TH-POS-I1 THE BRAIN AS A TASK-ORIENTED, SELF-ORGANIZING SYSTEM: A CLUE TO LATERAL ASYMMETRY AND A PHYSIOLOGICALLY REALIZABLE QUASI-DISTRIBUTED MEMORY SCHEME. Lawrence E. Mallach Department of Biophysical Sciences, State University of New York at Buffalo, Amherst, NY 14226.

The brain is considered as a task-oriented, self-organizing system with limited genetic determinism whose functional capabilities are delimited by environmental influence. Utilizing a general systems approach, we develop a functional scheme for sensory integration, learning and memory, and are able to demonstrate a mechanism for lateral asymmetry which emerges naturally as a result of two complimentary, but structurally incompatible modes of information processing. These modes, which correspond to left and right brain function, we call deductive and inductive to refer to their logical forms. The weak genetic specificity and strong environmental influence, coupled with an associative hypothesis, leads naturally to a distributed memory scheme which is consistent with behavioral, developmental, neuropathological, and serial lesion data. The remarkable property of this memory scheme is that it is quasi-local, in the sense that colonies of neurons compete for actualization of the task to be accomplished, and the colony which is completed first forms the local memory, or function generator. However, in the process many colonies which are almost complete form partial memories, and in fact may be actualized with repeated stimulus presentation. These scattered colonies form the distributed memory, and account for serial lesion data. The local memories are then linked to resolve more complicated tasks, but retain their individuality for use in other problem settings. Dynamical realization is shown to lead to inertia and depend heavily upon feedforward principles, or the corollary discharge of Sperry and Teuber. Incompatibility with reductionism is discussed, and experimental predictions noted.

TH-POS-I2 VOLTAGE DEPENDENCE OF DESENSITIZATION ONSET AND RECOVERY AT THE NEUROMUSCULAR JUNCTION. B. Scubon-Mulieri & R.L. Parsons*. Dept. Physiology and Biophysics, Univ. of Vermont, Burlington, VT 05401

The influence of voltage on the time course of desensitization onset and recovery was compared under voltage clamp conditions in potassium-depolarized frog sartorius muscle preparations at temperatures of 13-17°C. The end-plate region was point clamped at either -40 mV or +40 mV while being activated by the microperfusion of carbachol during a constant flow of bathing solution (mM/L: K-propionate 122.5; $CaCl_2$, 1.29; Ca-propionate 0.51; THAM, 1.0, pH=7.0). Large, 100 μ diameter, perfusion pipettes and relatively high concentration of carbachol, 1 mM, were used to achieve maximum uniformity of the concentration profile. Spatial uniformity of voltage was determined with an independent electrode implanted approximately 100 μ distant from the current electrode. No measurable deviation was recorded at the command electrode and the deviation 100 μ distant was approximately 15% of the driving potential at both voltages. Desensitization onset and recovery developed exponentially with onset being considerably faster than recovery at both potentials. Both desensitization onset and recovery were voltage dependent. The onset time constant was 10.7 ± 2.1 (SEM) sec (n=4) at -40 mV and 31.9 ± 6.0 sec (n=4) at +40 mV. In these same fibers the extent of recovery after a 120 second wash interval (expressed as a percent of the initial current magnitude) was 81.5 ± 8.0% at -40 mV and 62.5 ± 2.1% at +40 mV. At both voltages, desensitization developed more rapidly during the second carbachol perfusion than with the initial carbachol application; the onset time constant being shortened to 52.0 ± 2.5% and 21.8 ± 5.0 of the initial time constant at -40 and +40 mV, respectively. Supported by NIH Grant NS-07740.

TH-POS-I3 ISOLATED NICOTINIC RECEPTORS FROM TISSUE CULTURED CHICK MUSCLE. F. Sachs and G. Kemp, Dept. of Pharmacology, SUNY, Buffalo, NY 14214 and Dept. of Expl. Biology, Roswell Park Memorial Institute, Buffalo, NY 14203.

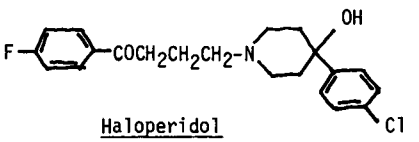
Tissue cultures of chick skeletal muscle, purified with cytosine arabinoside to remove fibroblasts, were extracted with 1% Triton X-100 detergent. In this way is was possible to solubilize 90% of the nicotinic acetylcholine receptor (AchR) in 5-10 minutes. Each 60 mm diameter dish contains approximately 0.2 pMoles of bungarotoxin binding sites and contains approximately one mg of protein. It is possible to use this Triton X-100 solubilized AchR to study competition between the binding of cholinergic agonists and antagonists and ^{125}I-α-bungarotoxin. Since the binding studies are performed within minutes of the time the receptor was incorporated within a living cell, it is expected that such preparations should constitute an ideal system in which to resolve questions regarding the properties of muscle AchR which are in dispute in the literature. These extracts in addition provide a source of AchR of relatively high initial specific activity that can easily be further purified by α-cobrotoxin affinity chromatography. The rate of binding shows two components with rate constants of 1 & 4×10^7 $mole^{-1}$ min^{-1}. Approximately 20% of the toxin binding is insensitive to inhibition by curare. Further results will be reported.

