Effects of neural modulators were studied in single cortical neurons that undergo adaptations that support learned behavior. Ionic conductances were measured directly, in vivo, using single electrode voltage clamp techniques. After extracellular application of acetylcholine and intracellular application of cyclic GMP dependent protein kinase decreases in fast outward membrane current were found. These modulatory agents are thought to regulate simple forms of learning in cortical cells. Other experiments studied effects of cyclic AMP and cyclic GMP on neuronal properties and identified regions of the hypothalamus that, when stimulated, increased rates of acquisition of simple learned behavior. Single units of the motor cortex and their adaptive properties were studied before and after rapid conditioning and extinction of conditioned motor responses. The results provide insights into the design and operation of elements that serve adaptive neural networks within the brain needed for some forms of learning.
STATEMENT OF WORK OBJECTIVES

1. Characterize in identified cortical neurons the effects of neurotransmitters potentially involved in accelerating rates of conditioning.

2. Examine the role of specific neuromodulators, such as cyclic GMP dependent kinases in controlling cellular adaptations supporting learned behavior.

3. Identify specific regions of the hypothalamus that when stimulated affect the rates of conditioning.

4. Define the patterns of unit activity and postsynaptic potentials evoked in cortical neurons by hypothalamic stimulation.

5. Conduct on-line data analysis and identify those cortical neurons showing response to hypothalamic stimulation.

STATUS OF RESEARCH
(1986-87, Year 2 of three years)

1. Outward currents were measured in neurons of the pericruciate cortex of awake cats using single electrode voltage clamp techniques. Holding currents ranged from -65 to -95 mV with depolarizing steps of 10 to 40 mV. Iontophoretic applications (90-95 nA, 30 sec) of 2 M acetylcholine (ACh), extracellulary, produced decreases in net transient, early outward currents (with currents measured 25-64 ms after onset of depolarizing steps subtracted from currents measured 0-24 ms after onset) in each of six cells that were tested and reduced early outward currents (averaged over the period 0-24 ms after onset of depolarizing steps) by more than 2 standard errors of the mean initial values in three of the cells. One additional cell tested showed a decrease in the early outward current but spiking was too great to permit quantification of the magnitude of the changes. Iontophoretic applications (90-95 nA, 30 sec) of 2 M saline did not significantly reduce the early outward currents in any of 13 cells tested. Intracellular pressure injections of cyclic GMP dependent protein kinase (cGPK) mixed with 10 uM cGMP decreased the net transient, early outward currents (3-12 minus 15-24 ms period) in each of 5 cells tested. Decreases in early currents in response to ACh and cGPK averaged 1.01 and .94 nA with respect to changes after saline. The average net change after saline was +0.18 ± 0.14 (sem) nA, after ACh -0.28 ± 0.06 nA, and after cGPK -0.56 ± 0.2 nA. Though preliminary in number, these results demonstrated significant reductions in outward currents in single neurons in response to ACh and to cGPK plus 10 uM cGMP. Nonparametric statistical analyses (Fisher) of the numbers of cells showing changes disclosed significant differences in effects on outward currents between ACh or cGPK and saline (p < 0.05). Parametric analyses (t tests) of the magnitudes of change in current showed significant differences in early and net transient, early outward currents between the same groups (p < 0.05). The findings demonstrate that the single electrode voltage clamp method can be used to detect changes in conductance in cortical neurons, in vivo, and provide direct evidence in support of the hypothesis. (Krnjevic, et al., J. Physiol., 1971; Woody, et al., Brain Res., 1978; Woody, et al., Exp. Neurol., 1986) that one of the actions of muscarinic cholinergic agents and their second messengers in cortical cells is to reduce an outward ionic conductance. (Woody and Gruen, Soc. Neurosci. Abstr., 12:725, 1986.)

