Review article

Acta psychiatr. scand. 1986:74:312–334

Key words: affective disorders; acetylcholine; cholinergic; depression; mania; stress; anticholinergics; muscarinic receptors; calcium; sodium.

Cholinergic mechanisms in affective disorders

Future directions for investigation

S. C. Dilsaver

Clinical Studies Unit for Affective Disorders, Department of Psychiatry, Mental Health Research Institute, and Neuroscience Laboratory, University of Michigan, Ann Arbor, U.S.A.

ABSTRACT - Advances in clinical and basic research methodology combined with clearly articulated concepts create new opportunities for researching the roles of cholinergic mechanisms in the pathophysiology of affective disorders. Areas for study include: 1) roles of cholinergic mechanisms in mediating effects of stress and cholinergic mechanisms linking the pathophysiologies of affective and panic disorders, 2) use of pharmacologic agents to produce cholinergic system supersensitivity in modeling biologic aspects of affective illness, 3) use of multigenerational intrapedigree studies of cholinergic markers associated with affective disease, 4) research into the neurobiology of lithium and ECT as they pertain to muscarinic cholinergic mechanisms, 5) study of the interrelationship of sodium, calcium and lithium ion metabolism and their relationship to cholinergic-monoaminergic interaction, 6) the development of brain imaging strategies and techniques, e.g., positron emission tomography (PET), to measure changes in cholinergic receptor density and affinity as a function of clinical state, 7) identification and validation of a peripheral model of the central muscarinic receptor, 8) study of the pharmacology of abusable substances and its relationship to mechanisms regulating mood, affect, psychomotor function and other variables related to the affective disorders, and 9) development of *in vitro* and *in vivo* models useful in studying the physiology and biochemistry of the interaction of cholinergic and monoaminergic neurons. These models may allow us to bridge the traditional cholinergic and monoamine hypotheses of affective disorders.

Received September 21, 1985; accepted for publication April 5, 1986

Research into the pathophysiology of affective disorders tends to focus on disturbances of cholinergic or monoaminergic mechanisms. "In the limit" investigations must consider interaction of these neurotransmitter systems but for practical and heuristic purposes it is possible to isolate them. This article highlights areas for future attention by those interested in cholinergic mechanisms involved in the pathophysiology of affective disorders.

1. *Cholinergic mechanisms as mediators of the effects of stress and affective disorders*

The dichotomization of depression as biological *or* psychological is problematic. The stereotypical melancholic or endogenous episode can occur without situational provocation. However, subjects with affective disorders may have an unsituationally related episode of melancholia at

one point in life and another occurring in strong association with a major life event at another. Both can be biological *and* psychological events. Weiss et al. (1) reported that an uncontrollable stressor, a phenomenon often reductionistically conceived as being "psychological in nature," produced a fall in the concentration of the brain biogenic amines. This is supposed to be a feature of a "biological" depression. Lloyd (2) reviewed studies examining the hypothesis: life events precipitate depressive disorders. He found that relative risk of illness is increased five- to six-fold in the 6 months after a major life event. Kennedy et al. (3) found a two-fold increase in life events during the 4 months preceding hospitalization in a cohort of 20 manic patients. Events carrying a significant negative impact were more common among manic subjects independent of clinical status. Rasmussen et al. (4) observed a case of "loss-induced" depression in an adult Macaque monkey after a miscarriage. The animal developed anorexia, psychomotor retardation, weight loss and poor grooming. After several weeks without improvement, she was treated with amitriptyline, 5 mg/kg. Within a week she became active and resumed eating and grooming. The "stress" of reintroduction into her usual social environment was associated with a recurrence, but symptoms abated within 2 weeks. Her closest companion was then removed from the colony. Once again she developed features of melancholia (5). These points highlight the difficulty of categorically distinguishing the psychological and biological. What appears to have a psychological cause can have a biological basis. Further, published data and clinical experience both suggest that affective disorder subjects are at an increased risk for development of manic or depressive episodes in the face of stresses due to their neurobiological peculiarities.

Janowsky and associates (6-8) proposed that effects of acute and chronic stress are partially mediated by central muscarinic systems with capacities to activate adrenergic networks. Acetylcholine is unique among known neurotransmitters in its ability to simultaneously produce behavioral, cardiovascular, neuroendocrine and noradrenergic effects typifying stress. Aberrant cholinergic mechanisms are also involved in the pathophysiology of the affective disorders and regulation of mood (9, 10). This includes genesis of the phenomenology and neuroendocrinology and polysomnographic abnormalities of these conditions. Secondly, central cholinergic systems participate in regulation of blood pressure and heart rate (11-13). Thus, affective, neuroendocrine, sleep and autonomic changes following acute or chronic stress could have a cholinergic component.

The reason stress increases the frequencies of depressive and manic episodes is unknown. However, this observation can be explained within the context of a cholinergic-monoaminergic interaction theory (9). This model predisposes that an abiding supersensitivity of cholinergic systems renders affective disorders subjects liable to developing depressive *and* manic episodes. Primary components in this account are an inhibitory cholinergic system and activatory monoaminergic network. Depressive phases are marked by "cholinergic overdrive" and mania by a hypocholinergic-hypermonoaminergic state. However, cholinergic systems are unusually perturbable in every phase in bipolar subjects. During depressed phases, there is a drive toward compensatory down-regulation and subsensitivity of cholinergic and up-regulation and supersensitivity of monoaminergic systems. The reverse characterizes the switch from mania to depression. This model emphasizes dysfunction of *homeostatic* mechanisms and imbalance between the cholinergic and monoaminergic systems as pathogenic forces. Stressors further activating overly driven cholinergic systems can incline the organism to development of mania due to dysfunction of these homeostatic mechanisms. That is, the more effective cholinergic systems are in provoking compensatory or restorative responses in cholinergic systems (down-regulation and subsensitivity) the greater the probability of a "paradoxical" mania occurring.

Aberrant cholinergic mechanisms, affective disorders and panic disorder may be related. Nearly 50 years ago, Lindemann & Finesinger (14) reported that norepinephrine and methacholine produced panic attacks. Thus, both cholinergic and aminergic drugs have been shown to produce panic. These observations are consis-

tent with our understanding of the physiology of the autonomic nervous system. For example, muscarinic agonists precipitate release of catecholamines from the adrenal medulla and cardiac tissue (15). This cholinergic effect is so potent that what is initially a hypercholinergic state can terminate in a hyperadrenergic state. It is quite possible panic disorder has a cholinergic component. The literature pertaining to this topic was recently discussed elsewhere (16).

The major affective and anxiety disorders are statistically associated. Major depression combined with panic disorder in index probands is associated with a marked increase in risk for both major depression and panic disorder in first degree relatives (17). Leckman et al. (17) suggested that panic disorder and major depression may have a common diathesis. While not the same disease, they may have a coinherited component.

The pathophysiology of panic could involve the central nervous system, peripheral nervous system or both. First, a marked increase in cholinergic transmission has several effects on the cardiovascular system. Vasodilatation typically occurs with an ensuing drop in blood pressure, decreased heart rate and a reduction in myocardial contractility (15). These initial changes are generally blocked by acetylcholine-mediated release of catecholamines from cardiac and extracardiac tissues and a reflex blunting of the direct effects of cholinergic overdrive. For example, small doses of intravenously administered acetylcholine produce a fall in blood pressure due to generalized vasodilatation. However, this is accompanied by reflex tachycardia and followed by vasoconstriction via a baroreceptor response in animals. In sufficient doses, acetylcholine stimulates the release of catecholamines from cardiac stores and the adrenal medulla and activates sympathetic ganglia. At this juncture the animal is no longer in a hypercholinergic state - cholinergic overdrive has produced secondary adrenergic overdrive. Both panic episodes and cardiac arrhythmias, features of great stress, might be expected to result from this. This model accords with findings that catechoaminergic agents precipitate panic attacks (18).

Cholinergic mechanisms also mobilize brain aminergic systems (Section 9). Acetylcholine receptors mediate release of biogenic amines. Central adrenergic systems (9), some of which are subject to cholinergic influences, are involved in regulation of blood pressure, pulse, and anxiety. Cholinergic mechanisms may be important in the pathophysiologies of stress, anxiety and affective illness (19–33). There may be a pathophysiological overlap between the various anxiety and affective disorders. We now need to find means of biologically characterizing subjects who have *both* anxiety and affective disorders. Finally, the question of whether there are variables that distinguish patients in various diagnostic groups from those in others is important.

2. *Pharmacological perturbation strategies*

Pharmacologic perturbation strategies can be employed to study mechanisms affecting phenomenological, physiological, biochemical and receptor binding variables. Deviation of these variables from statistically defined norms can result from up-regulation or supersensitivity of cholinergic systems, abnormalities of cholinergic-monoaminergic interaction or both. These pathophysiological states can be safely and inexpensively induced in laboratory animals and man. The clinical foundation, neuropharmacology, and principles giving a research program based upon induction of cholinergic system up-regulation or supersensitivity were discussed elsewhere (36).

"Pharmacologic perturbation strategies" are based on the safety and effectiveness of producing pharmacological movement of neural systems from a pretreatment baseline for the purpose of modeling pathophysiology. These strategies are parts of multivariate programs involving measurement of behavioral, psychological, physiological, biochemical and receptor binding variables. This multivariate emphasis and "pharmacologic perturbation" are conceptually distinct components of one program.

The ability to establish correlations among clinical or phenomenological variables (mood, affect, psychomotor function, hedonia) (19–35), physiological measures (e.g., dexamethasone suppression test (DST) results, absolute post-dexamethasone plasma cortisol, ß-endorphin, and

adrenocorticotropin hormone (ACTH) levels (32, 33, 37–40), polysomnographic parameters (REM latency and density, etc.) (41–48), pupillometric data (49–52), biochemical indices (phospholipid turnover, rate of cyclic GMP generation) (52–58), and B_{max} and K_D for binding of muscarinic receptor ligands *in vivo* (59–70) enhances experimental versatility. It also provides a powerful means of studying the same phenomenon from the multiple aspects which define or determine it.

Drugs increasing the sensitivity of cholinergic systems to acetylcholine are useful for affecting pharmacological perturbation. Antimuscarinic agents (63–70), opiates (71–73), cannabinoids (74–78), and barbiturates (83–86) up-regulate and supersensitize these networks. Despite this, these drugs are not used to model the pathophysiology of affective disorder in animals. Both classical antimuscarinic agents (62–66) and tricyclics (67–69) up-regulate (62–70) and supersensitize (52, 87–95) cholinergic systems and are appropriate for use in human subjects.

"Pharmacologic perturbation" was used to study the pathophysiology of polysomnographic abnormalities of depressive disorders. Sitaram et al. (86) and Gillin and associates (93) gave scopolamine to normal subjects for three consecutive mornings and withheld the drug on the fourth. Decreased REM latency and increased REM density was observed the following night. Dilsaver & Greden (94, 95) found that withdrawal of tricyclic antidepressants is associated with limbic-hypothalamic-pituitary-adrenal (LHPA) dysregulation. This was shown by an increased frequency of positive DSTs and elevation in the absolute post-dexamethasone plasma cortisol level during the withdrawal phase. A definitive study would involve the random assignment of subjects to treatment with an antimuscarinic, a tricyclic and placebo, and determination of polysomnographic status before, during and after drug treatment.

"Pharmacologic perturbation strategies" can be used in conjunction with the measurement of multiple physiological endpoints subject to cholinergic regulation. This is based on the assumption that the function of neurotransmitter systems can be quantified by measuring effects of agonists and antagonists on physiological variables. Many measures are appropriate. These include polysomnographic, neuroendocrine, psychomotor, pupillary, electroencephalographic (EEG), thermoregulatory, salivary, and sweat parameters.

REM sleep and temperature activity rhythms are coupled both in depressive illness and health. This association may be adventitious or obligatory. Cholinomimetics and anticholinesterases produce hypothermia in animals (90, 91, 96–99) Chronic administration of scopolamine to mice results in a supersensitive hypothermic response to pilocarpine (90, 91). Conversely, chronic anticholinesterase treatment blunts the hypothermic response to cholinergic challenge (99). This capacity for manipulating cholinergic systems raises possibilities. For instance, Snider & Dilsaver (100) recently showed that chronic treatment with amitriptyline produces increased drops in core temperature in response to oxotremorine challenge. The effectiveness of oxotremorine as a hypothermic agent was increased 10- to 20-fold by treatment with amitriptyline, 20 mg i.p. twice daily. The meaning of this is highlighted by clinical investigations.