TH-POS-I4 FLUORESCENT-LABELED ACETYLCHOLINE RECEPTOR MOTION AND LOCALIZATION IN CULTURED MUSCLE FIBER MEMBRANES. Daniel Axelrod, Peter Ravdin‡ E. L. Elson‡ W. W. Webb, and T. R. Podleski‡ School of Applied & Engineering Physics, Section of Neurobiology & Behavior, Department of Chemistry, Cornell University, Ithaca, N.Y. 14853

We have made direct, quantitative measurements of the lateral motion and age-dependent distribution of acetylcholine receptors (AChR) on the surface of rat myotubes in primary culture. AChR were fluorescent-tagged with tetramethyl rhodamine-labeled α-bungarotoxin (TMR-αBgt) and AChR lateral motion was measured by the Fluorescence Photobleaching Recovery (FPR) technique. We found two co-existing distinct classes of AChR: (1) mobile, uniformly distributed AChR which appear on all myotubes shortly after fusion from myoblasts; and (2) immobile, dense, highly granular AChR in patches of 10-60μm size which appear shortly after fusion and disappear after myotubes have become extensively interconnected. Immobilization of concanavalin A receptors by cross-linking with concanavalin A slows AChR lateral motion. Evidence of TMR-αBgt-AChR turnover is seen in the gradual internalization of surface fluorescence within 36 hours after labeling. Evidence for new synthesis of AChR is seen in the gradual recovery of TMR-αBgt labeling several hours after AChR blockage with unlabeled αBgt. Various disruptive agents and fluorescent-labeled antibodies against cytoplasmic contractile proteins were used to investigate cellular structures that might regulate AChR motion and localization. We also present a protocol for preparing mono-labeled TMR-αBgt and illustrate its binding to electroplax AChR and mouse motor endplates.

TH-POS-I5 HALOPERIDOL - A NEW SPECIFIC INHIBITOR OF SODIUM CONDUCTANCE IN MYELINATED NERVE. T. L. Pencek and C. L. Schauf. Departments of Physiology and Neurological Sciences, Rush Medical College, Chicago, Illinois, 60612.

Haloperidol was the first drug in the butyrophenone series of major tranquilizers and presently is widely employed as an anti-psychotic. On the voltage-clamped node of Ranvier of Rana catesbeiana its action resembles that of tetrodotoxin, producing a highly specific decrease in sodium permeability at concentrations as low as 10^{-8}M with no apparent effect on sodium activation or inactivation kinetics. The dose/response relation is described by a simple Langmuir adsorbtion isotherm with an apparent dissociation constant of 2×10^{-7}M. There is no consistent effect on potassium permeability or leak at concentrations sufficiently high to completly block sodium permeability. The effect of haloperidol is not readily reversible on frog myelinated nerve. In contrast to the effects on frog, haloperidol is without effect when externally applied to voltage-clamped Myxicola axons. Its structure (above) and low water solubility (14mg/L) suggests an interaction with the sodium channel which is fundamentally different from that responsible for the action of tetrodotoxin, and thus it may represent a new useful tool in studies of molecular mechanisms.



Haloperidol

TH-POS-I6 ACETYLCHOLINE CONTENT OF THE SQUID NERVE FIBER. Jorge Villegas and Donald J. Jenden*, Centro de Biofísica y Bioquímica, IVIC, Apartado 1827, Caracas 101, Venezuela and Department of Pharmacology, UCLA School of Medicine, Los Angeles, California 90024, U.S.A.

Gas chromatography/mass spectrometry was used to identify and measure acetylcholine and choline in extracts obtained from Sepioteuthis sepioidea peripheral nerves. Stellar nerves, isolated giant nerve fibers, extruded axoplasm, and sheaths attached to giant axons cut lengthwise were used. Both the retention times and the relative ion currents at the major peaks recorded with the tissue extracts were identical to those of authentic derivatives of acetylcholine and choline. Complete scans of some of the samples (both whole stellar nerve and 3-5 pooled giant axons) confirmed the presence of mass peaks at the most prominent masses, but did not yield evidence of the molecular ions. These compounds were quantitated in eight samples of giant axon weighing an average of 6.3 mg; 28.8 ± 4.5 pmol acetylcholine and 1.25 ± 0.34 nmol of choline were found. Average concentrations were 3.92 ± 0.69 and 159 ± 45 pmol mg^{-1}. The concentrations of acetylcholine and choline were not significantly correlated with each other ($P>0.2$), and neither showed a significant correlation with the time taken to dissect and freeze the preparations (10-17 min), or the time of storage between freezing and analysis (7-30 days). These experimental findings establish the presence of acetylcholine in the giant nerve fiber of S. sepioidea. This gives further support to the involvement of the cholinergic system in the mechanism responsible for the long-lasting Schwann cell hyperpolarizations following the conduction of nerve impulse trains by the axon, which had been previously observed.

TH-POS-17 IDENTIFICATION OF PARASYMPATHETIC PREGANGLIONIC NEURONS IN THE SACRAL SPINAL CORD OF THE CAT BY RETROGRADE AXONAL TRANSPORT OF HORSERADISH PEROXIDASE (HRP). I. Nadelhaft, C. Morgan*, W.C. de Groat*, and T. Schauble*, Departments of Neurosurgery, and Pharmacology, University of Pittsburgh School of Medicine and Veterans Administration Hospital, Pittsburgh, PA. 15261.

Cytoarchitectonic and electrophysiologic techniques have been used previously to determine the location of sacral autonomic neurons in the cat. In the present study the retrograde transport of HRP was used to provide a more definitive localization of these cells and to study their morphology. In adult cats anesthetized with pentobarbital a 25% aqueous solution of HRP (Sigma Type VI) was applied to sacral preganglionic axons in the central end of a transected pelvic nerve. The animals were sacrificed 22 to 48 hours later. Preganglionic neurons labeled with brown granules were identified in the intermediolateral region of the grey matter in the ipsilateral second and third sacral segments. The number of labeled neurons varied in different animals. However in the most successful experiments a total of 500 to 700 neurons were identified in the entire sacral cord; and it was not uncommon to find 5 to 9 neurons in one 30 μm section. Labeled neurons occurred most frequently in lamina VII on the lateral edge of the grey matter opposite the central canal. The neurons were often distributed in a narrow band 100-150 μm wide extending dorsoventrally for distances of 400-700 μm. Smaller numbers of cells were scattered more medially in the grey matter and a few were located in the lateral funiculus. Labeled cells were either spindle shaped (30-50 μm long, 12-15 μm wide) or round (15-30 μm). In summary, the HRP technique seems to be very effective for labelling sacral preganglionic neurons and should be useful in future experiments for localizing functionally distinct cell groups (i.e., colon, bladder, penile cells) in the sacral autonomic nucleus.

