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13. ABSTRACT (Maximum 200 words)

The goal of the project is to define the mechanisms responsible for inducing, expressing, and stabilizing long-term synaptic potentiation (LTP), a form of physiological plasticity that is likely to be responsible for the encoding of memory in telencephalic networks. Studies in the past year defined the cellular changes likely to be responsible for expression. The nootropic ("cognitive enhancing") drug aniracetam prolongs the open time of post-synaptic receptors mediating fast synaptic transmission. LTP changes the effect of the drug on synaptic responses in hippocampus; manipulations that enhance responses by increasing release do not interact with the drug. By far the most plausible explanation of this result is that LTP modifies receptors. This conclusion is supported by negative results from experiments testing the hypotheses that LTP is due to changes in release, receptor number, or spine resistance. These results combined with other findings described in this and previous Progress Reports provide a still tentative but reasonably complete hypothesis regarding how synapses are modified by patterns of physiological activity associated with learning.

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SUMMARY

1. Experiments completed during the past year indicate that expression of long-term potentiation (LTP) is probably not due to changes in the biophysics of dendritic spines or to an increase in the number of receptors. Further evidence that increases in transmitter release are not involved was also obtained. By exclusion, this work points to a change in receptor properties as the agent of LTP.

2. Direct evidence that modified glutamate receptors are responsible for LTP expression was obtained in the past year. Aniracetam, a drug that reversibly modulates the AMPA subclass of these receptors, has different effects on synaptic responses following induction of LTP. The only plausible explanation for the interaction between drug and potentiation is that they act at a common locus, namely the fast glutamate (AMPA) receptor.

3. Experiments using newly introduced and more selective inhibitors of calpain provided additional evidence that the calcium activated protease plays a central role in the induction of LTP. It was also found that blockade of receptors for the platelet activating factor (PAF), a locally synthesized trophic factor, prevents stable expression of LTP.

4. Estimates of the time required for the stabilization (consolidation) of LTP were obtained using two disrupting conditions applied after the induction of potentiation. Stable LTP appears within 2-5 minutes of high frequency stimulation. Efforts to identify the stabilization process were begun. Pharmacological studies have implicated a class of transmembrane adhesion receptors known as integrins. Biochemical experiments then showed that synaptic membranes are greatly enriched in still uncharacterized proteins that may be related to the appropriate integrin subgroup.

5. A reasonably complete, though still tentative, model of how LTP is induced, expressed, and stabilized can now be formulated:
   a. disorganization of the synaptic membrane cytoskeleton by elevated calcium and calpain activation;
   b. relaxation of adhesive relationships of the synaptic junction;
   c. changes in the synaptic membrane environment with corresponding enhancement of glutamate receptor conductance;
   d. re-establishment of adhesive connections via newly exposed integrin receptors followed by re-stabilization of the membrane cytoskeleton.

   Given the evidence linking LTP to learning, this model can also be seen as an hypothesis regarding the substrate of memory.

PROJECT DESCRIPTIONS

The goal of the program is to define the mechanisms responsible for the induction, expression, and stabilization of long-term potentiation (LTP). Since there is considerable evidence that LTP is a substrate for memory encoding in cortical and hippocampal networks, it is reasonable to assume that progress in this line of research will lead to a new understanding of learning and cognition.

Expression of LTP: Negative Results for Release, Receptor Number, and Spine Resistance

Dramatic progress was made in the past year in resolving the nature of the changes that express LTP. There is currently an intense controversy surrounding this question. Based on experiments using quantal analysis techniques, some have argued that an increase in release is responsible. This interpretation of the data is debateable (see Larson *et al.*, in press, for a review). Experiments supported by the AFOSR indicate that increased release as well as several other possible candidates are not likely to be involved in LTP. These findings are summarized briefly below:

1. Manipulations that increase the probability of release do not interact with LTP: If LTP were due to changes in release, it would be expected to interact with treatments that promote release. This does not occur across a wide variety of such treatments. (Cf. 6; Muller *et al.*, 1990, Proc. Nat. Acad. Sci.)