Avery et al. (101) studied a sample of nine affective disorder subjects and concluded that decreased REM latency, duration of the first REM period, elevated REM density and body temperature retain their typical relationships during a depressive episode. Does antimuscarinic treatment produce simultaneous phase advance of the REM and sleep-temperature cycles in normal man? This would implicate a cholinergic mechanism in *both* the pathophysiology of REM sleep and altered temperature activity cycle in the affective disorders. The associations of the REM-nocturnal cortisol latency (102) and sleep temperature cycle (103) are also open to study using this strategy. These cycles are coupled in normal and depressed subjects. Cholinergic mechanisms may well be important to this, and pharmacological perturbation techniques provide a way to study this.

Pupillometry (49–52) allows study of the sensitivity of a cholinergic system. Kelwala (49) found affectively ill subjects exhibit supersensitive miotic responses to pilocarpine relative to

control subjects. Responses to adrenergic agents did not distinguish groups. Dilsaver & Greden (50) concluded desipramine produced cholinergic system supersensitivity in depressed subjects. Twenty-one days of desipramine treatment, but not 7, produced enhancement of the miotic response to pilocarpine (51, 52). This could be due to either of two mechanisms. Desipramine may have sufficient antimuscarinic potency to produce supersensitivitiy to a cholinomimetic consequent to post-synaptic receptor blockade or noradrenergic properties of desipramine could shift the cholinergic-adrenergic status of the iris sufficiently to cause a functional denervation of cholinergic neurons. Nomura et al. (104) reported that isoproterenol caused up-regulation of muscarinic receptors and supersensitivity of cholinergic neurons in rat heart. This accords with the capacity of adrenergic systems to inhibit the release of acetylcholine and to thereby indirectly block post-synaptic muscarinic acetylcholine receptors.

Multiple indices of the status of cholinergic systems might provide a useful profile of cholinergic functions. This could serve several purposes. Neuroendocrinologists suggested this approach enhances the specificity, sensitivity and predictive value (105, 106) of laboratory tests (107, 108). Second, profiles might identify patient subgroups with unique clinical features, courses or responsiveness to treatment. Thus, a profile can become a nosologic instrument which distinguishes groups. Third, profiles might identify subgroups of affectively ill patients whose disorder is associated with mechanisms which differentiate them from other subgroups. Finally, a profile of cholinergic functions may be useful in isolating "homogeneous" subgroups of patients.

Pupillometry, the DST, measurement of absolute post-dexamethasone, ß-endorphin and ACTH levels, polysomnographic measurements, measures of mood, psychomotor function e.g., speech pause time (109, 110) or limb motility (111), hedonic capacity (defined by behavioral indices or scores on standard rating scales) (112), provide an array of variables which are both subject to cholinergic regulation and pertinent to affective disorders research. Some of these, e.g., mood, are now used as independent variables. However, categorization of variables is relative to knowledge, purpose and intent. As the strength of association of variables with affective disorders increases, the tendency to employ variables, previously categorized as dependent measurements, as independent measures increases. That is, the boundary between independent and dependent variables is malleable or fluid. Hence, as new and exciting research strategies become available, the pool of validated independent variables should increase, since many measures now regarded as dependent are expected to shift categories. When this occurs they are also useful in efforts to validate outstanding dependent variables. In this setting the pool of useful dependent variables also increases. Positron emission tomographic (PET) scanning is illustrative. This technique allows measurement of receptor binding variables. Prior to PET, the idea of using density of muscarinic receptors in the living human brain as a dependent variable was practically meaningless (though of theoretical value) but PET may make *in vivo* muscarinic receptor binding variables important dependent measures (59–61).

In summary, antimuscarinic agents induce up-regulation and supersensitivity of cholinergic systems. Measurement of change in behavioral, physiological, biochemical, and muscarinic receptor binding variables with cholinergic determinants provides means of quantitating effects of muscarinic receptor blockade. Incentive to use pharmacological perturbation strategies comes from evidence that cholinergic mechanisms participate in the pathogenesis of affective disorders. Dependent variables subject to change by cholinergic activating agents include mood, limb motility, speech pause time, hedonic capacity, rate of cyclic GMP (53–55) and phosphatidylinositol generation (56–58) body temperature, pupillary responsivity, time to onset of the first and second REM periods, DST status, absolute post-dexamethasone plasma cortisol, ß-endorphin and ACTH levels, and the regional distribution of mean EEG frequency (113, 114). Imaging strategies may soon create more possibilities.

Effects of antimuscarinics can be measured in

normal man by measuring dependent variables before, during and after pharmacological perturbation. These data could be compared to those characterizing an affective disorder sample while depressed, manic or euthymic, or analyzed in strict accordance with the possibilities suggested by the ABA design. Given this design and muscarinic receptor binding density and receptor affinity as dependent variables, and clinical state as an independent measure, six (depressed, B_{Max}; manic, B_{Max}; euthymic, B_{Max}; depressed, affinity, etc.) dependent-independent combinations arise for use in PET studies. There are other combinations, such as affective disorder categories versus other diagnostic groups and normal subjects. This illustrates the interrelationship between technological, methodological and conceptual advances in biomedical research.

Variables associated with affective disorders should be valuable dependent measures if they are sensitively and reliably measured, "quasi specific" to these disorders and associated with parameters which have previously been validated as markers of affective disease. This involves a process in which validation of variables now classified as dependent measures justifies their use as independent variables. Hence, former dependent measures can come to be used as standards in our efforts to validate as yet unvalidated dependent variables.

Adverse effects of muscarinic receptor blockade are not so frequent or severe as to prevent its use in normal human subjects. Antimuscarinic agents have been administered to man in doses sufficient to produce desired experimental effects without untoward effects (114–120).

3. *Affective disorders, associated psychopathology and cholinergic parameters: family history strategies*

Large pedigrees offer a means of studying the association of affective illness and other forms of psychopathology in the context of relative homogeneity. Researchers agree that there is considerable heterogeneity in a research population. This follows (in part) from the necessity to essentially limit criteria for affective disorders to cross-sectional features. This promotes variety, but variance can obscure differences between samples of a population and can complicate efforts to design experiments yielding meaningful data. Important differences among individuals are minimized. Search for biological traits or markers in a large pedigree with a high frequency of affective disorders in which the index proband has the feature of interest might minimize this problem.

Suppose 2% of all subjects with bipolar disorder have a given feature, Y, but that 2% of the general population also has this feature. In this situation no association of bipolar disorder and Y could be established. However, even if only 10% of the first degree relatives of subjects with bipolar disorder and feature Y have bipolar disorder and if 5% of these exhibit feature Y, while only 2% of the relatives without bipolar disorder have this characteristic, the investigators would establish association between Y and bipolar disorder if they have sufficient sample size.

Physiological parameters sensitive to cholinergic agents can be used to study the psychobiology of psychiatric syndromes clustering in a family. These include, 1) pupillometric (49–52), neuroendocrine (32, 35–40) and polysomnographic (41–48) responses to cholinomimetic challenge, and 2) changes in mood, affect and psychomotor function on physostigmine challenge (19–35). Restricting investigations of characteristics subject to cholinergic regulation to pedigrees with high incidences of affective disorders facilitates determining whether it is a trait or state marker regularly associated with the disorder of interest. It also allows assessment of modes of inheritance and degree of vulnerability associated with peculiarities of cholinergic systems.

Family history strategies are attractive, but there is difficulty in finding large pedigrees. However, it does not appear that there have been major research thrusts to study both the phenomenology and psychobiology of illness in subjects of large pedigrees. These pedigrees certainly do exist. For instance, diagnostic data was recently presented on 37 subjects in one family spanning three generations (121). A pedigree such as this provides means of studying the associations of cholinergic paramenters and affective illness. Complete specificity is too much to

expect. Markers would likely be observed both in ill and well relatives but tend to be associated with illness. If such a marker is a reliable and validated finding, i.e., a real trait, degree of risk associated with its presence can be measured. The association of this trait with other markers might represent a clustering of traits, all of which are seen in well relatives, but which when associated with one another confer increasing risk of illness. Mathematical models could also be used to study mode of inheritance of disorders and degree of heritability.

4. *The effects on muscarinic cholinergic systems of electroconvulsive therapy (ECT), lithium and tricyclic antidepressants*

ECT, lithium and the tricyclic antidepressants all act upon cholinergic systems in interesting ways. However, confounding variables interfere with efforts to interpret data pertaining to these actions. This is true both of measurements of function and receptor binding variables. Dilsaver (122) recently suggested that it is heuristically useful to hypothesize that the lithium ion stabilizes muscarinic cholinergic systems, i.e., renders them less perturbable in the face of endogenously generated or exogenously arising assaults apt to induce supersensitivity and up-regulation of cholinergic systems. ECT may have similar properties. Thus, antidepressant, antimanic and anticycling properties of lithium and ECT may be related to their proclivity to prevent or abort cholinergic system supersensitivity.

Cholinergic systems are plastic. Pharmacological induction of cholinergic system overdrive and muscarinic receptor blockade produce compensatory responses within these systems. For instance, cholinesterase inhibition reduces the density of muscarinic receptor radioantagonist binding sites (123–133) and subsensitivity of cholinergic networks to muscarinic agonists as determined by measuring cholinergically regulated behavioral, physiological and biochemical variables. In contrast, antimuscarinics increase muscarinic radioantagonist binding sites and induce supersensitivity of cholinergic systems to muscarinic agonists. These cholinotropic effects of psychotropics may be relevant to their mode of action.

Levy et al. (134) treated two groups of rats with a centrally active anticholinergic agent. One group also received lithium. The latter exhibited a significant reduction in the density of tritiated [^{3}H]-QNB binding sites, but the lithium did not prevent the 16% reduction in radioantagonist binding sites associated with disopropofluorophosphonate (DFP) treatment. Further, interperitoneally administered lithium prevented the nicotinic cholinoceptor up-regulation occurring at neuromuscular junctions after denervation of the soleus muscle in frogs. The mean density of extrajunctional nicotinic receptors in the lithium treated animals was only 39% that of controls ($P < 0.001$). There was also a significant reduction in the density of acetylcholine receptors at neuromuscular junctions of innervated muscle ($P < 0.005$).

Demonstration of the effectiveness of modalities to change the density of acetylcholine receptor binding sites may require modeling the pathology of disorders the drug is used to treat. For instance, lithium's capacity to decrease the density, i.e., prevent up-regulation, of muscarinic receptor ligand binding sites may require a maneuver causing muscarinic receptor up-regulation. There is evidence for this. Maggi & Enna (135) gave lithium to rats for 21 days via addition to chow. They were unable to find a change in the density of QNB binding sites in cortex, hippocampus and striatum. Unlike Levy et al. (134) and Pestronk & Drachman (136) they did not perform a manipulation which causes cholinoceptor up-regulation. The mode of action of lithium at cholinergic synapses may well not involve inspecific changes in muscarinic receptor binding variables and cholinergic physiology. If its effects on these variables relate to its therapeutic properties, showing this should require that the investigator reproduce an approximation of the pathophysiological state it corrects. Artificially increasing the density of muscarinic receptor binding sites prior to studying the capacity of lithium to produce muscarinic receptor down-regulation could be just such an approximation.

Dilsaver & Greden (9, 122) suggested that rather than producing nonspecific cholinoceptive effects, lithium decreases the probability of endogenous neurobiological events and exogen-

ous assaults apt to induce muscarinic receptor up-regulation or cholinergic system supersensitivity will actually do so. A model of possible neurochemical events involved in the pathogenesis of rapid-cycling bipolar disorder was developed. This model has many variants, distinguished more by their sphere of application than essential properties. It was posited as a theory useful in bringing the neurochemistry, physiology and phenomenology of affective disorders together into a unified framework. This theory predicts that supersensitization of cholinergic systems may induce rapid-cycling in man or an approximation of this in animals. They presented tricyclics as agents with particular potential for doing this. Jones et al. (137) subsequently reported, in full accordance with these predictions, that withdrawal of imipramine produced rapid-cycling (9).