TH-POS-18 CALCIUM CURRENTS IN MOLLUSCAN NEURONS. N. Akaike,* K. S. Lee,* and A. M. Brown (Intr. by F. H. Rudenberg), Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Two types of voltage-dependent calcium currents have been reported in molluscan neurons. One is activated by small depolarizations, has a slow time to peak, and does not inactivate (Eckert & Lux, 1975). It has been implicated in oscillating membrane potentials. The other is faster, has a peak transient and is reported to inactivate with hyperpolarizing and depolarizing voltage clamp pulses (Geduldig and Gruener, 1970). It is probably responsible for the calcium action potential of these neurons. We examined the calcium currents of individual Helix aspersa neurons which were isolated using a suction pipette. The suction pipette allowed us to combine voltage clamp and intracellular perfusion as described in the accompanying abstract (Lee, Akaike & Brown, 1977). We separated the Ca^{2+} current by blocking the outward K^+ current with internal Cs aspartate and blocking the Na^+ current with TTX or Tris substitution for external Na^+. At small depolarizations a slow non-inactivating inward current appeared and an inward tail current was present. At larger depolarizations a faster current with a peak transient appeared and the inward tail current persisted. The reversal potential for the peak transient current was about +80mV and was dependent upon external and internal Ca^{2+} levels. The peak current was inactivated by depolarizing prepulses but hyperpolarizing inactivation was not observed. The slow current did not appear to be affected by depolarizing prepulses. In addition to differences in voltage effects, pharmacological differences between the two calcium currents were observed.

TH-POS-19 A SPECIFIC ACTION OF TRYPSIN ON NEURONAL MEMBRANES. K. S. Lee,* N. Akaike,* and A. M. Brown, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

The proteins responsible for membrane excitability are unknown although some insight into their nature has been obtained using enzymes and group specific chemicals. We used this approach on individual Helix aspersa neurons. The voltage-dependent membrane currents were separated using a newly-developed suction pipette which allows isolation of the nerve cell body, intracellular perfusion and voltage clamp. Resting potentials of -60mV and action potentials with overshoots of +20-30mV may be recorded for upwards of six hours. Intracellular exchanges are complete in about five minutes. The shunt resistance is between 50 and 100 MΩ, the series resistance is about $10^4\Omega$ and the leakage resistance is about 5MΩ. The current transient under voltage clamp has a time constant of less than 200μsec. The delayed outward K^+ current was blocked by internal perfusion with Cs aspartate 110mM. The inward Ca^{2+} current was blocked by substituting Co^{2+} for Ca^{2+} or Verapamil 1×10^{-5}M. This left an inward Na^+ current which was blocked reversibly by TTX in doses of 10^{-5}M or by substitution of Tris ion for Na^+ in the snail Ringer. Exposure to 0.1% trypsin in the extracellular fluid for about 2-4 minutes abolished TTX sensitivity without affecting the magnitude or time course of the Na^+ current. However, the effects of Tris persisted and the reversal potential for the inward Na^+ current was unchanged. Under these conditions external trypsin had no effect on the inward Ca^{2+} current. More prolonged exposure to trypsin increased resting membrane conductance and reduced Ca^{2+} and Na^+ currents. Therefore, the TTX receptor contains arginine or lysine residues or both, is more accessible to the extracellular medium and is probably distinct from the selectivity and gating sites for the Na^+ channel.

TH-POS-I10 TRANSMITTER RELEASE AT THE NEUROMUSCULAR JUNCTION: SELECTIVE CHANGES PRODUCED BY SR^{++} AND BA^{++}. J.E. Zengel and K.L. Magleby, Dept. of Physiology and Biophysics, Univ. of Miami School of Medicine, Miami, Florida 33152.

At least four processes can act to increase transmitter release during and following repetitive stimulation at the neuromuscular junction: a first and a second component of facilitation which decay with time constants of about 50 and 300 msec, augmentation which decays with a time constant of about 7sec, and potentiation which decays with a time constant of tens of seconds to minutes. It has previously been difficult to distinguish among these processes except on the basis of their time constants of decay. We now report that Sr^{++} and Ba^{++} can have selective effects on facilitation and augmentation, thus providing a new way to distinguish among some of the processes involved in transmitter release. E.p.p.s were recorded from the frog neuromuscular junction under conditions of low quantal content (0.5 mM Ca^{++}, 5 mM Mg^{++}). The nerve was conditioned with 10 impulses at 20/sec, followed by a single testing impulse applied from 50 msec to 10 sec after each conditioning train. When the Ca^{++} was replaced with 1-1.2 mM Sr^{++} the magnitude of the second component of facilitation following the conditioning train was almost doubled and its time constant of decay increased about 1.5 times. Sr^{++} had little effect on the first component of facilitation or on augmentation. When 0.15-0.25 mM Ba^{++} was added to the low Ca^{++} Ringer the magnitude of augmentation following the conditioning train was increased 1.8 times. Ba^{++} had little effect on the time constant of decay of augmentation or on facilitation. The actions of Sr^{++} and Ba^{++} on transmitter release could be accounted for by assuming that Sr^{++} selectively increased the magnitude and time constant of the increment of the second component of facilitation added by each impulse, and that Ba^{++} selectively increased the magnitude of the increment of augmentation added by each impulse. The selective effects of Sr^{++} on the second component of facilitation and of Ba^{++} on the magnitude of augmentation were also present following longer conditioning trains of 100-400 impulses. Sr^{++} and Ba^{++} had little effect on potentiation.

