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ACUTE AND CHRONIC LITHIUM ADMINISTRATION TO THE RAT: RELATIONSHIP TO DRUG DISTRIBUTION AND BEHAVIORAL EFFECTS.

The Ohio State University, Ph.D., 1977
Pharmacology

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ACUTE AND CHRONIC LITHIUM ADMINISTRATION TO THE RAT:
RELATIONSHIP TO DRUG DISTRIBUTION AND BEHAVIORAL EFFECTS

DISSERTATION

Presented in Partial Fulfillment of the Requirements
for the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Donald Ray Britton, B.A.

* * * *

The Ohio State University
1977

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To my wife, Karen, I owe special thanks for her unique ability to provide emotional support as a loving partner and informed criticism as a colleague.

I will always be grateful to my first and most influential teachers - my mother and father.
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Work in Progress:

Effects of endorphins and enkephalins on opiate receptors (to be continued at U.C.S.D.)
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INTRODUCTION

In the late 1940's J.F.J. Cade was working in a small Australian mental hospital studying possible metabolic relationships to affective disorders. In testing the hypothesis that an abnormal metabolite might be associated with such disorders and that this metabolite might be present in greater quantities in the urine of manic-depressive patients, Cade injected concentrated urine into guinea pigs. He found that the urine of manic-depressive patients was more toxic than concentrates from control patients. As there was no reason to suspect changes in the amount of urea in the urine of manic patients, he looked for possible interactions with uric acid. In order to work with a water soluble form of urate, the salt lithium urate was used for the injections. Finding that lithium urate injections not only did not potentiate the toxic effects of the concentrated urine but actually provided a degree of protection, Cade looked at the effect of another lithium salt, lithium carbonate. Both lithium salts produced the protection against urine toxicity. Additionally, lithium salts alone produced a calming effect in the guinea pigs. This later finding caused Dr. Cade to look more fully at the effect of lithium itself. Following a series of self-administrations of lithium carbonate which he found to be without significant deleterious effects, Cade began the first clinical trial of lithium in the treatment of psychiatric disorders. In the September,
1949 issue of the *Medical Journal of Australia* Cade reported the results of these trials. Of the nineteen patients given lithium, three were chronically depressed, six were schizophrenic and ten were manic-depressive. Patients in the first two categories were generally unresponsive. All ten manic patients showed marked improvement. This initial report was soon followed by others and the efficacy of lithium therapy for manic-depressive illness was thus established.

In the nearly thirty years which have elapsed since Cade's first report of lithium's therapeutic effect in manic-depressives, this small ion has been the focus of considerable attention from both clinical and basic science researchers. Between 1949 and 1971, over sixteen hundred papers on the biological and pharmacological properties of lithium have appeared in the world's scientific literature (Kline, 1973).

**LITHIUM IN MEDICINE**

*Medical Use of Lithium (pre-1949):* While the modern therapeutic use of lithium is usually considered to have begun with the publication of the work of Cade (1949), lithium has a much longer history in medicine. Kline (1973) notes that alkaline waters (some of which presumably contained lithium) were discussed as treatments for behavioral disturbances as early as the 2nd century A.D. by the Greek writer Soranus of Ephesus. Hemmelhock (1976) noted that many of the mineral springs of Europe contain lithium in concentrations sufficient to provide therapeutic plasma levels upon ingestion of large quantities of the water and suggested a possible connection with the cures at these spas. The official introduction of lithium into rational therapy was based on the work of Garrod
in 1859 showing that the lithium salt of urate was the most soluble of the urate salts tested. This finding suggested the possible usefulness of lithium in promoting uric acid excretion (Johnson and Cade, 1975). This led to the use (albeit unsuccessful) of lithium in the treatment of gouty arthritis and eventually to Cade's selection of lithium as a carrier for uric acid in his studies of the metabolic factors associated with mania. By the early part of the twentieth century, lithium in various forms was being used for treatment of a variety of disorders. In their text Pharmacotherapeutics, Solis-Cohen and Githens (1928) refer to several lithium preparations in use for a variety of purposes: lithium carbonate as one of a variety of alkaline solutions used as an antiseptic, lithium benzoate as an antibacterial agent, lithium citrate as a diuretic and lithium bromide as a "nerve depressant". Although the recommended daily dose of lithium bromide (0.6 to 1.2 grams per day) is within the range of dosages used for lithium in current psychiatric therapy, its place in the neuropsychopharmacology of the time was clearly due to the bromide ion and its sedative effects. The notion of using lithium as a solubilizer of urate crystals in gouty arthritis had already been largely discontinued. Thus, the clinical use of lithium in the early 20th century was largely incidental, with lithium being only one of many alkaline metals used in preparations of various salts, the therapeutic value of which were attributed to the alkalinity of the solution or the active anion.