Some form of cholinergic-monoaminergic interaction theory may be of utility in studying any type of affective disorder with a neurobiological component. Stress is upon *utility*. An aspect of utility is the capacity to generate questions for clinical and basic inquiry. *Truth* of a theory in the popular sense, i.e., its accurate presentation of the status of neural systems in the affective disorders, is not essential to value. Value in directing study is a more fundamental standard. Effort to accord the model broad explanatory scope stems from their concept of the nature and purpose of scientific theories. The authors maintain the measure of any theory's value is its capacity to explain an array of phenomena *and* direct study. The mechanism of action of treatments is an example. The cholinergic-monoaminergic interaction theory explains why tricyclics would induce rapid-cycling and why lithium and ECT are effective anticycling agents.

The concept that *presence* of affective symptomatology or of a propensity to develop it, i.e., presence of an increased vulnerability to the phenotypic expression broadly labeled "affective illness", is due to abnormalities in the interaction of cholinergic and monoaminergic systems, is central to the theory. Defective interregulation of these networks is fundamental to any version of a cholinergic-monoaminergic interaction theory. Thus, the modalities which are most successful in preventing recurrent affective episodes are those which decrease the perturbability of cholinergic and monoaminergic systems. A corollary of this is "those modalities preventing or decreasing the depth and frequency of mood oscillations in subjects with a tendency to cycle are apt to minimize the future perturbability of cholinergic and monoaminergic systems". This follows from the hypothesis that monoaminergic overdrive states can cause cholinergic system up-regulation and supersensitivity, and vice versa. These states (endogenously occurring cholinergic and monoaminergic overdrive states) provide the relative "pathophysiological basis" for affective illnesses. However, the ultimate defect sustaining oscillation is neither monoamingergic nor cholinergic overdrive but an instability or wobble preventing rapid restoration of cholinergic and monoaminergic systems to a state compatible with adaptation in the spheres of thought, mood and behavior.

Agents preventing perturbations by endogenously arising "pharmacological" assaults can abort development of affective episodes. Spontaneous development of cholinergic system up-regulation and supersensitivity is likened to a pharmacological assault or perturbation, although one which is endogenously generated. These points reconcile the data of Levy and associates (134), that chronic lithium treatment prevented antimuscarinic induced cholinceptor up-regulation, with that of Maggi & Enna (135) who found that lithium did not affect muscarinic receptor binding in experiments in which perturbation of cholinergic systems was not employed.

Lerer et al. (138, 139) have reported that lithium renders animals supersensitive to cholinergic agents. This raises the possibility that lithium has cholinomimetic-like properties. However, other data are inconsistent with this conclusion (122). Whether lithium has cholinomimetic-like effects may not be relevant to the issue of its capacity to stabilize cholinergic-monoaminergic systems. It could have both effects.

ECT (140–143), or approximations to it, and calcium channel blockade (144) have also been reported to down-regulate cholinergic receptors. In contrast to the effects of lithium, ECT and,

verapamil, tricyclic antidepressants induce rapid-cycling (145), up-regulate muscarinic receptors and produce muscarinic system supersensitivity. The internal structure of any cholinergic-monoaminergic interaction theory suggests that tricyclic antidepressants will precipitate an increase in the frequency of depressions and manias in subjects with a bipolar diathesis. The hypothesis is "tricyclic antidepressants induce muscarinic cholinergic system supersensitivity and thus increase the probability that monoaminergic systems will undergo compensatory changes (up-regulation and supersensitivity), as a result of the partial denervation produced by a cholinergic system overdrive, and vice versa." Further, the model predicts persistence of muscarinic cholinergic system up-regulation and/or supersensitivity into periods of euthymia. This constitutes an abiding defect which makes its bearer subject to the development of depressions and manias. This perspective is consistent with but also conceptually different from reports that affective disorder patients exhibit supersensitive behavioral, neuroendocrine and polysomnographic responses to cholinergic challenge even while symptom-free.

Despite the documented efficacy of ECT and lithium in the treatment of both mania and depression, we have yet to account for their mode of action. The cholinotropic effects of these treatments may have explanatory significance. Cholinergic-monoaminergic interaction theories are useful in studying these and other treatments of depression and mania – a noteworthy attribute. Theory can be helpful in structuring study, and this is one area in which cholinergic-monoaminergic interaction models might be valuable.

5. *Interaction of lithium, sodium and calcium ions in the regulation of cholinergic mechanisms and the pathophysiology of affective disorders*

Cholinergic mechanisms and effects of sodium, calcium and other ions on membrane and receptor events can *in principle* be integrated into a comprehensive model useful in studying the biology of affective disorders. We do not yet possess the knowledge required to devise this model, but expectancy might hasten the fulfillment of this need. First, cholinergic system up-regulation and supersensitivity may be epiphenomena – occurrences unessential to the pathophysiology of affective illness. Receptor events are gross or "macroscopic" occurrences regulated by "microscopic" (neurochemical and molecular) processes which include ionic events. At this point we can perceive the interaction of receptor phenomena, sodium, calcium and lithium metabolism, and membrane biology. Table 1 summarizes components of the interaction between receptor and ionic events.

Lithium and calcium ions interact. These ions have similar ionic radii and charge densities (146). The neuron is exquisitely sensitive to changes in the intracellular concentration of calcium ion even in the micromolar range, and it is conceivable that lithium produces physiologically important changes in the intracellular calcium ion concentration. Increased extracellular calcium ion concentration prevents lithium-induced inhibition of norepinephrine release from brain slices (146), and lithium decreases the active transport of calcium ions or otherwise antagonizes its effects in neural tissue (147–151). This is a characteristic of agents interfering with intracellular actions of calcium (146, 152–157). Lithium may also produce indirect blockade of intracellular calcium-dependent processes by blocking the influx of calcium, potentiating calcium efflux or enhancing intraneuronal sequestration of this ion (156, 157). Hindrance of alimentary absorption of calcium by lithium may also be relevant (158). However, most intriguing is the competition of lithium and calcium at intraneuronal sites. Lithium ion may act as an agonist at these sites but, if so, it is considerably less potent than calcium. Consequently it would have the pharmacological properties of a competitive antagonist. Calcium is compartmentalized intraneuronally, and neurochemical events are dramatically affected by its release from these locales. Given that lithium can substitute for calcium ion in these compartments, the competitive antagonism of lithium and calcium can be significant.

Lithium causes a functional ("for all practical purposes") hyperparathyroidism (159–165) by rendering the parathyroid gland less sensitive to

Table 1

Receptors are dynamic structures mediating "messages carried" by neurotransmitters or other agonists by allowing their translation into physiologically relevant occurrences. For instance, activation of the nicotinic receptor promotes the influx of Cl-; an event producing those effects associated with receptor stimulation. Similarly, activation of the muscarinic receptor can activate the phosphotidylinositol (PI) cycle. Products of the PI cycle (arachadonic acid and inositol trisphosphate) promote the accumulation of intracytosolic Ca^{++} in particular compartments. This alone can mimic effects of muscarinic receptor activation

Cascade mechanisms (Second messsengers)
Principle: Cell-surface receptors are joined to mechanisms which translate agonist-receptor coupling into physiological responses. For example,
1. Ionic flux
2. Generation of intracellular signals, e.g., Δ in [cyclic nucleotide]$_i$ or [Ca^{++}]$_i$ followed by a cascade of events amplifying the 1° signal

extracellular calcium. However, the parathyroid defect is intracellular. This results in a lower threshold for release of any given quantity of parathyroid hormone (163, 164). That is, lithium causes the cell to behave as though there were interference with intracellular mechanisms required to accurately "read" the extracellular calcium ion concentration.

Lithium ions may also competitively displace sodium ions intraneuronally (166) and thus decrease the effective intraneuronal sodium ion concentration (167). Intracellular calcium ion and sodium ion concentrations positively covary (168). Lithium-induced reductions in the availability of intracellular sodium ions should be accompanied by a reduction in the intracellular calcium ion concentration. Lithium treatment causes an increase in the sodium-potassium ATPase activity in bipolar subjects *in vivo* (169, 170). Naylor (171) reported that this correlated with clinical improvement in manics. This is consistent with the observation that changes in interneuronal sodium and calcium ion concentrations parallel each other and that a decrease in calcium ion concentration accompanies transitions from mania to euthymia. Further, lithium produces an increased electrochemical potential across the neuronal membrane. This suggests decreased intracellular sodium ion concentration (172, 173). An increase in the activity of the sodium-potassium ATPase activity during lithium prophylaxis was also reported to predict a lower rate of recurrences (170, 171, 174). Erythrocytes from bipolar patients provide *in vivo* evidence that lithium enhances sodium-potassium ATPase activity. Non-bipolar subjects (175) do not show this.

Sodium and calcium ions, and sodium-potassium and calcium ATPase activities are pertinent to cholinergic mechanisms. Synthesis and release of acetylcholine requires calcium ions. Calcium is also involved in the transduction processes initiated by activation of the muscarinic receptor. It also increases the activity of tyrosine hydroxylase, the enzyme governing the rate-limiting step in catecholamine synthesis, by binding and activating the calcium dependent regulator protein (calmodulin). The calcium ion-calmodulin complex also enhances cyclic AMP generation by activating calmodulin-sensitive calcium ATPases.

Calcium is required for synthesis and release of norepinephrine and 5-hydroxytryptamine (176—181). Calcium also activates a phosphodesterase, an enzyme catabolizing cyclic AMP. Hence, cyclic AMP generation and destruction are both regulated by calcium ion-sensitive mechanisms. The relative rates of these anabolic and catabolic processes regulate the rate of norepinephrine synthesis. Finally, phosphorylation by calcium-activated phosphorylases of membrane elements may induce conformational changes in the postsynaptic membrane which render α_1 and α_2 receptors subsensitive. In conclusion, calcium is essential to the regulation of both monoaminergic and cholinergic systems. Table 2 summarizes some of calcium's neuronal functions.

Recovery from depressive episodes is accompanied by changes in ion metabolism. Coppen et

al. (182) reported that remission is accompanied by a reduction in the intracellular sodium ion concentration. Cox et al. (183) found that residual sodium is greater in depressed patients compared to control subjects. Erythrocytes from affectively ill patients demonstrate a fall in sodium ion concentration as subjects pass from depressed to euthymic phases (184, 185). Similarly, CSF calcium concentration declines (186). Sodium pump activity increases simultaneously. Changes in sodium metabolism also occur on passing from the manic to euthymic state. These include a reduction in residual sodium, and enhancement of erythrocyte sodium pump (185) and sodium-potassium ATPase activities.

Activity of the neuronal sodium pump is partially regulated by the intracellular sodium ion concentration. Naylor et al. (185) concluded that increased intraerythrocyte sodium ion concentration in cells from bipolar subjects caused increased sodium-potassium ATPase activity *in vitro*. Erythrocytes of normal control subjects did not exhibit this. Thus, erythrocytes from bipolar patients responded to increased intracellular sodium concentration by pumping sodium across the membrane in exchange for sodium, whereas cells from normal subjects demonstrated enhanced passive diffusion of sodium ions out of and potassium ions into the cell.

Linnoila et al. (187) reported that calcium ATPase activity was higher in erythrocyte membrane fragments of 8 affective disorder patients compared to 12 control subjects. Calcium ATPase activity was greater during hypomanic and manic episodes than euthymia. The activity of this enzyme appeared to covary with changes in mood in 4 of 8 patients.