2. Unit activity and excitability of neurons of the cat pericruciate cortex were studied after rapid acquisition of conditioned blink responses. Conditioned eyeblink responses with short (16-60 ms) onset latencies developed rapidly, within 5-50 trials, after pairing click CS, glabella tap US, and electrical stimulation of the hypothalamus (HS) at an interstimulus interval of 570-10 ms between CS and US-HS. (Pairings of the same CS and US without HS require hundreds of trials, over days, for equivalent levels of conditioning.) Longer latency (80-240 ms) eye blink responses developed later after further application of conditioning trials. When CSs were presented alone after conditioning, the number of CRs decreased gradually; spontaneous recovery of CRs occurred between extinction sessions given for 1-5 days (learning savings). Another control paradigm in which HS was given 2.5 s before each CS-US pairing ("backward HS") did not produce rapid acquisition of CRs.
The amount of neuronal activity elicited by the CS increased with conditioning. The discharges preceded blink responses with latency sufficient to control production of the learned response. During extinction, neuronal responses to the CS decreased but remained greater than in the naive state. Threshold levels of current needed for spike elicitation were significantly lower after than before conditioning in each of 5 cats tested. The "backward HS" paradigm was less effective in increasing neural excitability and did not result in significant differences in excitability before and after these sessions in each of 4 cats. After extensive extinction, the threshold level of spike initiation increased toward the level in the naive state in 3 cats but remained at a level comparable to that in the conditioned state in 2 cats. The latter 2 cats showed more persistent spontaneous recovery of CRs during extinction than did the former 3 cats. (Aou, Birt and Woody, Soc. Neurosci. Abstr., 12:555, 1986.)

3. Specific regions of the hypothalamus were identified that when stimulated increased rates of conditioning as described above (Fig. 1).

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Fig. 1. Locii of the hypothalamus at which electrical stimulation was applied to produce accelerated rates of conditioning. (Some animals were stimulated on left as well as right sides, each side unilaterally, in separate experiments.) Cd, caudate nucleus; Ch, optic chiasm; Cl, internal capsule; En, entopeduncular nucleus; Fx, fornix; GP, globus pallidus; LH, lateral hypothalamus; MB, mamillary body; Th, thalamus; TO, optic tract; VA, anterior ventral thalamic nucleus. (Numbers are anterior stereotaxic planes in mm, Snider and Klener's atlas.)
4. Intracellular injections of cyclic AMP (cAMP) and horseradish peroxidase (HRP) were made in neurons of the motor cortex of awake cats. Eighty-six percent of injected cells responded to cAMP and HRP with a rapid decrease in input resistance. The decreases in input resistance occurred immediately after injection and began to return toward baseline two to three minutes later. The decreases were significantly greater than the small decreases in input resistance normally seen in un.injected cells held for two minutes or more after penetration and exceeded comparably small decreases in input resistance seen after control injections of 5' AMP plus HRP. Pyramidal cells of layer V were identified as responding to cAMP with a decreased input resistance. A spiny stellate cell of layer III and a pyramidal cell of layer VI were also identified that showed similar responses. The cells also showed increased rates of discharge after penetration with electrodes containing cAMP, but significant changes in input resistance were not found in association with the increased rates of discharge. After pressure injection of cAMP, the rates of discharge fell toward more normative levels. Our findings indicate that cAMP has an effect on cortical neurons similar to that found in some types of invertebrate (molluscan) neurons and dissimilar to the effect of cyclic guanosine monophosphate. (Woody and Gruen, Exp. Neurol., 1986.)

5. Cyclic cGMP and horseradish peroxides (HRP) were injected intracellularly in neurons of the motor cortex of awake cats. Fifty-four percent of injected cells responded to cGMP and HRP with an increase in input resistance within 30 sec after injection. None of a control group of cells injected with HRP without cGMP so responded. In cells given intracellular depolarizing current sufficient to produce repeated spike discharge at the time of injection, the increase in input resistance after cGMP persisted for as long as the cells could be held. There was no significant increase in firing rate after injection of cGMP. Cells responding to cGMP with an increased input resistance were identified as pyramidal cells of layer V. One inverted pyramidal cell of layer VI also showed an increase in input resistance in response to cGMP. Previous studies have suggested that muscarinic cholinergic agents produce an increased input resistance (thought to reflect a decreased potassium conductance) underlying an increased rate of discharge in neocortical neurons. Our results favor a dual action of muscarinic cholinergic transmission in mammalian cortical neurons -- the increase in input resistance in layer V pyramidal cells being mediated by cGMP, the increase in rate of discharge being otherwise mediated. (Woody et al., Exp. Neurol., 1986.)