This largely innocuous history preceded the introduction of lithium chloride as a salt substitute for patients with restricted salt intake in the 1940's. Although lithium seemed the logical choice for a sodium
substitute as it had both the taste and appearance of the sodium salt, problems soon arose. The population most in need of a substitute for sodium chloride consisted of patients with various forms of cardiovascular disease. This fact predisposed those using lithium to the cardiotoxic actions of the ion. The reports of serious toxicity and even death resulting from lithium use led to the discontinuation of lithium chloride for this purpose. It was official designation as a poisonous substance in 1950. Lithium was not reintroduced as an FDA approved therapeutic agent in the United States until 1970 and then with the restrictive clause that the sole indicant for the use of lithium was the treatment of manic episodes associated with manic depressive psychoses.

Modern Psychiatric Use of Lithium: Although Cade's first report of lithium's efficacy in treating manic depressive disorders was, emperically, an auspicious debut for any psychopharmacologic agent, the acceptance of lithium into the therapeutic arsenal of psychiatry was something less than overwhelming. Kline (1973) notes that in the five years immediately following Cade's publication only eight additional papers on the psychiatric use of lithium appeared in the world's literature. By 1964 (15 years after the initial report) there was an average of only four papers per year published on the clinical uses of lithium. In the five year period from 1959 to 1964 the only publication from the United States on the clinical uses of lithium was a letter to the editor appearing in the Journal of the American Medical Association.

Several factors have been suggested as contributing to the initially slow acceptance of lithium. Firstly, its psychiatric use began shortly after widespread concern over the toxicity of the agent during its use
as a salt substitute. It is understandable that the path from being officially designated a poisonous substance to its present position as the treatment of choice for manic episodes is a long one fraught with concern for the patients well-being. The low therapeutic index of lithium still demands that considerable attention be given to the management of patients undergoing such treatment. Secondly, it was in the decade immediately following Cade's initial report that other drugs began to make inroads in psychiatry which drastically reduced the number of institutionalized psychiatric patients and gave birth to the era of clinical psychopharmacology. Delay and Deniker (1952) are credited with the first reports of the antipsychotic properties of chlorpromazine. Meprobamate was introduced by Berger in 1954 as an antianxiety agent. The tricyclic antidepressants were introduced in 1958 with the report by Kuhn of the antidepressant effects of imipramine. With these and several other drugs newly available to the psychiatrist it is not surprising that the discredited panacea, lithium, should remain in the background. Another factor noted by Johnson and Cade (1973) as contributing to lithium's slow acceptance is an economic one. Drug companies, having invested substantially in the development of the aforementioned psychoactive agents, could hardly be expected to divert major efforts to proselytize on behalf of a drug which was selling for 67¢ per pound in 1959 and could not be patented.