McDonald et al. (188) studied the sensitivity of erythrocyte membrane-bound calcium ATPase to calcium ion and calmodulin in 12 bipolar and 23 control subjects. Membrane-bound enzyme activity displayed more variation in patients. This was not due to variation in the quantity of calmodulin present in the fragments. Unresponsiveness to calmodulin was significantly related to calcium ATPase activity, even at low concentrations of calcium ion. Samples demonstrating high sensitivity to calmodulin were also calcium sensitive, i.e., there was essentially no calcium ATPase activity in these fragments at low calcium ion concentrations. There was a linear relationship between calcium ion and calmodulin sensitivity which was qualitatively similar in experimental and control subjects. However, statistical analyses were not provided to allow a comparison of the groups. Variable responsiveness of the membrane calcium ATPase to calmodulin resembles effects observed when purified forms of the enzyme are inserted into liposomes of various phospholipid composition. Niggli et al. (189, 190) reported that incorporation of calcium ATPase into liposomes containing phosphidylserine rendered it calmodulin sensitive and produced considerable enzymatic activity even at low calcium ion concentrations, e.g., 10^{-7} molar. In contrast, insertion of the enzyme into liposomes rich in phosphatidylcholine confered high sensitivity to calmodulin and calcium ion dependence. The authors hypothesized that differences in calcium ATPase activity levels might be due to a variation in the phospholipid composition of the membranes rather than intrinsic differences in the enzyme.

Roelofsen (191) proposed that a calcium ATPase is an endogenous regulator of the sodium-potassium pump. This agrees with findings that intracellular calcium and sodium ion concentrations covary positively. Linnoila et al. (187) graphically showed the activities of the calcium and sodium-potassium ATPases without providing statistical analyses. The graphs reveal no obvious relationship between the activities of the two enzymes. Roelofsen's (191) hypothesis can be consistent with this, however. A mechanism coupling calcium and sodium-potassium pump activity (192) could be sensitive to variation in the conditions of measurement. There could be a threshold effect such that when calcium ATPase activity reaches a critical point sodium pump activity does not increase. Calcium ATPase activity in membrane fragments studied by Linnoila et al. (185) might have exceeded this threshold. This is relevant to the observation that sodium-potassium ATPase activity is depressed in affective disorder patients relative to control subjects and increases in the process of recovery. This threshold effect is also relevant to the observation that calcium ATPase activity is elevated

Table 2
Ca^{++} has a crucial role in cellular physiology. It is an obligatory ion for synthesis and release of several neurotransmitters, regulates the activity of tyrosine hydroxylase, the enzyme governing the rate-limiting step in catecholamine synthesis, activates and modulates calmodulin, the calcium dependent regulatory protein, affects receptor sensitivity and number (e.g., Ca^{++} produces muscarinic receptor down-regulation - an effect of muscarinic receptor activation) and activates regulatory enzymes, such as phosphorylases.

Calcium ion-related events
Neurotransmitter synthesis and release (e.g., Ach, NE, 5HT)
Increases the activity of tyrosine hydroxylase
Increases the activity of calmodulin
Calcium ion influx → muscarinic receptor down-regulation
Activition of phosphodiesterase
Activation of cyclases (e.g., ↑ cGMP)
Modulation of the sensitivity of α_1, α_2 receptors by phosphorylation of membrane

during depressed phases relative to manic episodes in bipolar patients. The critical point for not promoting further increase in sodium-potassium ATPase activity could be too low. When calcium ATPase activity reaches this set-point, the activity of the sodium pump may not increase appropriately. Alternatively, the calcium ATPase activity could be related to the activity of sodium-potassium ATPase. That is, an abnormally low sodium-potassium ATPase activity and an increased intracellular sodium ion concentration could cause development of increased calcium ATPase activity. In the first scheme, an abnormally low set-point for "turning off" renders the sodium-potassium ATPase insensitive to increases in the calcium ATPase activity, i.e., the depressed phase set-point is causal or explanatory of the elevation in the calcium ATPase activity. In the second situation, depression of sodium-potassium ATPase activity, despite increased intraneuronal sodium ion concentration, would be the factor accounting for the elevated calcium ATPase activity during depressed relative to manic phases.

Receptor function predisposes the integrity of ionic events. They also mobilize ionic currents. This is certainly true of the nicotinic acetylcholine receptor and sometimes true of muscarinic receptor activation. Calcium, sodium, and lithium all influence the synthesis and release of acetylcholine. Integration of seemingly disparate perspectives is indeed a challenge. We now have cholinergic, norepinephrine, serotonergic, sodium and calcium hypotheses of affective disturbance. These may be reconcilable viewpoints, subject to synthesis into a comprehensive, unified whole. This type of model accords with the nature of neuronal function, it is a complex of interrelated particulars. Such a model would also encourage the study of affective illness from an increasingly mechanistic and molecular viewpoint.

6. *Positron emission tomography scanning*

Study of the pathophysiology of psychiatric diseases has been limited by the inability to measure brain function *in vivo*. The advent of computer axial tomographic (CAT) scanning made the anatomy of neurological and psychiatric disorders amenable to description. Investigation of affective disorders would profit even more from the measurement of function. Functions capable of being measured in affective disorders subjects are: cerebral blood flow, oxygen and glucose utilization, and receptor binding variables. Measurement of function using PET is discussed here.

The statistical power of tests used to examine

group data can be minimal. Studies with greatest promise may be those in which individuals can be their own controls, i.e., those using ABA designs. These studies can employ affective disorder patients or normal control subjects treated with a drug creating the pathophysiology of some disorder. Rapidly cycling bipolar patients are ideal. Their clinical state can change rapidly, even within the course of a day. Subjects frequently display switches between states within days and weeks. Many behavioral and physiological variables change concomitantly. These include mood and other clinical features scored on standard rating scales (e.g., the Hamilton Rating Scale for Depression includes 17 variables), hedonic capacity (112), speech pause time and limb motility (109–111), REM latency, density, and activity of REM sleep during the first half of the polysomnographic reading, sleep efficiency (42, 45), DST status, absolute postdexamethasone plasma cortisol (107, 108), ACTH and ß-endorphin levels (35, 40), nocturnal cortisol latency (102), measures of thermoregulation (36, 101, 103), pupillometric variables (49–51), and measures provided by brain area electrical mapping (BEAM) (113). These various clinical and physiological measures include over 40 variables to be used in conjunction with novel experimental techniques such as PET. This provides numerous opportunities to demonstrate the association of PET variables with validated measures, i.e., those associated with a given disease entity. This is not only important but essential because the imaging variables do not possess intrinsic validity. Recurrent association of PET variables with previously validated clinical and physiological measures can provide *prima facie* validation of the former. Some methods of measuring a variable may not be adequate for comparing samples owing to intersubject variance, but may be useful if subjects are used as their own controls, i.e., measurements are made in the same individual under different conditions. Limb motility and speech pause time are illustrative. Indeed, it is possible to devise profiles of variables allowing the measurement of clinical and physiological changes in subjects participating in imaging studies.

Cholinergic neurons are extremely sensitive to antimuscarinic agents. This allows the modeling of aspects of affective illness in normal subjects. Sitaram et al. (88) and Gillin et al. (93) did this using scopolamine. This agent apparently caused cholinergic system supersensitivity and thereby produced changes in REM sleep characteristic of primary depression (193). Normal subjects can be studied using a design stipulating predrug administration or baseline, drug treatment, acute withdrawal and long-term postwithdrawal phases. Variables sensitive to cholinergic manipulation can be measured and PET abnormalities occurring in relationship to them documented (36).

We do not yet have a radioligand suitable for *in vivo* PET studies in man, but one may be available soon. Preclinical work is now under way (59–61). PET or other imaging methods must take into account limitations of the technique, i.e., the degree of resolution, utility of the radioligand, and tracer kinetic models, etc. Testing specific hypotheses rather than "shotgun approaches", which attempt to find differences in patient and non-patient populations, should be the most useful strategy.

7. *Search for a peripheral model of the central muscarinic receptor*

Several peripheral tissues have muscarinic receptors. The fibroblast (194) is an example. However, it does not provide a high number of both stable and functional muscarinic receptors. Demonstration of up-regulation of a functional muscarinic receptor on a peripheral tissue in affectively ill patients would be an important finding, as would be the establishment of a peripheral model of the central muscarinic receptor. A peripheral model of the receptor was recently proposed but then invalidated (195, 196).

Epidemiological research would be encouraged if one can establish that affectively ill patients have muscarinic receptor up-regulation on a peripheral cell; i.e., muscarinic receptor up-regulation is a trait or a marker of disease. Subjects could be identified before onset of illness. This might allow primary prevention. In addition to phenomenological or behavioral,

physiological and biochemical variables, we would also have muscarinic receptor binding variables to which to relate other variables. The peripheral model could provide a reference. The interrelationship of variables in the same (e.g., phenomenological vs. phenomenological variables) and different (phenomenological vs. physiological, biochemical vs. muscarinic receptor binding variables) classes could mark or characterize disorders if a unifying principle accounting for their association is identified.

8. *Drug abuse data*

Classes of abusable substances with well-defined pharmacologies tend to either activate monoaminergic systems or inhibit cholinergic networks via the direct blockade of muscarinic receptors or presynaptically mediated inhibition of acetylcholine release. These effects accord with our knowledge of the regulation of mood and affect and drive reduction behavior. The pharmacology of abusable substances and neurobiology of mood and affect are logically separate but related. A model useful in accounting for many of

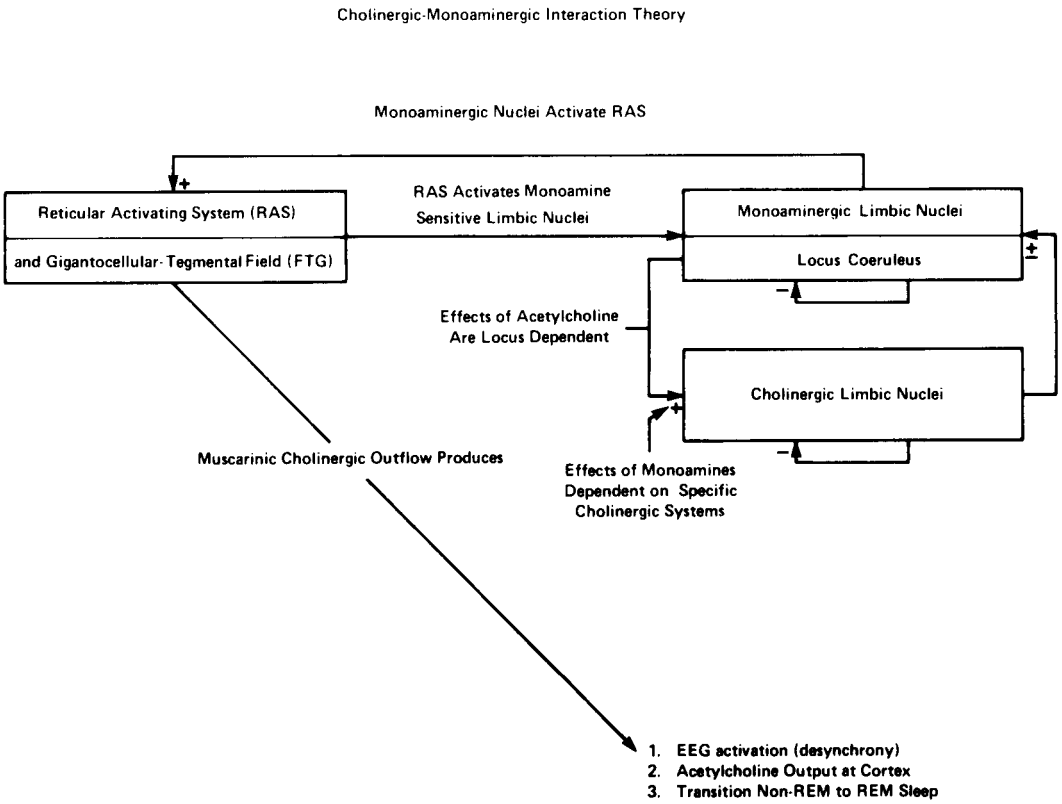

Fig. 1. This presents elements of the cholinergic-monoaminergic interaction theory. The reticular activating system (RAS) interacts with limbic aminergic nuclei involved in affecting behavioral arousal. Cholinergic pontine neurons (FTG) interact with adrenergic neurons on the locus coereolus similarly in the regulation of REM-nonREM transitions. The RAS and FTG both produce cholinergic outflow and the output of acetylcholine at the cortex. This is essential to development of the electrocortical desynchrony which normally accompanies behavioral arousal and the physiology of REM-nonREM shifts. Cholinergic limbic neurons interact with monoaminergic cells to regulate and modulate mood, affect, neuroendocrine functions, psychomotor status, and other variables relevant to the affective and substance abuse disorders. These systems are auto *and* interregulated. Thus, the value of monoaminergic variables is a function of the value of variables describing the cholinergic limbic nuclei, and vice versa. Please see text for further explanation. (Reprinted with permission of Brain Research Reviews, Ref. 10.)

the phenomena of affective illness, a cholinergic-monoaminergic interaction theory (9), is equally useful in explaining the mode of action of abusable drugs. Further, study of the pharmacology of substances of abuse may increase our understanding of affective disorders.

