding of memories. It should now be possible to develop principled explanations for some forms of amnesia and design biologically based approaches to enhancing learning. Some steps in this direction were taken during the tenure of AFOSR 89-0383. Certain benzodiazepines used clinically as anxiolytics produce a profound anterograde amnesia. These drugs were found to be potent inhibitors of LTP (37), most probably via subtle effects on GABA receptors. Analyses of this possibility are now in progress. Other work directed at enhancing LTP focussed on the events occurring during and between the short bursts of afferent activity used to induce the potentiation effect. Greater depolarization during a burst reduced the number of bursts needed to produce maximal potentiation while suppression of the between-burst hyperpolarization elevated the ceiling on maximal LTP (40). The drug aniracetam, which as noted prolongs AMPA currents, emerged from these studies as a selective tool for enhancing LTP formation. The drug is not sufficiently potent and metabolically stable to be useful in this regard following peripheral administration and this led to attempts to synthesize variants that could be used in behavioral experiments. Substantial progress has been made in this area as described in previous Progress Reports.

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