TH-POS-I11 INACTIVATION SHIFTS WITH THE NEUTRAL AND CHARGED FORMS OF LOCAL ANESTHETICS. W. Schwarz*, P. T. Palade*, and B. Hille, Physiology & Biophysics, University of Washington Medical School, Seattle, Washington 98195.

Local anesthetic (LA) molecules shift inactivation of Na channels so more channels are inactivated at resting potential. Shifts induced by neutral (benzocaine), amine (lidocaine, procaine), and internal quaternary (QX-314) LA were studied in frog muscle fibers under voltage clamp using the following extracellular/intracellular pH combinations: 9/8.1, 9/6.3, 6/6.3, 6/8.1. For all pH combinations, single testpulses revealed little shift with quaternary LA, while trains of testpulses induced large further shifts (use-dependent shift). With neutral LA, single testpulses already revealed a large shift and there was little further use-dependent shift. In the same test, ionizable amine LA acted like neutral LA at pH 9/8.1 and like quaternary LA at pH 6/6.3, showing for procaine and lidocaine (1) that both neutral and protonated forms can block Na channels, and (2) that the shift with the two forms develops with different kinetic properties. In addition, amine LA acted relatively like neutral LA with pH combination 9/6.3 (9 external) and like quaternary LA with 6/8.1 (6 external), showing that the form of drug acting on the receptor is controlled more by the external pH than by the internal pH. From other evidence, drug molecules are thought to approach the receptor from the axoplasmic side of the membrane and bind within the Na channel. We suggest, therefore, that the pH within Na channels is usually closer to the external pH than to the internal pH. The drug-channel reaction has additional pH dependence not attributable to ionization of the drug, since the total shift with neutral or quaternary LA increased when external or internal pH was lowered. (Supported by USPHS grants NS08174, NS05082, RR00374)

TH-POS-I12 THE INTEGRAL SPECTRUM: AN ADVANTAGEOUS APPROACH TO ANALYZING CONDUCTANCE FLUCTUATION DATA FROM SQUID GIANT AXON. B.A. Sokol and L.J. DeFelice, Anatomy Dept., Emory Univ., Atlanta, GA 30322

The integral spectrum (IS) method of membrane noise analysis (Biophys. J.16:827,1976) is extended to include multiple Lorentzian (relaxation) processes. Theoretical estimates for ISs and power spectral densities (PSDs) are calculated for two probabilistic models of conductance fluctuations from the squid giant axon, assuming finite data and perfect analyzers. 1/f noise encountered experimentally in membrane noise measurements are included in the calculations. The mean normalized error in the amplitude of the spectra, ε, is calculated for all spectra.

Using 5 Hz rectangular filters and a one-minute sampling time, the maximum difference between PSDs of the two models is 1.15ε for the potassium system and 1.28ε for the sodium system. ISs of the two models differ by 5.1ε at 10 Hz and 17.9ε at 100 Hz for the K and Na systems respectively. Although a distinction between models could not be made by visual examination of the PSD estimate, as is the case with the IS estimate, a difference could be detected by a complete statistical analysis of the independent PSD data points. Each IS point contains information from the entire frequency range and is therefore correlated to all other IS points. Statistically, the IS is a convenient and simple way of analyzing the data to show differences in the noise models.

These estimates, calculated by assuming perfect analyzers, represent an upper bound to the accuracy obtainable for the given data length. The accuracy of experimental spectra will depend upon how much of the original information was used and the approximations made in deriving the estimates.

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TH-POS-113 INVESTIGATIONS OF STEREOSPECIFICITY IN AXONAL MEMBRANE RECEPTORS. By J. K. Marquis, H. G. Mautner, and I. Tasaki. Dept. of Biochemistry & Pharmacology, Tufts Univ. School of Med., Boston, MA, and Lab. of Neurobiology, NIMH, Bethesda, MD.

Optically-active, conformationally-defined neuroactive ligands and fluorescent derivatives of these ligands have been used to investigate the stereospecificity of axonal membrane receptor macromolecules. Enantiomeric axon labelling reagents were synthesized previously from the optical isomers of amphetamine. R and S-p-amino- and p-nitro (2-dimethylaminopropyl) benzene are structurally similar to some local anesthetics. Their local anesthetic potency was tested in spider crab walking leg nerves. All the compounds blocked conduction in millimolar concentrations, but none exhibited any stereospecificity. The effects of both enantiomers of the more active p-nitro derivative proved to be poorly reversible. Fluorescence signals from optically-active conformationally-defined dyes were analyzed in externally stained crab leg nerves. Dansyl-R- and dansyl-S-amphetamine, dansyl-R- and dansyl-S-phenylalaninol, and the danthoyl(carboxamido) analogs of these ligands (P. Quain & H. G. Mautner, Abstr. Am. Chem. Soc., Sept., 1976, BIOL-199) exhibited no measurable stereospecificity. Signals of equivalent intensity were produced by the dyes upon electrical stimulation of nerve fibres. These findings are not compatible with the idea that local anesthetics are attached to specific receptors in axonal membranes. (Supported in part by NINDS (NS 09608) and a fellowship from the Medical Foundation, Inc.)

TH-POS-114 CHEMOTAXIS TOWARD OXYGEN IN EUGLENA GRACILIS. G. Colombetti* and B. Diehn, Department of Chemistry, The University of Toledo, Toledo, Ohio 43606.