Fig. 1 illustrates a model integrating the neurobiologies of both substance abuse and affective illness. First, cholinergic and monoaminergic systems are in dynamic interaction, and perturbation of one affects the other. The reticular activating system (RAS) is an electrical activating network in continuity with limbic structures involved in producing behavioral activation. Evidence for separateness of the electrical and behavioral activating systems is provided by observations that massive lesioning of the RAS produces "electrical" coma, despite allowing behavioral arousal should the rostral portion of the hypothalamus be directly stimulated. Evidence for anatomic separation of electrical and behavioral acting systems and phylogenetic reasons for this were presented previously (9).

Activation of the RAS in the intact animal activates limbic nuclei containing monoaminergic cells. These nuclei form the neural substratum of behavioral arousal, drive reduction behavior and other phenomena associated with favorable psychic states. Activation of the RAS, e.g., by an exogenously delivered electrical impulse, activates cholinergic systems involved in producing the electroencephalographic (EEG) features of arousal. This is accompanied by release of acetylcholine at the cortex. Blockade of cholinergic systems prevents this EEG pattern. Either direct or indirect activation of the monoaminergic nuclei activates cholinergic systems. This is evidenced by the cortical release of acetylcholine and electrocortical activation following the systemic or iontophoretic administration of adrenergic drugs. Hence, there is constant interaction between cholinergic and monoaminergic systems and the RAS in the healthy animal.

Drug-induced cholinergic overdrive produces depression, whereas agents activating monoaminergic systems such as amphetamine and cocaine cause euphoria and behavioral activation. However, aminergic agents also activate cholinergic systems and cholinergic activation affects compensatory responses within monoaminergic networks. Drugs of abuse may tip cholinergic-monoaminergic balance in the direction of monoamine excess, while preventing an adequate compensatory response by cholinergic systems. For instance, antimuscarinic agents are substances of abuse (197–201). They directly bind to postsynaptic muscarinic receptors and presumably thereby "ablate" unpleasant mood or cause euphoria. Cannabinoids (74–78), opiates (71–73), ethanol (73–82), and barbiturates (83–86) are all potent inhibitors of the release of acetylcholine. Hence, one category of drug acts postsynaptically, the classical antimuscarinic agents (trihexythenidyl, benztropine, scopolamine, biperidine, etc.) and the second presynaptically. All are liable to abuse. These agents have other pharmacological effects, but their effects on cholinergic systems are unifying.

According to the model presented in Fig. 1, both postsynaptic blockade of muscarinic receptors and decreased release of acetylcholine enhance monoaminergic transmission. Agents acting on the cholinergic side should produce euphoria. Agents acting on the monoamine side do the same but by a different path. Both cause activation of limbic monoaminergic nuclei exceeding the capacity of cholinergic systems to make adequate compensatory response.

In conclusion, a model explaining many phenomenological and physiological features of affective illness is also useful in explaining the mode of action of several classes of substances of abuse. A unifying theory can be developed. Substances of abuse produce favorable psychic states, i.e., they appear to act on mechanisms involved in the regulation of affect, mood, drive reduction behavior and psychomotor function. The integrative study of abusable drugs and the psychobiology of the affective disorders may be a useful strategy.

9. *Cholinergic-monoaminergic interaction*

Interaction of cholinergic and monoaminergic systems could be basic to the pathophysiology of affective disorders. This view contrasts with the perspective or tacit assumption that analysis of monoaminergic or cholinergic systems is suffi-

cient for gaining understanding of the psychobiology of affective disorders. Dilsaver & Greden (9) proposed that this reductionistic approach to the neurobiology of the affective disorders is inadequate and suggested that "interaction" between neurotransmitter systems is paramount. Their model maintains that many neurochemical systems come together to create pathophysiological conditions. Cholinergic and monoaminergic systems are focused upon most because knowledge is sufficient to discuss their interrelationship.

The basic premise of the Dilsaver-Greden model (9) is that neurotransmitter systems do not, indeed cannot, exist in isolation. The query, "In which direction does the face of the Roman god Janus face?" misses the point. It is based on the assumption that his face is directed in some one direction. The question is amiss because its premise does not capture the essence of the situation. Similarly, our questions regarding the neuropharmacology of events may be amiss if we improperly assume "either/or" or "one but not both" of the alternatives is reasonable. Sorscher & Dilsaver (201) recently reviewed this conceptual problem and presented several examples of the ways in which faulty or reductionistic assumptions produce questionable conclusions in neuropsychopharmacology. Cholinergic networks have an impact on monoaminergic systems, and conversely: cholinergic overdrive and hypoactivity produce decreases (down-regulation) and increases (up-regulation) in the density of muscarinic binding sites, and subsensitivity and supersensitivity to cholinergic agonists; monoaminergic systems regulate muscarinic receptor binding and the release, synthesis, and intraneuronal concentrations of acetylcholine. Cholinergic mechanisms regulate the activity of tyrosine hydroxylase, and the synthesis and release of monoamines and monoamine receptor binding (9). Kazic (203) reported that physostigmine produced a significant drop in hypothalamic and brain stem norepinephrine content within 15 min of infusion. This is compatible with a massive release of norepinephrine. Within this short frame, there was also an increase in the turnover of [^{14}C] tyrosine, the precursor of norepinephrine. Muscarinic receptor stimulation also produced decreased release of norepinephrine in the hypothalamus of the rat (203). Similarly, acetylcholine can decrease the release of norepinephrine from adrenergic neurons (204) in the periphery. Cholinergic (205-207) and dopaminergic agonists (208, 209) can also increase the turnover of dopamine and acetylcholine, respectively. This implies increased rates of neurotransmitter synthesis. The phenomenon is well described for the striatum.

In summary, cholinergic and monoaminergic systems interact in the regulation of receptor binding density, synthesis of enzymes involved in neurotransmitter synthesis, and the synthesis, turnover, and release of neurotransmitters. The idea that we may one day be able to study the interaction of these systems in affective disorder patients versus normal subjects, is not only attractive but important to the development of this field. This is clearly a direction for future research!

Conclusions

Progress in biomedical research is often hastened by simultaneous clinical and basic thrusts in the study of the same disease entity. This is certainly true of the topic focused upon in this article. Description, natural history, treatment, genetics, biochemistry, physiology and pharmacology are all aspects of a medical approach to disease. The affective disorders have been described but we are still learning about their boundaries, i.e., how they are to be distinguished from other diseases (e.g., panic and other anxiety disorders, variants of the borderline personality disorder, etc.), natural histories, treatment and mode of inheritance. While we have biological correlates of these illnesses, we do not yet know their pathophysiologies. However, we do know enough to be encouraged in our pursuits. Our current knowledge base situates us so that we have a reasonable probability of successful study. We have an idea of where it might pay to look. "Cholinergic mechanisms" is certainly one of these "places". Clinical investigations of the roles of cholinergic systems in these illnesses

have created many opportunities for basic scientists interested in modeling the pathophysiology of affective disorders.

All of us are both beneficiaries and victims of our assumptions. An illustration is helpful. Thus far, biological psychiatrists have assumed that if there is a cholinergic abnormality in the affective disorder, it involves the postsynaptic muscarinic receptor. Whether this assumption is correct may not have been relevant to this point. Investigation may not have proceeded differently. However, study of "mechanisms" - the "how of a thing" is a luxury basic advances might provide. If this assumption is faulty, it may soon make a real difference. Cholinergic abnormalities could involve the postsynaptic receptor, abnormal pre- or postsynaptic neural membrane, intracytosolic (cascade or amplifying mechanisms), or defective presynaptic receptors. Cholinergic system overdrive could, for example, be the consequence of presynaptic muscarinic receptor down-regulation or subsensitivity (210). There are also other possibilities. Progress demands we ask critical questions and examine those unarticulated assumptions which shape our field. We may do well to discard some of our presuppositions or acquire new ones. The issue is: "How and why we think the way we do".

Study of cholinergic mechanisms involved in the pathophysiology of affective disorders should benefit from efforts to foster a close interdependence of the clinical and basic sciences. Clinical and basic researchers not only study different aspects of the same dimension but think differently and thus ask different questions. Basic scientists do not merely possess an ability to use techniques that clinical researchers do not, but, more essentially, use different concepts and values in evaluating problems. A sojourn into a laboratory and close association with basic researchers cannot but transform clinicians intellectually. A distinct attitude toward the study of phenomena permeates these environments. Similarly, clinical investigators and clinical research units have characters of their own. I suggest that the future of the topic we have been discussing, "cholinergic mechanisms in the affective disorders", rests with our ability to hold to the ideal of simultaneous clinical and basic research and to accept the tensions and compromises that this ambition brings.

Acknowledgement

Supported in part by Physician-Scientist Development Award, Grant #SRCIK11 MH0055301 (Muscarinic Receptor Abnormalities in Affective Illness), National Institute of Mental Health.

References

1. Weiss J M, Goodman P A, Losito B G, Corrigan S, Charry J M, Bailey W H. Behavioral depression produced by an uncontrollable stressor: Relationship to norepinephrine, dopamine and serotonin levels in various regions of rat brain. Brain Res Rev 1981:*3*:167-205.
2. Lloyd C. Life events and depressive disorder: II. Events as precipitating factors. Arch Gen Psychiatry 1980:*37*: 541-548.
3. Kennedy S, Thompson R. Stancer H C, Roy A, Persad E. Life events precipitating mania. Br J Psychiatry 1983: *142*:398-403.
4. Rasmussen K L R, Reite M. Loss-induced depression in an adult Macaque monkey. Am J Psychiatry 1982: *139*:681-697.
5. Diagnostic and Statistical Manual of the American Psychiatric Association, 3rd ed. (DSM III), 1980.
6. Janowsky D S, Risch S C, Huey L, Judd L, Rousch J. Central physostigmine-induced cardiovascular and behavioral changes: Toward an acetylcholine hypothesis of stress. Psychopharmacol Bull 1983:*19*:675-682.
7. Janowsky D S, Risch S C, Huey L Y, Kennedy B, Zigler M. Effects of physostigmine on pulse, blood pressure, and serum epinephrine levels. Am J Psychiatry 1985: *142*:738-740.
8. Janowsky D S, Risch S C. Cholinomimetic and anticholinergic drugs used to investigate an acetylcholine hypothesis of affective disorders in stress. Drug Dev Res 1984:*4*:125-142.
9. Dilsaver S C, Greden J F. Antidepressant withdrawal-induced activation (hypomania and mania): Mechanism and theoretical significance. Brain Res Rev 1984:*7*:29-48.
10. Dilsaver S C. Cholinergic mechanisms in depression. Brain Res Rev (in press).
11. Brezenoff H E, Caputi A P, Camilleri B: Reduction of blood pressure in spontaneous hypertensive rats by atropine and intracerebroventricular injection of hemicholinium-3. Pharmacologist 1979:*21*:255.
12. Brezenoff H E, Giuliano R. Cardiovascular control by cholinergic mechanisms in the central nervous system. Ann Rev Pharmacol Toxicol 1982:*22*:341-381.
13. Brezendoff H E. Centrally induced pressor responses to intravenous and intraventricular physostigmine evoked via different pathways. Eur J Clin Pharmacol 1973: *23*:290-292.
14. Lindemann E, Finesinger J E. The subjective response of psychoneurotic patients to adrenaline and mecolyl. Psychosom Med 1940:*2*:231-246.