6. Studies were concluded on effects of pressure injecting purified, cyclic GMP-dependent protein kinase (cGPK) into neurons of the cat precruciate cortex. Input resistances increased within seconds after injection and remained elevated for two minutes or longer. The increases were larger when cGPK was injected in a mixture with 10 uM cGMP than when injected alone. Injections of heat-inactivated cGPK, with or without 10 uM cGMP, failed to produce increases in input resistance. The results indicate that injection of activated cGPK into neurons of the mammalian motor cortex can mimic actions of extracellually applied acetylcholine and intracellurally applied cGMP, the latter in hundred-fold higher concentrations than those used here, in neurons of the same cortical areas. Since the effects of acetylcholine, cGMP and cGPK are identical with the increases in excitability and input
resistance produced in similar (layer V) cortical pyramidal neurons after conditioning, it is possible that these are the modulations that mediate the conditioning change in these neurons. Voltage clamp studies suggest that cGPK acts by decreasing an outward potassium conductance. (Woody et al., Brain Res., 1986.)

7. Intracellular effects of CS and US presentations were studied in cells of the motor cortex of awake cats. Behaviorally, conditional stimuli (CS) are distinguished from unconditional stimuli (US) by the ability of the US to produce an unconditioned motor response. Appropriate pairing of a CS with a US results in the development of a conditioned response (CR) to the CS, but pairing one CS with another CS does not. An important issue in studying the neural basis of conditioning is to determine how stimuli which serve as USs differ from stimuli which serve as CSs at the cellular level. Glabella tap and click have been used extensively as US and CS in eyelink conditioning. Cells of the motor cortex have been shown to be necessary for blink conditioning to occur with these stimuli. Intracellular recordings were obtained from 92 cells in 8 awake cats of the response to tap US and from 55 cells in a separate group of 8 cats of the response to click CS. Averaged spike histograms made from these two groups of cells showed differences in the magnitude of evoked discharges in response to click and tap. Peak rates of firing elicited by tap-US were significantly larger (t test p < .01) than those elicited by click-CS and the proportion of cells responsive was higher for tap than click (chi square p < .05). Averages of postsynaptic potentials prepared by digitizing the intracellular recordings of membrane potential, digitally removing spikes, averaging all trials for each cell, and then averaging results from all cells showed a greater depolarization in response to tap than to click (t test p < .05). Analysis of spike histograms and PSPs in single cells also disclosed inhibitory responses which were not apparent in the overall averages. When analyzed cell by cell, the magnitude of reduced discharges seen in spike histograms was greater for click than tap (t test p < .01) as was the proportion of cells showing such reductions. (Birt, Aou and Woody, Soc. Neurosci. Abstr., 12:555, 1986.)


9. The above results provide the first measurements of cellular mechanisms that might directly support mammalian learning since the mechanisms have been studied in the context of a specific model of operational behavioral learning and have been found in cells necessary for the acquisition of the learned behavior. Further studies of these mechanisms are being pursued during conditioning of single cortical units.
LIST OF PUBLICATIONS

1. Publications Supported by Current AFOSR Research (Contract No. F49620-85-C-0100)


II. Publications Supported by Earlier AFOSR Research, 1976-1985


PROFESSIONAL PERSONNEL ASSOCIATED WITH THE RESEARCH EFFORT

Charles D. Woody, M.D.
Shuji Aou, M.D., Ph.D.
Attila Baranyi, Ph.D.
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William Holmes, Ph.D.*
Dorwin Birt, Ph.D.

*Ph.D. in Biomathematics awarded January 1986 for thesis titled: "Cable Theory Modeling of the Effectiveness of Synaptic Inputs in Cortical Pyramidal Cells."
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