In 0.1 mm thick suspensions illuminated with photosynthetically inactive light, the motile unicellular alga Euglena spontaneously aggregates in random locations. These clumps of motile cells then move radially outward from the point of aggregation, forming ring-like patterns that propagate with a velocity which depends on the initial cell concentration in the suspension. The patterns are reproducibly altered by illumination with monochromatic red and blue light. An analysis of these changes, and of the effect on them of adding to the suspension an inhibitor of photosystem II of photosynthesis, leads to the conclusion that the observed pattern formation reflects a chemosensory response of Euglena toward oxygen. The mechanism of this response will be discussed in terms of the cells' stimulus/response system and motor apparatus.

TH-POS-115 AN IMPROVED DYNAMIC MODEL FOR SLOWLY ADAPTING STRETCH RECEPTORS. G.N. Franz and D.G. Frazer*, Department of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

The model partitions receptor action into four aspects: mechanical filtering of the stimulus, mechano-electric transduction at the sensory membrane, spike generation, and the action of an electrogenic pump. Mechanical filtering accounts for the viscoelastic component of receptor adaptation as evident from the different dynamic responses to mechanical and electrical stimulation. The model excludes frequently proposed mechanical models of the receptor-substrate complex as incompatible with experimental data. Mechano-electric transduction is assumed to be the consequence of surface area changes of the sensory neural membrane. On the basis of morphological information several functions relating receptor deformation and surface area change are considered, two of which have the necessary nonlinear characteristics to explain the time course of adaption typical for slowly adapting stretch receptors, i.e., creep and stress relaxation of tissue proportional to log t are transformed into responses of the firing frequency of the form t^{-k}. Conductance changes of the sensory membrane are modelled as functions of surface area change. The spike generator is represented by a "leaky" integrator; this is compatible with experimentally observed "minimal, adapted firing frequencies". Dynamic asymmetries are accounted for by nonlinearities in mechanical filtering, in mechano-electric transduction and spike generation, and by the action of an electrogenic pump.

TH-POS-116 SPATIAL DECAY OF THE RESPONSES TO STRETCH AND TO INJECTED CURRENT IN THE COXAL RECEPTORS OF THE CRAB SCYLLA. M. Mirolli, Medical Sciences Program, Indiana University School of Medicine, Bloomington, Ind. 47401

The sensory dendrites measure 0.8 to 1.0 cm in length and 0.008 to 0.012 cm in diameter. The diameter is not constant, being smaller at the proximal end. In thirteen experiments the response to stretch was studied with two microelectrodes, one inserted distally and one proximally. The response to stretch consisted of a spike-like initial deflection followed by a sustained depolarization. Both components spread electrotonically in the dendrites. The sustained depolarization decayed with a length constant, lambda (assuming the infinite cable model), of between 3.5 to 6.5 cm; the spike-like component was much more attenuated. In ten experiments a third pipette was used to inject current. The steady state response to current injected distally decayed with a lambda of 7 to 9 cm and that to current injected proximally with lambda of 2.3 to 4.0 cm. The input resistance varied between 0.9 to 2.0 MΩ. The apparent reversal potential of the steady state response to stretch was about +20 mV.

TH-POS-117 ASSOCIATION OF GUANYLATE CYCLASE WITH THE AXONEME OF BOVINE RETINAL RODS. Darrell Fleischman, Dan Raveed* and Michael Denisevich*, Charles F. Kettering Research Laboratory, Yellow Springs, Ohio 45387.

Retinal rod outer segments previously have been shown to display especially high levels of guanylate cyclase and cyclic GMP-specific phosphodiesterase activity. We have found that treatment of bovine rod outer segments with a 2% solution of the non-ionic detergent triton X-100 (at pH 7.0 and in the presence of 5mM Mg^{++}) does not solubilize the cyclase activity. After separation of the detergent-dispersed material by isopycnic centrifugation in a detergent-containing sucrose density gradient, the cyclase activity is found to be localized in a turbid band at about 55% sucrose. Electron microscopy reveals that the band contains the rod axonemes, along with attached basal body, rootlets and centrioles. SDS polyacrylamide gel electrophoresis indicates that the major proteins present have molecular weights of about 56,000 d and 45,000 d, and are present in 1:1 molecular ratio. The former protein co-migrates with tubulin from Euglena flagella. The cyclase cannot be solubilized by washing the isolated axonemes with additional triton X-100 solution, or with 100 mM or 600 mM KCl solutions.

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TH-POS-118 BIREFRINGENCE GRADIENTS IN FROG ROD OUTER SEGMENTS. M.W. Kaplan, P.A. Liebman, and M.E. Deffebach*, Neurological Sciences Institute, Good Samaritan Hospital, Portland, OR 97210 and Dept. of Anatomy, Univ. of Pennsylvania, Philadelphia, PA 19174.

Birefringence (Δn) measurements of R. pipiens rod outer segments (ROS) reveal microstructure inhomogeneities not seen using other techniques. In the basal 20-30 µm length of the ROS, there is a nearly linear axial gradient in Δn of $\simeq -2 \times 10^{-5}$/µm (eg. $\Delta n = .0009 \rightarrow .0005$). A much smaller gradient of $\simeq -4 \times 10^{-6}$/µm is usually seen in the distal 20-30 µm (eg. $\Delta n = .0005 \rightarrow .0004$). Occasionally a highly birefringent ($\Delta n \simeq .004$) band 2 µm long is seen at the basal end of the ROS, possibly where the envelope membrane folds to form new disk membranes. The measured Δn is the sum of a positive intrinsic Δn (due to aligned anisotropic molecules in the disk membranes) and a negative form Δn (due to the regular layering of the disk membranes in the cytoplasm matrix). Using a glycerol imbibition technique to separate the intrinsic and form components (M.W. Kaplan & P.A. Liebman, J. Physiol, in press), the basal Δn gradient is seen to be a gradient of intrinsic Δn only. Such imbibition measurements also show that the membrane volume fraction is uniform along the cell, so the Δn gradient must be due to a gradual change in the organization of the disk membranes as they get farther from the inner segment. Since axial distance from the inner segment is proportional to the time since the disk membrane was formed, the Δn gradient may reflect an aging process. The possibility exists that this membrane aging may play a role in regulating the removal of disk membranes at the distal end of the ROS. (Supported by NIH grant EY01779).