The eventual widespread interest in lithium was almost certainly supported by two factors. On the clinical side, the reports which were made were fairly consistent in showing improvement in 60 to 90% of patients diagnosed as manic phase manic depressive psychotics. A second factor contributing to the acceptance of lithium is the role it played
in the development of theoretical concepts of the etiology of affective disorders. The work of Schildkraut and others (Schildkraut et al, 1966, 1967 and 1969) demonstrated that acute effects of lithium in animals included a decrease in the amount of norepinephrine available for release in the brain. These findings along with the observed effects of reserpine, the monoamine oxidase inhibitors and other psychoactive agents were supportive of the "catecholamine hypothesis" of affective disorders as first proposed by Schildkraut in 1965. The central theme of this hypothesis is that states of elevated activity, heightened mood and inappropriately heightened affect are associated with an increase in the relative contribution made by the catecholamines norepinephrine and dopamine to the neurotransmitter balance in the brain while states of depression are associated with relatively low levels of catecholamine activity. It was thus proposed that states of manic excitement resulted from excess amounts of catecholamines being active in the central nervous system and that lithium, by reducing the availability of catecholamines, exerted its therapeutic action resulting in a normalization of the patient.

Following its somewhat laborious ascent from the quagmire of folk medicine when it had been tried as a remedy for everything from gout to diabetes to bacterial infections, lithium has become something of a focal point for those interested in the biochemical bases of mental disorders. In the five years between 1966 and 1970, a bibliography prepared by Schou (1972) shows there were close to 900 papers published on the pharmacology and biology of lithium.
While the use of lithium in the treatment of manic phase manic depressive patients has received the greatest attention, a recent report of the American Psychiatric Association Task Force for the study of lithium therapy (APA Task Force, 1975) supports the contention of many psychiatrists that lithium is also effective in the prophylactic treatment of depressive episodes associated with manic depressive illness. This aspect of lithium treatment is reviewed by Schou and Thomsen (1975).

Other disorders in which lithium treatment has been evaluated include hyperkinesis in children (Greenhill et al, 1973; Whitehead and Clark, 1970). The results of these two studies were not supportive of a place for lithium in hyperkinesis therapy.

Lithium has been shown to be effective in the treatment of aggressive behavior in some individuals. Sheard (1971) found lithium was effective in reducing various manifestations of aggression in a group of prison mates with a history of violent and aggressive behavior. Favorable responses to lithium have also been reported in the treatment of premenstrual tension (Sletten and Gashon, 1966), tardive dyskinesia (Simpson, 1973). Lithium treatment has also been evaluated in the treatment of epilepsy, sociopathy and alcoholism (reviewed by Kline and Simpson, 1975).

**Lithium and Manic Depressive Symptomatology:** Current estimates of the incidence of manic depressive disorders range approximately from 1.0 to 1.6% based on lifetime expectancy. As is the case with other forms of affective disorders, the incidence is higher among females than among males. In both sexes the peak incidence occurs relatively late in life, between the ages of 55 and 64 for males and 60 to 69 for females, (Klerman and Barrett, 1973). However, the disorder is not restricted to this
age group and manic episodes responsive to lithium have been observed in patients as young as eleven (Huerta, personal communication).

As previously noted, lithium is currently regarded as the treatment of choice for manic phase manic-depressive patients. This form of affective disorder has been long recognized under various nosological systems. With the evolution of these systems the syndrome of the manic depressive disorder has defined and re-defined reflecting the philosophical and scientific notions of the time.

Among the nosological systems currently in use is the Research Diagnostic Criteria (RDC) described by Spitzer et al (1975). Using this system, diagnoses are made on the basis of the presence of specific symptoms and the absence of others. The checklist for the diagnosis of mania is shown below.