2. LTP has very different effects on the currents mediated by the two classes (AMPA and NMDA) of co-localized post-synaptic glutamate receptors. This result, which has been confirmed in a second laboratory, was described in previous Progress Reports. During the past year, we extended our initial observations by analyzing the circumstances under which the second class of glutamate receptors (the NMDA variety) can be potentiated and by providing evidence that this does not occur under circumstances sufficient to produce LTP of AMPA receptor mediated responses (Muller *et al.*, Hippocampus, 1991). The selectivity of LTP is strong evidence against a release hypothesis.

3. LTP does not increase binding to AMPA receptors: This was established using autoradiographic binding techniques in slices of hippocampus (Kessler *et al.*, Brain Res., 1991).

4. LTP is not due to a reduction in voltage saturation at spine heads because of a change in spine biophysics: Two separate paradigms failed to confirm predictions arising from spine resistance hypotheses of LTP (Jung *et al*, Synapse, 1990; Larson *et al.*, Brain Res., 1991). These experiments also indicate that dendritic spines are not voltage saturated by LTP.

Expression of LTP: Evidence that Increased Receptor Conductance is Responsible

By exclusion, the above experiments point to changes in receptor properties, most probably involving conductance, as the substrate of LTP. Tests of this became possible with the discovery by Ito and co-workers (J. Physiol., 1990) that the nootropic drug aniracetam increases glutamate triggered currents mediated by AMPA receptors without affecting NMDA or GABA receptors. We subsequently found that the drug causes a slight reduction in glutamate binding to AMPA receptors (Xiao *et al*, Hippocampus, 1991) indicating that its facilitatory actions on AMPA currents must be due to enhanced conductance (e.g., greater open time). If LTP changes AMPA receptors, then it is reasonable to expect that it would change the response of the receptors to aniracetam. This result was obtained in the past year. Aniracetam increases the amplitude of control responses by 25-30% but has only half this effect on potentiated responses. As expected, manipulations that increase release show no interaction with aniracetam (Staubli *et al.*, Psychobiol., 1990; Xiao *et al.*, Hippocampus, 1991). The drug changes the shape as well as the amplitude of the synaptic response, a finding which strongly suggests that it prolongs the open time of channels. LTP substantially modifies this effect of the aniracetam. This result was obtained with extracellular recording, intracellular recording, and with cells patched clamped at -70 mV (Staubli *et al.*, Science, submitted).

We have built a mathematical model of synaptic currents and used this to pinpoint possible receptor changes that could account for the LTP-aniracetam interaction. (Release and receptor numbers have no interaction with the drug in the model.) The model reproduced the interactions when LTP was simulated as an increase in receptor conductance (Staubli *et al.*, submitted).

To summarize, the effects of a drug targeted at AMPA receptors are changed by LTP; the only plausible explanation for this is that LTP itself changes the receptors.

Aniracetam as a "cognitive enhancer"

The nootropic family of drugs to which aniracetam belongs are used as cognitive enhancers; they have been extensively tested in humans without side effects but their efficacy in promoting intellectual performance is very controversial. Aniracetam and the very similar drug AHP are the only members of the family we have found to enhance AMPA receptor functioning and these drugs are effective only at concentrations (1.0 mM) in excess of what might reach the brain from the periphery. Despite this, aniracetam could be used as a lead compound to search for drugs that facilitate transmission in brain regions underlying human cognition. Such drugs would be of great scientific interest and might indeed enhance intellectual performance.

Induction and stabilization of LTP

The induction of LTP is known to involve NMDA receptors and increases in post-synaptic calcium levels (see previous Progress Reports). During the past year we showed that stimulation of the glycine site on the NMDA receptor is an obligatory event in inducing LTP (Oliver *et al.*, Synapse, 1990) and that this site promotes calcium entry into cells via the NMDA receptor (Oliver *et al.*, Neurosci. Lett., 1990). We have proposed that a critical step beyond calcium is the activation of the calcium sensitive protease calpain followed by the partial disorganization of the cytoskeleton (previous Progress Reports). During the past year we found that recently introduced and more

selective inhibitors of calpain block induction of LTP (del Cerro *et al.*, Brain Res., 1990) and this has been confirmed by other groups.

In an effort to further define the mechanisms responsible for induction and stabilization, we sought to determine how much time is required for stable LTP to develop. Studies with hypoxia as a disrupting agent indicated that 2-3 minutes are needed to produce stable LTP (Arai *et al.*, Brain Res., 1990); subsequent work showed that intense stimulation of adenosine receptors can reverse LTP during this point (Arai *et al.*, Neurosci. Lett., 1991).