TH-POS-119 ^{3}H-OUABAIN BINDING TO FROG RETINA. C.E. Stirling, Department of Physiology, University of Washington, Seattle, Wa. 98195

Measurements of extracellular potentials with paired microelectrodes indicate that in the absence of light there is a flow of current between the inner and outer segments of vertebrate photoreceptors. Ion substitution experiments further suggest that this dark current is carried by Na^+. From these observations (Hagins & Yoshikami, Ann. N.Y. Acad. Sci. 264: 314, 1976) one might predict a high density of Na^+ pump sites on the inner segment cell membrane and an absence or low density of sites on the outer segment cell membrane. This hypothesis was tested using ouabain as a marker of Na^+-K^+ transport sites in the isolated frog (Rana Pipiens) retina and high resolution autoradiography (Stirling, J. Microscopy 106: 145, 1976) to localize retinal bound cardioglycoside. In accordance with the above hypothesis, a high density of ouabain binding was found in the inner segments and synaptic pedicles of rods. The outer segment exhibited little or no binding. Other regions of the retina also bound ouabain and in general, the density of binding was highest where the cell membrane density was highest (ie. synaptic regions). No significant difference in binding distribution was noted in the dark adapted vs. the light adapted retina. The presence of a limited number of binding sites was demonstrated by raising the ouabain concentration from 2μM to 500μM with unlabelled glycoside. The specificity of binding was demonstrated using high K^+ (50mM) incubation medium to slow the rate of ouabain binding. Supported by PHS grant AM-13182.

TH-POS-120 A LIGHT-ACTIVATED GTPase IN RETINAL ROD OUTER SEGMENTS. W.E. Robinson and W.A. Hagins, Laboratory of Chemical Physics, National Institutes of Health, Bethesda, Maryland 20014.

Both theory and experiment indicate that a single photon absorbed in a rod outer segment must cause the intracellular release of hundreds of transmitter molecules to produce a sensory response. Since the free energy in the photon is insufficient by itself to release or reaccumulate such a large number of transmitter molecules, some other source of free energy seems required in rod excitation, at the stage preceding control of the inward Na^+ current at the plasma membranes. It has been previously shown that endogenous ATP in isolated frog rod outer segments decreases in light when the plasma membranes are broken [Robinson, Yoshikami and Hagins, Biophys. J. 15, 168a (1975), Carretta and Cavaggioni, J. Physiol. 257, 687 (1976), Robinson and Hagins, Fed. Proc. 35, 1555 (1976)]. The biochemical basis of this effect has been studied in rod discs using as substrates ATP and GTP labeled with ^{32}P in the γ phosphate and with ^{3}H in the base. GTP decreases in the light, producing equal amounts of GDP and Pi. The light-activated decrease in ATP does not produce Pi. Instead, the γ phosphate of ATP is apparently transferred to GDP to regenerate GTP. The sequence of reaction is thus:

$$GTP \xrightarrow{h\nu} GDP + Pi \qquad\qquad ATP + GDP \rightleftharpoons ADP + GTP$$

There is little or no GTPase in dark adapted rods, but bleaching 1 rhodopsin in 5000 activates the enzyme 70%. The action spectrum is that of rhodopsin. At low light exposures (1 rhodopsin bleached in 5000) and at a substrate concentration of 0.2 μM, the GTPase splits more than 15 GTP per absorbed photon within 4 secs. While the significance of this reaction is not yet clear, it could yield the large amounts of free energy needed for rod excitation.

TH-POS-121 EFFECTS OF INTRACELLULAR PROTON AND METAL BUFFERS ON LIGHT RESPONSES OF RETINAL RODS. W.A. Hagins, NIAMDD and S. Yoshikami, NEI, N.I.H., Bethesda, Md. 20014

Ca^{++} has been proposed as an intracellular transmitter of visual excitation from rod disks to the plasma membrane of rod outer segments, where it binds reversibly and transiently reduces the inward dark Na^+ current (Yoshikami & Hagins, Biophys. J. 11, 47a (1971)). To test whether an increase in Ca^{++} activity in rod cytoplasm is an essential step in excitation, EGTA, CDTA and other metal and proton buffers have been introduced into rods at ∿10μM concentrations by the vesicle fusion technique using a fluorescent dye to monitor the extent of intracellular buffer transfer. (Weinstein, et al. Biophys. J. 15, 104a (1976)). Both EGTA and CDTA reduce the size of extracellularly recorded electrical responses of rat rods to weak light flashes (<100 photons absorbed rod^{-1} $flash^{-1}$) but the responses to bright flashes are not affected. Lowering external [Mg] from 1 MM to 50μM increases the desensitizing effect of CDTA but not EGTA. Proton buffers had no effect on the light responses. Analysis of the amplitude-intensity curves for retinas containing EGTA and CDTA suggest (1) neither protons nor Mg^{++} ions act as excitatory transmitters, (2) that the free Ca^{++} activity in dark adapted outer segments is 0.5 to 1μM, (3) that a single absorbed photon releases 400 to 1000 free Ca^{++} ions in the outer segment cytoplasm.