A. Either is required:
   1) Euphoria
   2) Irritability

B. If euphoric, at least three (if irritable, four) of the following are required. (One less for hypomania):
   1) Hyperactivity (at work, socially, sexually or physically)
   2) Pressure to keep talking
   3) Flight of ideas
   4) Grandiosity (may be delusional)
   5) Decreased need for sleep
   6) Distractability
   7) Reckless behavior (e.g. spending sprees, sexual indiscretions, foolish investments, reckless driving)
C. At least one of the following is present (none for hypomanic):
   1) Meaningful conversation is impossible
   2) Serious impairment socially, at school, at home or at work
   3) Hospitalization
D. Duration at least one week (any duration if hospitalized)
E. Does not meet criteria B for schizo-affective disorder
   (Criteria B for schizo-affective disorder involves the presence of delusions of being controlled and/or hallucinations)

As is the case in much if not all of clinical psychopharmacology, one cannot speak in terms of cure but rather must deal with controlling symptoms. It is relevant then to ask which symptoms of mania are most often controlled with lithium therapy. This question is not easily resolved due to a number of methodological problems. Very often reports in the clinical literature specify only the number of patients showing improvement and the rating scale(s) if any by which the patient's status was evaluated. Additionally, such patients are often treated with multiple drugs introducing further variables to be considered. This type of report sheds little light on the action of lithium on specific symptoms. In a review of the effects of lithium in the treatment of mania Gershon (1970) describes the limitations of such studies and discusses three studies which he considered most informative. Of the three, one study (Johnson et al, 1968) of 42 patients (28 manic and 14 schizoaffective) reports a 78% remission rate for manic patients with lithium compared to 36% with chlorpromazine. Both drugs were found to decrease the elevated motor activity of manic patients with chlorpromazine showing a faster onset of action in this respect. Normalization of affect and ideation were also
reported. In this same review Gershon notes what other reports have confirmed; that lithium is not generally found to be superior to placebo in schizophrenic states. Hemmelhock (1976) has likewise noted the effect of lithium (particularly when used in conjunction with haloperidol) on the motor behavior of manic patients, in fact suggesting this to be the most pronounced evidence of therapeutic action. This reduction in motor activity is not immediate, but may be seen within 3 to 7 days following initiation of treatment.

**Lithium Dosages, Absorption and Excretion:** As previously mentioned, lithium has a relatively low therapeutic index indicating therapeutic levels are often quite close to toxic levels. The dose typically recommended may range from 0.8 to 1.8 g per day depending on a number of factors including the age, weight, physical condition and psychiatric state of the patient. The gastrointestinal absorption of lithium is quite rapid and peak blood levels occur within 2-4 hours following oral administration. The serum half-life for lithium in humans is approximately 24 hours (Bergner et al, 1973).

There is a great deal of intraindividual variability in the dosages required to produce a given blood level and in the blood levels required to produce a therapeutic response. However the optimal serum lithium levels for manic patients at 12 hours after the last dose range from 0.7 to 1.4 meq/liter (Prein et al, 1971 and Schou et al, 1971). Following the rapid absorption of lithium into the circulation, there is considerable variation in the rate at which it distributes to various tissues (Schou, 1958; Birch and Jenner, 1973; Bond et al, 1975). Lithium appears to equilibrate relatively slowly with brain tissue reaching equilibrium with
plasma approximately 24 hours following administration. Regional differences in the distribution of lithium within the brain have also been reported (Bond et al, 1975).

The elimination of lithium is almost entirely in urine (Trautner et al, 1955) with less than 1% of the ingested dose appearing in feces. The actual rate of elimination again varies considerably among individuals. Among the more interesting aspects of this variability is the finding that manic patients tend to retain lithium to a greater extent than do normals or schizophrenics. Table 1 taken from the work of Almy and Taylor (1973) shows that in their study manic patients excreted lithium at a rate less than 60% that of normal controls. Other studies have reported similar observations noting that during the manic phase patients both tolerate and require dosages which become toxic once the manic attack breaks (Kerry, 1975).
TABLE 1

Urinary lithium excretion in manic patients and normal control subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total urinary excretion of lithium in 36 hours, mEq mean ± SD</th>
<th>Total urinary volume in 36 hours, Liters mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>16.84 ± 2.13</td>
<td>2.04 ± 0.08</td>
</tr>
<tr>
<td>Manic Patients</td>
<td>9.84 ± 1.76</td>
<td>2.04 ± 0.33</td>
</tr>
<tr>
<td>P (normal vs. manic)</td>
<td>0.001</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