15. Taylor P. Cholinergic agonists. In: Gilman A G, Goodman L S, Gilman A, eds. The pharmacological basis of therapeutics, 6th ed. New York: Macmillan, 1980:93.
17. Leckman et al.: Panic disorder and major depression. Arch Gen Psychiatry 1983:*40*:1055–1060.
18. Oradell N J. Medical economics. Physician's Desk Reference, *34*, 1980:688–690.
19. Bowers M B, Goodman E, Sim V M. Some behavioral changes in man following anticholinesterase administration. J Nerv Ment Dis 1964:*138*:383–389.
20. El-Yousef M K, Janowsky D S, Davis J M, et al. Induction of severe depression by physostigmine in marijuana intoxicated individuals. Br J Addict 1973:*68*:321–325.
21. Gershon S, Shaw F H. Psychiatric sequelae of chronic exposure to organophosphorus insecticides. Lancet 1961:*1*:1371–1374.
22. Grob D, Harvey A M, Langworthy O R, Lilenthal J L. The administration of diisopropylfluorophosphonate (DFP) to man. Johns Hopkins Hosp Bull 1947:*81*:257–266.
23. Janowsky D S, Davis J M, El-Yousef M K, et al. Acetylcholine and depression. Psychosom Med 1973:*35*:568.
24. Janowsky D S, Davis J M, El-Yousef M K, et al. Cholinergic antagonism of methylphenidate-induced stereotyped behavior. Psychopharmacology 1972:*27*:295–303.
25. Janowsky D S, Davis J M, El-Yousef M K, et al. A cholinergic adrenergic hypothesis of mania and depression. Lancet 1972:*2*:632–635.
26. Janowsky D S, Davis J M, El-Yousef M K, et al. Parasympathetic suppression of manic symptoms by physostigmine. Arch Gen Psychiatry 1973:*28*:542–547.
27. Janowsky D S, Risch S C, Judd L L, Parker D C, Kalin N H, Huey L Y. Behavioral effects of physostigmine in affective disorder patients. In: Clayton P J, Bennet J R, Eds. Treatment of depression. New York: Raven, 1982.
28. Janowsky D S, Risch S C, Parker D, et al. Increased vulnerability to cholinergic stimulation in affect disorder patients. Psychopharmacol Bull 1980:*16*:29–31.
29. Janowsky D S, Risch S C, Parker D, et al. Behavioral-neuroendocrine effects of physostigmine. Collegium Internationale Neuropsychopharmacologium 13th C.I. N.P. Congress, Jerusalem, Isreal, 1982:349.
30. Janowsky D S, El-Yousef M K, Davis J M, Sekerke H J. Antagonistic effects of physostigmine and methylphenidate in man. Am J Psychiatry 1973:*130*:1370–1376.
31. Risch S C, Cohen P M, Janowsky D S, et al. Physostigmine induction of depressive symptomatology in normal human subjects. J Psychiatr Res 1981:*4*:89–94.
32. Risch S C, Cohen P M, Janowsky D S, Kalin N H, Murphy D L. Plasma ß-endorphin and cortisol elevations accompany the mood and behavioral effects of physostigmine in man. Science 1981:*209*:1545–1546.
33. Risch S C, Kalin N H, Janowsky D S. Cholinergic challenges in affective illness: Behavioral and neuroendocrine correlates. J Clin Psychopharmacol 1981:*1*:186–192.
34. Rowntree D W, Neven S, Wilson A. The effect of diisopropylfluorophosphonate in schizophrenia and manic depressive psychosis. J Neurol Neurosurg Psychiatry 1950:*13*:47–62.
35. Risch S C, Kalin N H, Janowsky D S. Cholinergic challenges in affective illness: Behavioral and neuroendocrine correlates. J Clin Psychopharmacol 1981:*1*:186–192.
36. Dilsaver S C: Pharmacologic induction of cholinergic system up-regulation and supersensitivity in affective disorders research. J Clin Psychopharmacol 1986:*6*:65–74.
37. Carroll B J, Greden J F, Haskett R, et al. Neurotransmitter studies of neuroendocrine pathology in depression. Acta Psychiatr Scand 1980:*61*:Suppl 280, 183–199.
38. Doerr P, Berger M. Physostigmine-induced escape from dexamethasone in normal subjects. Biol Psychiatry 1983:*18*:261–268.
39. Risch S C, Kalin N H, Janowsky D S, et al. Co-release of ACTH and ß-endorphin immunoreceptivity in human subjects in response to central cholinergic stimulation. Science 1983:*222*:77.
40. Risch S C. ß-endorphin hypersecretion in depression: Possible cholinergic mechanisms. Biol Psychiatry 1982: *17*:1071–1079.
41. McCarley R W. REM sleep and depression-common neurobiological control mechanisms. Am J Psychiatry 1982:*139*:565–570.
42. Gillin J C, Sitaram N, Duncan W C, et al. Sleep disturbance in depression: diagnostic potential and pathophysiology. Psychopharmacol Bull 1980:*16*:40–42.
43. Sitaram N, Mendelson W B, Wyatt R J, Gillin J C. The time dependent induction of REM sleep and arousal by physostigmine infusion during normal human sleep. Brain Res 1977:*122*:562–567.
44. Sitaram N, Nurnberger J, Gershon E, et al. Faster cholinergic REM sleep induction in euthymic patients with primary affective illness. Science 1980:*208*:200–202.
45. Sitaram N, Nurnberger J, Gershon E, Gillin J C. Cholinergic regulation of mood and REM sleep: potential model and marker of vulnerability to affective disorders. Am J Psychiatry 1982:*139*:571–576.
46. Sitaram N, Wyatt R J, Dawson S J, Gillin C J. REM sleep induction by physostigmine infusion during sleep. Science 1976:*191*:1281–1283.
47. Hill S Y, Reyes R B, Kupfer D J. Imipramine and REM sleep: cholinergic mediation in animals. Psychopharmacology 1980:*69*:5–9.
48. Hill S Y, Reyes R B, Kupfer D J. Physostigmine induction of REM sleep in imipramine treated rats. Community Psychopharmacol 1979:*3*:261–266.
49. Kelwala S. Enhanced pilocarpine miosis in depressed patients. Presented at the 137th Annual Meeting of the American Psychiatric Association in Los Angeles, California, May 5–11, 1984: NR25.
50. Shur E S, Checkley E, Delgado I. Failure of mianserin to affect autonomic function in the pupils of depressed patients. Acta Psychiatr Scand 1983:*67*:50–55.
51. Shur E, Checkley S: Pupil studies in depressed patients: an investigation of the mechanism of outcome of desipramine. Br J Psychiatry 1982:*140*:181–184.
52. Dilsaver S C, Greden J F. Antidepressant withdrawal syndromes: evidence for supersensitivity of the cholinergic systems as an etiologic factor. J Clin Psychopharmacol 1983:*3*:330.
53. Kanba S, Richelson E. Antimuscarinic effects of lithium. N Engl J Med 198?:*310*:989–990.

54. El-Fakahany E, Richelson E. Effects of some calcium antagonists on muscarinic-mediated cyclic GMP formation. J Neurochem 1983:*40*:705–710.
55. Kanba S, Richelson E. Lithium affects muscarinic function. Presented at the 39th Annual Convention an Scientific Program of the Society of Biological Psychiatry. Los Angeles, California, May 2–6, 1984.
56. Fischer S K, Klinger P D, Agranoff B W (1982): Muscarinic agonist binding and phospholid turnover in brain. J Biol Chem *258*:7358–7363.
57. Fisher S K, Agranoff B W. The biochemical basis and functional significance of enhanced phosphatidate and phosphainositide turnover. In: Eichberg J, ed. Phospholipids in nervous tissues. New York: John Wiley, 1985.
58. Fisher S K, Van Rooijen L A A, Agranoff B W. Renewed interest in the polyphosphainositides. Trends Neurosci 1984:*7*:53–56.
59. Frey K A, Ehrenkaufer R L L, Agranoff. Quantitative *in vivo* receptor binding. II. Autoradiographic imaging of muscarinic cholinergic receptors. Neuroscience 1985 (in press).
60. Frey K A, Hichwa R D. Ehrenbaufer R L L, Agranoff R W. Quantitative *in vivo* receptor binding. III. Tracer kinetic modeling of muscarinic cholinergic receptor binding. Neuroscience (submitted).
61. Frey K A, Ehrenkaufer S, Beaucage S, Agranoff B W. Quantitative *in vivo* receptor binding. I. Theory and application of the muscarinic cholinergic receptor. J Neurosci 1985:*5*:421–428.
62. Ben-Barak Y, Dudai Y. Scopolamine induces an increase in muscarinic receptor level in rat hippocampus. Brain Res 1980:*193*:309–313.
63. Ehlert F J, et al. The nature of muscarinic binding. In: Iversen L L, Iversen S D, Snyder S N, eds. Handbook of psychopharmacology, Vol. 17. New York: Plenum, 1983.
64. Takeyasu K, Uchida S, Nogcchi Y, et al. Changes in brain muscarinic acetylcholine receptors and behavioral responses to atropine and morphine in chronic atropine treated rats. Life Sci 1979:*25*:585–592.
65. Wise B C, Shoji M, Koo J F. Decrease or increase in cardiac muscarinic cholinergic receptor number in rats treated with methacholine or atropine. Biochem Biophys Res Commun 1980:*92*:1132–1142.
66. Yamada S, Iogai M, Okudaira H, Hayashi E. Regional adaptation of muscarinic receptors and choline uptake in brain following repeated administration of diisopropylflourophosphonate and atropine. Brain Res 1983: *268*:315–320.
67. Goldman M E, Erickson C K. Effects of acute and chronic administration of antidepressant drugs in the central cholinergic nervous system: Comparison with anticholinergic drugs. Neuropharmacology 1983:*22*:1222–1285.
68. Rehavi M, Ramot O, Yavetz B, et al. Amitriptyline: Long-term treatment elevates α-adrenergic and muscarinic receptor binding in mouse brain. Brain Res 1980:*194*:443–453.
69. Nomura Y, Kajiyma H, Okai K. Influence of repeated administration of dysmethylimipramine on ß-adrenergic and muscarinic cholinergic receptors and $^{45}Ca++$ binding to sarcoplasmic reticulum in the rat heart. J Pharmacol Exp Ther 1982:*223*:834–840.
70. Nomura Y, Kajiyama A, Segawa T. Possible influence of noradrenalin on ß-adrenergic and muscarinic receptors in rat heart: Effects of 6-hydroxydopamine, isoproterenol and desmethylimipramine. In: Segawa T, et al., eds. Molecular pharmacology of neurotransmitter receptors. New York: Raven Press, 1983:83–90.
71. Jhamandas K, Tinsley C, Phillis J W. Effects of morphine and its antagonists on release of cerebral cortical acetylcholine. Nature 1970:*228*:176–177.
72. Domino E F, Wilson A E. Effects of narcotic analgesic agonists and antagonists on rat brain acetylcholine. J Pharmacol Exp Ther 1973:*184*:18–32.
73. Jhamandas K, Sutar M, Bell S. Modification of precipitated morphine withdrawal syndrome by drugs affecting cholinergic mechanisms. Eur J Pharmacol 1973:*24*:296–305.
74. Kumbarachi M N, Nastuk W L. Effect of Δ^9-tetrahydrocannabinol on excitable membranes and neuromuscular transmission. Mol Pharmacol 1980:*17*:344–345.
75. Layman J M, Milton A S. Some actions of Δ^1-tetrahydrocannabinol and cannabinoids at cholinergic functions. Br J Pharmacol 1971:*41*:379P–380P.
76. Yoshimura H, Fujiwara M, Ueki S. Biochemical correlates in mouse-killing behavior of the rat: brain acetylcholine and acetylcholinesterase after administration of Δ^9-tetrahydrocannabinol. Brain Res 1974:*81*:567–570.
77. Dilsaver S C, Leckrone J G, Greden J F. An anticholinesterase-like syndrome precipitated by psychotropic withdrawal in a chronic abuser of marijuana. Psychosomatics 1984:*8*:632–634.
78. Rosenblatt J E, Janowsky D S, Davis J M. The augmentation of physostigmine toxicity in the rat by Δ^9-tetrahydrocannabinoid. Res Commun Chem Pathol Pharmacol 1972:*3*:479–481.
79. Carmichael F J, Isreal Y: Effects of ethanol on neurotransmitter release by rat brain cortical slices. J Pharmacol 1974:*193*:824–834.
80. Tabakoff B, Muroz-Marcus M, Fields J Z: Chronic ethanol feeding produces an increase in muscarinic cholinergic receptors in mouse brain. Life Sci 1979:*25*:2173–2180.
81. Rabin R A, Wolfe B B, Dibner M D, et al. Effects of ethanol administration and withdrawal on neurotransmitter receptor system in 157 mice. J Pharmacol Exp Ther *213*:491–496.
82. Smith T L: Influence of chronic ethanol consumption of muscarinic cholinergic receptors and their linkage to phospholipid metabolism in mouse synaptosomes. Neuropharmacology 1983:*22*:661–663.
83. Wahlstrom G. Estimation of brain sensitivity to the convulsive effects of choline and changes induced by chronic barbital treatments in the rat. Eur J Pharmacol 1978:*51*:219–227.
84. Wahlstrom G, Nordberg A. Sensitivity to an active synaptosomal uptake of choline in the abstinence after chronic barbital treatments. Acta Physiol Scand 1979: Suppl 473:65–203.
85. Nordberg A, Wahlstrom G. Changes in cholinergic func-