TH-POS-J1 ESR STUDIES OF HEME PROTEINS IN GREEN PLANT PHOTOSYNTHESIS. J. T. Warden, Biochemistry Program, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, N.Y. 12181

Cytochromes of the b-type have emerged recently as energy transducing candidates for phosphorylation in green-plant photosynthesis and mitochondrial respiration. However due to the low in-situ concentration of the Cytochromes b in the green plant chloroplast, as well as a requirement for cryogenic temperatures, an electron spin resonance (esr) study of the green plant hemoproteins has not been reported to date. This report summarizes the first esr identification of the b-type chloroplast cytochromes.

Examination of chloroplast fragments from spinach in the presence of 5mM ferricyanide at 9K revealed three esr resonances in the region of $g \sim 6$. These resonances ($g \sim 5.66$, $g \sim 6.12$ and $g \sim 6.74$) are characteristic of high-spin, ferric, heme-proteins. Incubation of the chloroplast fragments with dithionite eliminates all three resonances; however the mild reductant, ascorbate, quenches only the resonance at $g \sim 6.12$. These data suggest that the low-field esr resonances arise from oxidized heme proteins. Potentiometric analysis as well as a comparison of the chloroplast heme signals with that observed from purified, chloroplast Cytochrome b-559 indicate that the $g \sim 6.12$ resonance originates from low-potential ($E_m \sim 50$ mV), ferric Cytochrome b-559. The other esr resonances have been assigned tentatively to Cytochrome b_6. Complete characterization of these heme signals is in progress.

TH-POS-J2 PARTIAL CHARACTERIZATION OF NUCLEOTIDE BINDING TO CHLOROPLAST COUPLING FACTOR USING FLUORESCENCE ANALYSIS. Dave VanderMeulen and Govindjee, Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801

Measurements of cyclic and non-cyclic photophosphorylation, ADP or ATP inhibition of ferricyanide reduction, and ATP stimulation of proton uptake in isolated chloroplasts demonstrated that the fluorescent-modified analogs (εADP and εATP) substitute well (60-85%) for the unmodified nucleotides. Our results further show that (1) fluorescence polarization using a photon counting instrument can be used to study binding of εADP and εATP to isolated coupling factor (CF_1) in its ATP-synthetase form as these nucleotides bind well to CF_1, and (2) the cofactors of photophosphorylation (ADP, ATP, P_i, Mg^{++}) interact directly at the level of binding to CF_1. The affinity of εADP for CF_1 is dependent on the ionic content of the medium (30 mM Tricine-NaOH, pH 8) increasing in the order: buffer only <100 mM NaCl + 0.5 mM $MgCl_2$<100 mM NaCl + 5 mM $MgCl_2$<0.5 mM $MgCl_2$<5 mM $MgCl_2$. In the presence of 5 mM $MgCl_2$, both εADP and εATP exhibit two binding sites with dissociation constants (K_d) of ~0.5 μM and ~2 μM; with 5 mM $MgCl_2$ + 0.5 mM KH_2PO_4 the binding affinity is reduced, with $K_d \simeq 10$ μM. With 10 μM ATP the K_d's for the εADP binding sites are increased about tenfold, but with 10 μM ADP the K_d's for εADP were unmeasureably high, since the polarization (binding) was nearly zero at all concentrations. Analogously, 10 μM ADP increases the K_d's for the εATP binding sites by about an order of magnitude, whereas 10 μM ATP almost completely prevented binding of εATP. Binding of εAMP could not be detected: the polarization of εAMP fluorescence was essentially zero with $MgCl_2$, $MgCl_2$ + KH_2PO_4, $MgCl_2$ + ADP, or $MgCl_2$ + ATP. The involvement of changes in CF_1 conformation, monitored by changes in the polarization of fluorescence of tyrosine residues intrinsic to CF_1, will also be presented.

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TH-POS-J3 A COMPARISON OF THE KINETICS OF THE PHOTOREDUCTION OF CYTOCHROME b-559 AND PLASTOQUINONE IN SPINACH CHLOROPLASTS. J. Whitmarsh* and W. A. Cramer, Department of Biological Sciences, Purdue University, W. Lafayette, Indiana 47907.

The role of the chloroplast cytochrome b-559 in photosynthetic electron transport is not known. It has been shown that the amplitude of oxidation of b-559 by system I can be increased in the presence of low concentrations of PMS, and after preillumination with high intensity actinic light. Inhibition of these oxidation reactions by low concentrations of DBMIB implies the existence of a pathway to photosystem I. The rate of this photooxidation is slow, and the amplitude decreases with increasing far-red light intensity. However, in the presence of PMS it is shown that the quantum yield of cytochrome b-559 photooxidation by PS I is comparable to that of cytochrome f. We have studied the rate of the photoreduction of high-potential (i.e. hydroquinone reducible) cytochrome b-559 and plastoquinone. Cytochrome b-559 was oxidized with low intensity far-red light in the presence of PMS or after preillumination. Using long flashes of red light, the half-reduction time of cytochrome b-559 was found to be 100 ± 10 ms, compared to 6-10 ms for the photoreduction of the plastoquinone pool. The photoreduction is inhibited by DCMU and accelerated in the presence of DBMIB ($t_{\frac{1}{2}} \simeq 35$ms). The addition of uncouplers, which caused a stimulatory effect on ferricyanide reduction under the same experimental conditions, resulted in a decrease in the rate of cytochrome b-559 reduction. The relatively slow rate of cytochrome b-559 photoreduction implies that electrons can be transferred efficiently from PS II to plastoquinone without the involvement of cytochrome b-559 as an intermediate. These results indicate that it is unlikely that high-potential cytochrome b-559 functions as an obligatory redox component in the main electron transport chain joining the two photosystems. (Supported by NSF grant BM75-16037X).