(from Almy and Taylor, 1973)
Physical Properties of Lithium: Lithium is the lightest of the alkali metals with an atomic weight of 6.939. As one would anticipate from its position in the periodic table, lithium has many properties similar to the next heaviest of the alkali metals, sodium. This fact has prompted suggestions that lithium may exert its effects in biological systems by interfering with the normal role of sodium. A comparison of the properties of lithium with other cations, however, shows that lithium also resembles potassium and magnesium in certain respects. Table shows the values of some of these properties for lithium, sodium, potassium, magnesium and calcium. The acknowledged importance of these other cations in biological systems demands that the possibility of lithium substitution for these other ions not be overlooked.

Although the ionic radius of lithium is the smallest of any of the elements in Table 2, lithium readily becomes hydrated in solution. The hydrated radius of lithium, 3.40 Å, is actually larger than the radius of the hydrated sodium ion (2.76). Williams (1973) notes that the small size of the unhydrated lithium ion suggests a propensity for lithium to fit in a lattice of small anions thus reflecting Pauling's radius-ratio effect. The water molecule is one such molecule which readily accommodates lithium. While lithium will readily form complexes with other anions, these complexes have low stability and the only stable form of lithium in biological systems is the lithium ion (Millerup and Jorgensen, 1975). The similarity of lithium to both sodium and magnesium has prompted researchers to consider systems known to be dependent on these two endogenous cations as possible sites for competition by lithium.
Table 2

Physical Properties of Lithium

<table>
<thead>
<tr>
<th>Ion</th>
<th>Atomic Weight</th>
<th>Valence</th>
<th>Ionic Radius</th>
<th>Coordination Number</th>
<th>Hydrated Radius</th>
<th>Rate of Exchange of water from Hydrated Cations</th>
<th>Ionization Potential (ev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li⁺</td>
<td>6.94</td>
<td>1</td>
<td>0.60</td>
<td>4,6</td>
<td>3.40</td>
<td>$5.0 \times 10^{-8}$/sec</td>
<td>5.4</td>
</tr>
<tr>
<td>Na⁺</td>
<td>22.99</td>
<td>1</td>
<td>0.95</td>
<td>6</td>
<td>2.76</td>
<td>$1.0 \times 10^{-9}$/sec</td>
<td>5.1</td>
</tr>
<tr>
<td>K⁺</td>
<td>37.10</td>
<td>1</td>
<td>1.33</td>
<td>6</td>
<td>2.32</td>
<td>$1.5 \times 10^{-9}$/sec</td>
<td>4.3</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>24.31</td>
<td>2</td>
<td>0.65</td>
<td>6</td>
<td></td>
<td>$1.0 \times 10^{-5}$/sec</td>
<td>7.6, 15.0</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>40.08</td>
<td>2</td>
<td>0.99</td>
<td>6,8</td>
<td></td>
<td>$3.0 \times 10^{-8}$/sec</td>
<td>6.1, 11.9</td>
</tr>
</tbody>
</table>


Behavioral Studies with Lithium: One of the anomalies in the development of lithium therapy is that the initial findings of Cade of a calming, sedating effect in guinea pigs has often been difficult to replicate. Numerous studies have been made of the effects of lithium on locomotor activity in laboratory animals, yet the general results of these studies indicate that lithium has little if any effect on activity of animals except when lithium is administered very shortly before testing or the animal is made hyperactive with other drugs.