tion in the rat brain late in the abstinence after chronic barbital treatment. Drug Alcohol Depend:7:51-61.
86. Nordberg A, Sundwall A. The effect of sodium pentobarbital on the apparent turnover of acetylcholine in different brain regions. Acta Physiol Scand 1977:*99*:336-344.
87. Dilsaver S C, Kronfol Z, Greden J F, Sackellares J C. Antidepressant withdrawal syndromes: evidence supporting the cholinergic overdrive hypothesis. J Clin Psychopharmacol 1983:*3*:157-164.
88. Sitaram N, Moore A M, Gillin J C. Scopolamine-induced muscarinic supersensitivity in normal man: Changes in sleep. Psychiatr Res 1979:*1*:9-16.
89. Dilsaver S C, Greden J F. Antidepressant withdrawal phenomena. Biol Psychiatry 1984:*19*:237-256.
90. Friedman M J, Jaffe J. A central hypothermic response to pilocarpine in the mouse. J Pharmacol Exp Ther 1969:*167*:34-44.
91. Jaffe J H, Sharpless S K. Pharmacological denervation supersensitivity in the central nervous system. Research Publications Association, Res Nerv Ment Dis 1968: *46*:226-241.
92. Innes I R, Nickerson M. Atropine, scopolamine and related antimuscarinic drugs. In: Goodman L S, Gilman A, eds. The pharmacological basis of therapeutics, 5th ed. New York: Macmillan, 1975:520.
93. Gillin J C, Sitaram N, Duncan W C. Muscarinic supersensitivity: A possible model for the sleep disturbance of primary depression? Psychiatr Res 1979:7:17-22.
94. Dilsaver S C, Greden J F. Antidepressant withdrawal and hypothalamic-pituitary-adrenal regulation. Presented at the 137th Annual Meeting of the American Psychiatric Association in Los Angeles, California, 1984: NR, Abstract #26.
95. Dilsaver S C, Greden J F. The effects of antidepressant withdrawal on the DST status of endogenously depressed patients. Psychiatr Res 1985:*14*:111-122.
96. Lomax P, Foster R F, Kirkpatrick W E. Cholinergic and adrenergic interactions in the thermoregulatory centers of the rat. Brain Res 1969:*15*:431-438.
97. Lomax P, Jenden D J. Hypothermia following systematic and intracerebral injection of oxotremorine in the rat. Neuropharmacology 1966:*5*:353-359.
98. Morpurgo C. Interaction of imipramine and desmethylimipramine with tremorine and oxotremorine in mice. Life Sci 1967:*6*:721-731.
99. Overstreet D H, Kozar M P, Lynch G S. Reduced hypothermic effects of cholinomimetic agents following chronic anticholinesterase treatment. Neuropharmacology 1973:*12*:1017-1032.
100. Snider R M, Dilsaver S C. Amitriptyline induced supersensitivity to the hypothermic effects of oxotremorine. Presented at at the Annual Meeting of the Federation of the American Society of Experimental Biology (FASEB), St. Louis, MO, April, 1986.
101. Avery D, Wilschiotz G, Rafaelsen. REM latency and temperature in affective disorder before and after treatment. Biol Psychiatry 1982:*17*:463-470.
102. Asnis G M, Halbreich U, Sachar E J, et al. Plasma cortisol and REM latency in adult endogenous depression. Am J Psychiatry 1983:*140*:750-753.
103. Schultz H, Lund R. Sleep onset REM episodes are associated with circadian parameters of body temperature: A study in depressives and normal subjects. Biol Psychiatry 1983:*18*:1411-1426.
104. Nomura Y, Kajiyama H, Segawa T. Alteration in sensitivity to isoproterenol and acetylcholine in rat heart after repeated administration of isoproterenol. J Pharmacol Exp Ther 1982:*220*:441-445.
105. Galen R S. Application of the predictive value model in the analysis of test effectiveness. Clin Lab Med 1982: *685*:99.
106. Galen R S, Gambino S R G. Beyond normality: the predictive value and efficiency of medical diagnosis. New York: John Wiley, 1975.
107. Winokur A, Amsterdam J, Caroff S, Snyder P J, Brunswick D. Variability of hormonal reponses to a series of neuroendocrine challenges in depressed patients. Am J Psychiatry 1982:*139*:39-44.
108. Targum S D. The application of serial neurendocrine studies in the management of depressive disorders. Biol Psychiatry 1983:*18*:3-19.
109. Greden J F, Carroll B J. Decrease in speech pause time with treatment of endogenous depression. Biol Psychiatry 1980:*15*:575-587.
110. Greden J F, Albala A A, Smokler I A, Gardner R, Carroll B J. Speech pause time: a marker of psychomotor retardation among endogenous depressives. Biol Psychiatry 1981:*16*:851-859.
111. Greden J F, Carroll B J. Psychomotor function and affective disorders. Am J Psychiatry 1981:*138*:1441-1448.
112. Fawcett J, Clark D C, Scheftner W A, Hedeker. Differences between anhedonic and normally hedonic depressives states. Am J Psychiatry 1983:*140*:1027-1030.
113. Duffy F H, Burchfield J L, Lombroso C T. Brain electrical activity mapping (BEAM): A method for extending the clinical utility of EEG and evoked potential data. Ann Neurol 1979:*5*:309-332.
114. Sagelas T, Erill S, Domino E F. Differential effects of scopolamine and chlorpromazine on REM and NREM sleep in normal male subjects. Clin Pharmacol Ther 1969:*10*:522-529.
115. Callaway E, Band R I. Some physiological effects of atropine. Arch Neurol Psychiatry 1958:*79*:91-102.
116. English D C. Reintegration of affect and psychic emergence with Ditran. J Neuropsychiatry 1962:*3*:304-311.
117. Toyoda J, Sasaki K, Kurkhara M. A polygraphic study on the effect of atropine on human nocturnal sleep. Folia Psychiatr Neurol 1966:*20*:275-289.
118. Orkin L R, Bergman P S, Nathanson M. Effects of atropine, scopolamine and meperidine on man. Anesthesiology 1956:*17*:30-37.
119. Osteid A M, Aruguete A. Central nervous system effects of hyosine in man. J Pharmacol Exp Ther 1962:*137*:13-139.
120. Wangemen C P, Hawk M H. The effects of morphine, atropine and scopolamine on human subjects. Anesthesiology 1942:*3*:24-36.
121. Dilsaver S C, White K. Affective disorders and associated psychopathology. J Clin Psychiatry 1986:*47*:162-169.
122. Dilsaver S C. Lithium's effects on muscarinic receptor

binding parameters: A possible relationship to its therapeutic efficacy. Biol Psychiatry 1984:*19*:1551-1565.
123. Creese I, Sibley D R. Receptor adaptations to centrally acting drugs. Ann Rev Pharmacol Toxicol 1981:*21*:317-391.
124. Ehlert F J, Kukka N, Fairhurst A S. Altered (^{3}H) quinuclidinyl benzilate binding in the striatum of rats following chronic cholinesterase inhibition with diisopropylfluorophosphonate. Mol Pharmacol 1980:*17*:29-30.
125. Gazit H, Silaman I, Dudai Y. Administration of an organophosphate causes a decrease in muscarinic receptor levels in rat brain. Brain Res 1974:*174*:354-356.
126. Luqmanc Y A, et al. Depolarization-induced changes in muscarinic cholinergic receptors in synaptosomes. Nature 1979:*271*:481-483.
127. Pestronk A, Drachman D B, Stanley E F, et al. Cholinergic transmission regulates extrajunctional acetylcholine receptors. Exp Neurol 1980:*70*:690-696.
128. Russell R W, Overstreet D H, Cotman C W, et al. Experimental tests of hypotheses about neurochemical mechanisms underlying tolerance to the anticholinesterase diisopropylfluorophonate. J Psychopharmacol 1979:*192*:73-85.
129. Russell R W, Carson V G, Jope R, et al. Development of behavioral tolerance: A search for subcellular mechanisms. Psychopharmacology 1979:*66*:155-158.
130. Russell R W, Carson V G, Booth R A, Tenden D J. Mechanisms of tolerance to the anticholinesterase: DFP, acetylcholine levels and dynamics in the rat brain. Neuropharmacology 1981.
131. Schiller G D. Reduced binding of (3H)-quinuclydinyl benzilate associated with low acetylcholinesterase activity. Life Sci 1979:*24*:1159-1164.
132. Shifrin G S, Klein W L. Regulation of muscarinic acetylcholine receptor concentration in cloned neuroblastoma cells. J Neurochem 1980:*34*:993-999.
133. Siman R G, Klein W L. Cholinergic activity regulated muscarinic receptors in central nervous system cultures. Proc Natl Acad Sci 1979:*76*:4141-4145.
134. Levy A, Zohar J, Belmaker R H. The effect of chronic lithium treatment in rat brain muscarinic receptor regulation. Neuropharmacology 1982:*21*:1199-1201.
135. Maggi A, Enna S J. Regional alterations in rat brain neurotransmitter systems following chronic lithium treatment. J Neurochem 1980:*34*:808-892.
136. Pestronk A, Drachman D B. Lithium reduces the number of acetylcholine receptors in skeletal muscle. Science 1980:*210*:342.
137. Jones B D, Steinberg S, Chouinard G. Fast-cycling bipolar disorder induced by withdrawal from long-term treatment with a tricyclic antidepressant. Am J Psychiatry 1983:*19*:237-256.
138. Lerer B, Stanley M. Effect of chronic lithium on cholinergically medicated responses and (^{3}H) QNB binding in rat brain. Brain Res 1985:*344*:211-219.
139. Lerer B. Studies on the role of brain cholinergic systems in the therapeutic mechanisms and adverse effects on ECT and lithium. Biol Psychiatry 1985:*20*:20-40.
140. Dasheiff R M, Byrne M C, Patroni U, McNamara J O. Biochemical evidence of decreased cholinergic neuronal communication following amygdala kindled seizures. Brain Res 1981:*206*:233-238.
141. Dasheiff R M, Savage D D, McNamara J O. Seizures down-regulate muscarinic cholinergic receptors in hippocampal formation. Brain Res 1982:*235*:327-334.
142. Dasheiff R M, McNamara J O. Evidence for an agonist independent down regulation of hippocampal muscarinic receptors in kindling. Brain Res 1980:*195*:345-353.
143. Byrne M C, Gottlieb N, McNammara T O. Amygdala kindling induces muscarinic cholinergic recptor declines in a highly specific distribution within the limbic system. Symp Neurol 1980:*69*:85-98.
144. El-Fakahany E, Richelson E. Effect of some calcium antagonists on muscarinic-mediated cyclic GMP formation. J Neurochem 1983:*40*:705-710.
145. Wehr T A, Goodwin F K. Rapid cycling manic-depressives induced by tricyclic antidepressants, Arch Gen Psychiatry 1979:*15*:149-154.
146. Bunney W E, Murphy D L. The neurobiology of lithium. Neurosci Res Progr Bull 1976:14:110-186.
147. Pandey G N, Davis J M. Biology of the lithium ion. In: Rossof A H, Robinson W A, Eds. Lithium effects on granulopoiesis and immune function. New York: Plenum, 1980:15-59.
148. Mellerup E T, Plenge P, Ziegler R. Lithium effects on calcium metabolism in rats. Int Pharmacopsychiatry 1970:*5*:258-265.
149. Gotfredsen C F, Rafaelson O J. Effect of lithium and other psychopharmaca on rat electrolyte metabolism. Int Pharmacopsychiatry 1970:*5*:242-248.
150. Garret K M, Ross D H. Use of terbium fluorescence to probe calcium receptors on synaptic membranes. Pharmacologist 1978:*20*:321.
151. Rafaelson O J, Mellerup E T. Mechanisms of action of lithium salts: Biochemical aspects. Psychiatr Neurol Neurochir 1973:*76*:523-528.
152. Devis G, Somers G, Van Obberghen E, et al. Calcium antagonists and islet function. Diabetes 1975:*24*:547-551.
153. Freer R J, Smith A B. Lithium/Ca++ interactions in depolarized rat uterine smooth muscle. Fed Proc 1977:*36*:603.
154. Freer R J, Smith A B. Lithium dissociation of calcium- and angiotensin-induced contractions in depolarized rat uterus. Am J Physiology 1979:*236*:C171-C176.
155. Rink T J, Baker P F. The plasma membrane in the regulation of intracellur calcium. In: Wasserman R H, Corradino R A S, Carafoli E, eds. Calcium binding proteins and calcium function. New York: North-Holland, 1977:235-242.
156. Van Breeman C, Aaronson P, Loutzenhiser R, Meisheri K. Ca++ movements in smooth muscle. Chest 1980: *78*:157-165.
157. Baastrup P C, Christiansen C, Transbol I. Calcium metabolism in lithium-treated patients. Acta Psychiatr Scand 1978:*57*:124-128.
158. Christensson T A T. Lithium, hypercalcaemia, and hyperparathyroidism. Lancet 1976:*1*:144.
159. Davis B M, Pfefferbaum A, Krutzik S, Davis K L. Lithium's effect on parathyroid hormone. Am J Psychia-