TH-POS-J4 PARAMAGNETIC COUPLING BETWEEN THE REACTION CENTER INTERMEDIATE AND THE IRON-QUINONE COMPLEX IN PHOTOSYNTHETIC BACTERIA. D.M. Tiede*, R.C. Prince*, and P.L. Dutton* (Intr. by S. Goodgal), Dept. of Biochem. & Biophys., Univ. of Pennsylvania, Phila., PA 19174.

We have previously described the EPR spectra of the photochemically trapped, reduced reaction center intermediate, I^-, in Rps. viridis and C. vinosum. The spectra for I^- were shown to be composed of two signals easily distinguishable by relaxation properties into an isotropic free-radical g 2 signal and a pair of asymmetric absorbances split by ∿60 gauss in C. vinosum and ∿100 gauss in Rps. viridis, centered about g 2. We demonstrate here that the observed splitting of the I^- signal arises as a result of a paramagnetic interaction between I^- and the reduced "primary" acceptor, the quinone-iron complex Q^-Fe. The integrated intensities for the double I^- signal are shown to have temperature dependencies which follow exactly the unusual non-Curie law dependence of the Q^-Fe g 1.82 signal. This temperature dependence of the Q^-Fe has been interpreted in terms of thermally accessible QFe high spin states, in which the ground state is observed as the g 1.82 signal (J.C. Salerno, unpublished). Thus the coupling between I^- and Q^-Fe radicals as observed by the splitting of the I^- signal and prominent alterations of the QFe g 1.82 signal occurs only at low temperatures, corresponding to the population of the QFe ground state. In addition, experimental manipulation of the g 1.82 Q^-Fe signal in Rps. viridis, where chromatophores show prominent g 1.82 signals and I^- appears solely as the split signal and elimination of the g 1.82 signal results in I^- appearing entirely as the isotropic g 2 form, lends further support to the proposal that the splitting of the I^- signal represents an expression of spin interaction between I^- and Q^-Fe, and that this is the in vivo situation. Supported by NSF PCM 76-14209.

TH-POS-J5 THE EFFECT OF MANGANESE EXTRACTION ON THE MIDPOINT POTENTIAL OF CHLOROPLAST CYTOCHROME b-559 AND ON PHOTOSYSTEM II ACTIVITY. Peter Horton, Division of Cell and Molecular Biology, SUNY at Buffalo, Buffalo, NY 14214.

The role of cytochrome b-559 in photosystem II reactions has been investigated using treatments which extract manganese from the thylakoid membranes. Incubation of chloroplasts with hydroxylamine in darkness resulted in inhibition of photooxidation of H_2O (but not other electron donors) and loss of hydroquinone reducible cytochrome b-559. Loss of H_2O oxidizing activity perfectly correlated with the decrease in amplitude of reduction of b-559 by hydroquinone. Redox titration of b-559 after hydroxylamine treatment showed a b-559 component with $Em_{7.8}$ at approximately +180 mV compared to the control value of +370 mV. The Mn content had decreased by 55-65%. Incubation with hydroxylamine in the light further reduced the Mn content to 5-10% of the control and resulted in an inhibition of photosystem II mediated photooxidation of donors such as hydroxylamine, catechol, benzidine and hydroquinone but not ferrocyanide. The midpoint potential of cytochrome b-559 in these chloroplasts is decreased further to a value of +70 mV. It seems that the loss of photooxiding activity of photosystem II is associated with a two stage removal of membrane-bound Mn and a similar stepwise decrease in midpoint potential of cytochrome b-559. This work is supported by NSF grant PCM 76-09669.

TH-POS-J6 NMR STUDIES ON CHLOROPLAST MEMBRANES: FREQUENCY AND TEMPERATURE DEPENDENCE OF WATER PROTON RELAXATION RATES. T. Wydrzynski and Govindjee, Dept. of Physiology and Biophysics, S.B. Marks*, P.G. Schmidt and H.S. Gutowsky*, Dept. of Chemistry, University of Illinois, Urbana, Illinois 61801

Longitudinal (T_1^{-1}) and transverse (T_2^{-1}) proton relaxation rates of water in suspensions of chloroplast thylakoid membranes were measured as a function of nmr frequency and temperature in order to gain some insight into the relaxation mechanisms involved. A significant part of T_1^{-1} and T_2^{-1} in the chloroplast system can be attributed to the effects of membrane bound manganese. Extraction of the loosely bound fraction of Mn by exchange with Mg leads to decreases in T_1^{-1} values. The T_1^{-1} frequency dependance shows a broad maximum in the range 19-27 MHz, similar to the behavior observed in other systems containing bound Mn (II). The T_2^{-1} values increase with increasing nmr frequency up to 90 MHz. This frequency behavior suggests that the correlation time governing proton relaxation in the chloroplast system is frequency dependent and that it is dominated by the electronic relaxation time of bound paramagnetic ions. The values of T_1^{-1} and T_2^{-1} at 8, 27 and 64 MHz increase with decreasing temperature in the range 7-38° C, the rates leveling off at the lower temperature. Activation energies at the steepest part of the temperature curves are ~2.8 kcal/mole for T_1^{-1} and ~1.6 kcal/mole for T_2^{-1}. The negative temperature dependence, low activation energies and large T_1/T_2 ratio (~3) indicate that the observed rates are not in a slow exchange limit for the chloroplast system. Calculated values of the correlation times involved, based on a Solomon-Bloembergen-Morgan analysis of the experimental data, will be presented. This research was supported by NSF Grants GB 36751 to G. MPS 73-04984 to H.S.G. and NIH Grant GM 18038 to P.G.S.