Other work identified an agent which could serve to maintain calcium levels at an elevated state over the period of time needed for production of stable LTP. Synapses are greatly enriched in receptors for the platelet activating factor (PAF), a trophic substance generated from cell membranes by the activation of calcium sensitive phospholipases. PAF causes a very large increase in calcium in cultured neurons and this led us to test if antagonists of its receptors block LTP. This proved to be the case; we also obtained evidence that stimulation of PAF receptors increases intracellular calcium currents (del Cerro *et al.*, Behav. Neural Biol., 1990; Arai *et al.*, submitted). Beyond describing a mechanism that could enhance calcium for the time period needed to stabilize LTP, these results are of great interest because of the recent finding that certain widely used benzodiazepines (tranquilizers) block the PAF receptor. These drugs are known to produce varying degrees of reversible amnesia, a result which can now be explained in light of their effects on LTP. A paper describing the link between benzodiazepines and LTP has been submitted (del Cerro and Lynch, submitted).

Studies in our laboratory have shown that LTP can persist unchanged for weeks, the longest period over which testing is feasible (see previous Progress Reports). This extreme stability strongly implies that LTP changes the morphology of the synapse, something which would presumably require adjustments to the adhesive chemistries that maintain junctional connections. Previous work from the laboratory showed that LTP affects the morphology of the synaptic zone (see previous Progress Reports) and during the past year we concluded a much more detailed analysis of this effect (Schottler and Lynch, in prep.). We have also begun efforts to identify adhesion receptors and matrix proteins involved in the stabilization of LTP.

Two primary groups of receptors used in forming junctional contacts are CAMs and integrins. During the past year we found that calpain cleaves the cytoplasmic tail of N-CAM 180 (Sheppard *et al.*, Biochem. Biophys. Acta., 1991), an adhesive protein known to be concentrated in post-synaptic densities. This action of the protease could contribute to a "relaxation" of the synaptic junction. We also found that infusions of a small peptide known to block the interaction of a subclass of integrin receptors with matrix proteins prevents the stabilization of LTP (Staubli *et al.*, Behav. Neural Biol., 1990; Xiao *et al.*, NeuroRep., 1991). This experiment suggests that exposure of latent integrins followed by adhesion to matrix proteins is needed to produce irreversible potentiation. (Integrins in some types of cells are latent and become fully functional only after an activation event). This led us to begin a search for synaptic integrins. Immunochemical techniques using antibodies against known integrin receptors detected two polypeptides that were greatly concentrated in synaptic plasma membranes (Bahr *et al.*, NeuroRep., 1991a,b). One of these was subsequently found to adhere to a matrix protein and in a manner that is blocked by the same antagonist of integrin receptors used to prevent stabilization of LTP (Bahr *et al.*, in prep.).

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A test of the spine resistance hypothesis of LTP expression. *Brain Res* **538**: 347-350, 1991. Larson, J. and Lynch, G.

Activation of the glycine site associated with the NMDA receptor is required for induction of LTP in neonatal hippocampus. *Int J Dev Neurosci* **8**:417-424, 1990. Oliver, M., Larson, J., and Lynch, G.

An inhibitor of integrin receptors blocks long-term potentiation. *Behav Neural Biol* **53**:1-5, 1990. Staubli, U., Vanderklish, P. and Lynch, G.

Aniracetam has proportionately smaller effects on synapses expressing long-term potentiation: Evidence that receptor changes subserve LTP. *Psychobiology* **18(4)**: 377-381, 1990. Staubli, U., Kessler, M. and Lynch, G.

Anoxia reveals a vulnerable period in the development of long-term potentiation. *Brain Res* 511:353-357, 1990. Arai, A., Larson, J., and Lynch, G.

Antagonists of the platelet-activating factor receptor block long-term potentiation in hippocampal slices. Arai, A. and Lynch, G.

Antibodies to the $\alpha_v\beta_3$ integrin label a protein concentrated in brain synaptosomal membranes. *NeuroReport* (in press). Bahr, B.A., Vanderklish, P.W., Bakus, B.L., Capoldi, D., Sheppard, A. and Lynch, G.

Compartmentation and glycoprotein substrates of calpain in the developing rat brain. *Synapse* (in press). Sheppard, A., Wu, J., Bahr, B.A., and Lynch, G.