try 1981:*138*:489-492.

160. Dubovsky S L, Franks R D, Lifschitz M L, Coen P, Walker S H, Sutryan V. Hypocalciuric effect on lithium: A confirmatory study. Psychoneuroendocrinology 1982: *7*:355-359.
161. Franks R D, Dubovsky S L, Lifshitz M, Coen P, Subryan V, Walker S H. Long-term lithium carbonate therapy causes hyperparathyroidism. Arch Gen Psychiatry 1982: *39*:1074-1077.
162. Lanng Nielsen J, Christensen M S, Pedersen E B, Darling S, Amdisen A. Parathyroid hormone in serum during lithium therapy. Scand J Clin Lab Invest 1977:*37*:369-372.
163. Brown E M. Lithium-induced abnormal calcium-regulated PTH release in dispersed bovine parathyroid cells. Clin Res 1980:*28*:646A.
164. Shen F, Sherrard D J. Lithium-induced hyperparathyroidism: An alteration of the "set-point". Ann Intern Med 1982:*96*:63-65.
165. Carmen J S, Crews E, Bancroft A, et al. Calcium and calcium-regulating hormones in the biphasic periodic psychoses. J Operational Psychiatry 1980:*11*:6-17.
166. Singer I, Rotenberg D. Mechanisms of lithium action. N Engl J Med 1973:*289*:254-260.
167. Nelson R W, Cohen J L. Plasma and erythrocyte kinetic considerations in lithium therapy. Am J Hosp Pharm 1976:*33*:658-664.
168. Mullins L J, Brinkley F J. Calcium binding and regulation in nerve fibers. In: Wassenman R H, Corradino R A, Carafoli E, eds. Calcium binding protein and calcium function. New York: North-Holland, 1977:87-95.
169. Naylor G J, Dick D A T, Dick E G, Moody J P. Lithium therapy and erythrocyte membrane cation carrier. Psychopharmacology 1974:*37*:81-86.
170. Glen A I M, Ongley E C, Robinson K. Diminished membrane transport in manic-depressive psychosis and recurrent depression. Lancet 1968:*2*:241-242.
171. Naylor G J, Dick D A T, Dick E G, et al. Erythrocyte membrane cation carrier in mania, Psychol Med 1976: *6*:659-663.
172. Peet M. The potential difference across the rectal mucosa during depressive illness and lithium therapy. Br J Psychiatry 1975:*127*:144-148.
173. Naylor G J, Fleming L W, Stewart W K, et al. Plasma magnesium and calcium levels in depressive psychosis, Br J Psychiatry 1972:*120*:683-684.
174. Naylor G J, Dick D A T, Dick E G. Erythrocycte membrane cation carrier, relapse rate of manic-depressive illness and prediction of response to lithium, Psychol Med 1976:*6*:257-263.
175. Naylor G J, Smith A, Boardman L J, Dick D A T, Dick E G, Dick P. Lithium and erythrocyte membrane cation carrier. Psychol Med 1977:*7*:229-233.
176. Gutman D, Lichtenberg J, Cohen J, Boonyaviroj P. Increase and reduction of catecholamine release from adrenal medulla by liposomes loaded with various strimulants and inhibitors. Israel J Med Sci 1978:*14*: 998-999.
177. Nordmann J J. Hormone release and calcium uptake in the rat neurohypophysis. In: Carafoli E, Clementi F, Drabikowski W, eds. Calcium transport in contraction and secretion. New York: American Elsevier, 1975:285-286.
178. Cheung W Y. Calmodulin plays a pivotal role in cellular regulation. Science 1980:*207*:19-27.
179. Katz R I, Kopin I J. Release of norepinephrine-^{3}H and serotonin-^{3}H evoked from brain slices by electrical field stimulation - dependency and the effects of lithium, ouabain and tetrodotoxin. Biochem Pharmacol 1969:*18*: 1935-1939.
180. Rubin R P. Calcium and the secretory process. New York: Plenum Press, 1974.
181. Meyer S E. Neurohumoral transmission and the autonomic nervous system. In: Gilman A G, Goodman L S, Gilman A, eds. The pharmacological basis of therapeutics. New York: MacMillan, 1980:56-90.
182. Coppen A, Shaw D M, Malleson A, Costain R. Mineral metabolism in mania. Br Med J 1966:*1*:71-75.
183. Cox J R, Pearson R E, Speight C J. Changes in sodium, potassium, and body fluid spaces in depression and dementia, Gerontol Clin 1971:*13*:233-245.
184. Naylor G J, McNamee H E, Moody J P. Changes in erythrocyte sodium and potassium on recovery from a depressive illness. Br j Psychiatry 1971:*118*:219-221.
185. Naylor G J, Dick D A T, Le Poidevin D, White S F. Erythrocyte membrane cation carrier in depressive illness. Psychol Med 1973:*3*:502-508.
186. Jimerson D J, Post R M, Carman J S, et al. CSF calcium: Clinical correlates in affective illness and schizophrenia. Biol Psychiatry 1979:*14*:37-51.
187. Linnoila M, McDonald E, Reinila M, Leroy A, Rubinow D R, Goodwin F K. RBC membrane adenosine triphosphatase activities in patients with major affective disorders. Arch Gen Psychiatry 1983:*40*:1021-1026.
188. McDonald E, Rubinow D, Linnoila M. Sensitivity of RBC membrane Ca^{2+}-adenosine triphosphatase to calmodulin stimulation. Arch Gen Psychiatry 1984:*41*:487-493.
189. Niggli V, Adunyah E S, Penniston J T, Carafoli E. Purified (Ca^{2+}-Mg^2)-ATPase of the erythrocyte membrane. J Biol Chem 1981:*256*:395-401.
190. Niggli V, Adunyah E S, Carafoli E. Acidic phospholipids, unsaturated fatty acids, and limited protein mimic the effect of calmodulin on the purified erythrocyte Ca^2-ATPase. J Biol Chem 1981:*256*:8588-8592.
191. Roelofsen B: The (non)specificity in the lipid requirement of calcium and (sodium plus potasium)-transporting adenosine triphosphatases. Life Sci 1981:*29*:2235-2247.
192. Hokin-Naerverson M, Spiegel D A, Lewis W C. Deficiency of erythocyte sodium pump activity in bipolar manic-depressive psychosis, Life Sci 1975:*15*:1739-1748.
193. Feighner J P, Robins E, Guze S B, et al. Diagnostic criteria for use in psychiatric research. Arch Gen Psychiatry 1972:*26*:57-63.
194. Nadi N S, Nurnberger J I, Gershon E S. Muscarinic cholinergic receptors on skin fibroblasts in familial affective disorder. N Engl J Med 1984:*311*:225-230.
195. Lennox R H, Hitzemann R J, Richelson E, Kelsoe J R. Failure to confirm muscarinic receptors on skin fibroblasts in familial affective disorder. N Engl J Med

1984:*311*:225–230.

196. Kelsoe J R, Gillin J E, Janowsky D J, Brown J H, Risch S C, Lunkin B. Failure to confirm muscarinic receptors on skin fibroblasts. N Engl J Med 1984:*312*:861–862.
197. McVicar K. Abuse of antiparkinson drug by psychiatric patients. Am J Psychiatry 1977:*138*:809–811.
198. Pullen G P, Best N R, Maguire J. Anticholinergic drug abuse. A common problem? Br Med J 1984:*289*:612–613.
199. Kaminer Y, Munitz H, Wijsenbelc H. Trihexyphenidyl (artane) abuse: euphoriant and anxiolytic. Br J Psychiatry 1982:*140*:473–474.
200. Rubinstein J S. Abuse of antiparkinsonism drugs: feigning of extrapyramidyl symptoms to obtain trihexyphenidyl. J Am Med Assoc 1978:*239*:2365–2366.
201. Sorscher S M, Dilsaver S C. Antidepressant induced sexual dysfunction in males: Due to cholinergic blockade? J Clin Psychopharmacol (submitted).
202. Kazic I. Norepinephrine synthesis and turnover in the brain: acceleration by physostigmine. In: Usdin E, Snyder S H, eds. Frontiers in catecholamine research. Oxford: Pergamon Press, 1973:897–899.
203. Westfall T C. Effect of acetylcholine on the release of (^{3}H)norepinephrine by nicotine and potasium chloride from rat brain slices. In: Usdin E, Snyder S H, eds. Frontiers of catecholamine research. New York: Pergamon Press, 1973, 617–618.
204. Muscholl E. Regulation of catecholamine release. The muscarinic inhibitory mechanism. In: G Usdin, Snyder S H, eds. Frontiers in catecholamine research. New York: Pergamon Press, 1973:537–549.
205. Andersonn K, Fuxe K, Agnati L F. Effects of single injections of nicotine in the ascending dopamine pathways in the rat. Evidence for increases of dopamine turnover in neostriatal and mesolimbic dopamine neurons. Acta Physiol Scand 1981:*112*:345–347.
206. Jaeckle R S, Dilsaver S C. Covariation of depressive symptoms, parkinsonism, and post-dexamethasone plasma cortisol levels in a bipolar patient: simultaneous response to ECT and lithium carbonate. Acta Psychiatr Scand 1986:*74*:68–72.
207. Penny J B, Young A B. Speculations on the functional anatomy of basal ganglia disorders. Ann Rev Neurosci 1983:*6*:73–94.
208. Beani L, Bianchi C. Effect of amantadine on cerebral acetylcholine release and content on the guinea pig. Neuropharmacology 1973:*12*:283–289.
209. Beani L, Bianchi C. Effect of amantadine on cerebral acetylcholine release and content on the guinea pig. Neuropharmacology 1973:*12*:283–289.
210. Dilsaver S C: Pathophysiology of "cholinoceptor supersensitivity" in affective disorders. Biol Psychiatry 1986:*21*:813–829.

Address

Dr. *S. C. Dilsaver*
Mental Health Research Institute
205 Washfenaw Place
University of Michigan
Ann Arbor, MI 48109
U.S.A.