Development of hippocampal long-term potentiation is reduced by recently introduced calpain inhibitors. *Brain Res* 530:91-95 (1990). del Cerro, S., Larson, J., Oliver, M.W., and Lynch, G.

Distribution of calcium-activated protease calpain in rat brain. *J Comp Neurol* 296:269-276, 1990. Perlmutter, L.S., Gall, C., Baudry, M., and Lynch, G.

Effect of bromophenacyl bromide, a phospholipase A_2 inhibitor, on the induction and maintenance of LTP in hippocampal slices. *Brain Res* 537:49-53, 1990. Massicotte, G., Oliver, M.W., Lynch, G., and Baudry, M.

Evidence that matrix recognition contributes to the stabilization but not the induction of LTP. *NeuroReport* (in press). Xiao, P., Bahr, B.A., Staubli, U., Vanderklish, P.W., and Lynch, G.

Evidence that changes in spine neck resistance are not responsible for expression of LTP. *Synapse*, 7 (No.3), 216-220, 1991. Jung, M.W., Larson, J. and Lynch, G.

Factors governing the potentiation of NMDA receptor-mediated responses in hippocampus. Muller, D., Arai., A., and Lynch, G.

Failure to detect changes in AMPA receptor binding after long term potentiation. *Brain Res* (in press). Kessler, M., Arai, A., Vanderlish, P., and Lynch, G.

Fibronectin binding by brain synaptosomal membranes may not involve conventional integrins. *NeuroReport* 2:13-16, 1991. Bahr, B.A., Sheppard, A., and Lynch, G.

Glycine site associated with the NMDA receptor modulates long-term potentiation. *Synapse* 5:265-270, 1990. Oliver, M., Kessler, M., Larson, J., Schottler, F. and Lynch, G.

Inhibition of long-term potentiation by an antagonist of platelet-activating factor receptors. *Behav Neural Biol* 54:213-217, 1990. del Cerro, S., Arai, A., and Lynch, G.

Levels of mRNA for a putative kainate receptor are affected by seizures. *Pro Natl Acad Sci USA* 87:7643-7647, 1990. Gall, C., Sumikawa, K., and Lynch, G.

Modulation of DL-alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid-Quisqualate receptors by phospholipase-A_2: A necessary step in long-term potentiation. *Proc Natl Acad Sci (USA)* 88:1893-1897, 1991. Massicotte, G., Oliver, M., Lynch, G., and Baudry, M.

Protein kinase C activity is not responsible for the expression of long-term potentiation in hippocampus. *Proc Natl Acad Sci (USA)* 87:4073-4077, 1990. Muller, D., Buchs, P-A., Dunant, Y., and Lynch, G.

Proteolytic modification of neural cell adhesion molecule (N-CAM) by the intracellular proteinase calpain. *Biochimica Biophysica Acta* 1076:156-160, 1991. Sheppard, A., Wu, J., Rutishauser, U., and Lynch, G.

Re-evaluating the constraints on hypotheses regarding LTP expression. *Hippocampus* 1 (1):9-14, 1991. Lynch, G. and Baudry, M.

Role of NMDA and non-NMDA receptors in synaptic transmission in rat piriform cortex. *Exp Brain Res* 82:451-455, 1990. Jung, M.W., Larson, J., and Lynch, G.

Selective effects of aniracetam across receptor types and forms of synaptic facilitation in hippocampus. *Hippocampus* (in press). Xiao, P., Staubli, U., Kessler, M., and Lynch, G.

Synaptic modulation of N-Methyl-D-Aspartate receptor mediated responses in hippocampus. *Synapse* 5:94-103, 1990. Muller, D. and Lynch, G.

The effects of adenosine on the development of long-term potentiation. *Neurosci Lett* 119:41-44, 1990. Arai, A., Kessler, M., and Lynch, G.

The glycine site modulates the NMDA-mediated increase in intracellular free calcium from hippocampal cultures. *Neurosci Lett* 114:197-202, 1990. Oliver, M.W., Shacklock, J.A., Kessler, M., Lynch, G. and Baimbridge, K.G.

The protease inhibitor leupeptin interferes with the development of LTP in hippocampal slices. *Brain Res* 505:233-238, 1990. Oliver, M., Baudry, M. and Lynch, G.

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