Johnson (1971) reported that rats treated with LiCl (2.4 meq/kg/day) for four days were slightly less active on an activity wheel than were NaCl treated controls. This difference, however, failed to achieve statistical significance. Segal et al. (1975) reported that rats receiving a single injection of lithium (1.5 meq/kg) immediately prior to testing showed a significant reduction in locomotion. However, if the single injection was given 24 hours prior to testing or if the animals received eight daily injections with the last injection 24 hours prior to testing they did not differ significantly from the NaCl treated controls in spontaneous locomotion. Judd et al. (1975) likewise found no reduction in spontaneous activity in rats provided a diet containing 30 mmol Li/kg dry food. Similar results were obtained by Furukawa et al. (1975) using mice with lithium chloride (0.52, 1.58 or 4.72 meq/kg) for 1, 7, 14 and 21 days prior to activity testing in an open field situation. U'Prichard and Steinberg (1972) studied the effects of lithium on another measure of activity in animals. They injected mice with a single dose of LiCl (1.5 meq/kg, s.c.) and tested the animals four hours later. The activity measure consisted of counting the number of times the animal would poke
its nose into one of a series of holes in the floor of the testing apparatus. This response was not affected significantly by lithium. Other reports have confirmed a suppressive effect of lithium on activity in animals. Syme and Syme (1973) tested rats on activity platforms either alone or in pairs at either 20 minutes or 3 hours following the single injection of 3.0 meq/kg LiCl. They found a decrease in activity at both times under both testing conditions (alone vs. paired). Lithium was least effective in animals tested alone at 3 hours post-injection and greatest at 20 minutes post-injection for both pair tested and individually tested subjects. In one of the relatively few studies showing an inhibitory effect of lithium on locomotor activity Smith and Smith (1973) fed rats a diet containing 0, 20, 40 or 70 mmol Li/kg of food for a period of one week before testing in an open field. The three lithium diets resulted in serum lithium levels of 0.31, 0.63 and 0.92 meq/l for the low, medium and high lithium diets respectively. All three lithium treated groups showed a decrease in open field activity compared to pair fed controls. However, activity wheel scores for the lithium treated animals actually tended to be higher than the scores of animals given a normal diet with free access to food but lower than pair fed control animals. In both the open field tests and the activity wheel tests, the pair fed control group was significantly more active than the group receiving the normal diet ad lib. Thus, by using pair fed controls to compare with the lithium treated animals they established a higher level of "normal" activity from which to judge the suppressive effects of lithium. Given the consistent finding that hungry animals are more active under almost any situation, the validity of comparing lithium treated rats on an ad lib diet with animals on a
restricted diet may be questioned when comparing the results to those of other studies. In a later study, Smith (1975) injected rats twice daily with LiCl (1.5 meq/kg) for five days. The last injection was given approximately four hours prior to activity testing in the open field. This lithium regimen resulted in mean plasma lithium levels of 0.87 meq/l and a significant decrease in the number of squares entered in the three minutes test period. A recent study by Mackherjee et al (1977) investigated the effects of lithium in a single injection of 1.0, 2.0 or 3.0 meq/kg on motor activity in rats for a period of up to 72 hours. They found activity to be significantly reduced from 4 to 24 hours following lithium with no significant difference from controls after 24 hours. In this acute study, the alterations in locomotor activity were roughly inversely proportional to the lithium concentration in various brain regions.

The many inconsistencies found in these various studies of locomotor activity are somewhat surprising in light of the aforementioned clinical impression that reduced locomotion is perhaps the most striking of lithium's therapeutic effects.

There have been numerous reports of the interactions of lithium with other neuropharmacological agents in producing behavioral effects. Many of the studies previously mentioned used lithium in conjunction with other agents to ascertain the behavioral effects of lithium and some of the possible biochemical mechanisms underlying those effects. Other studies to be considered here reported only on the effects of lithium with these other agents and made no attempt to compare the effects of lithium alone to a control group.
Among the first behavioral effects of lithium to be studied was that resulting from its interaction with monoamine oxidase inhibitors (e.g. tranylcypromine and pargyline). Grahame-Smith and Green (1974) showed that rats treated for three days with lithium followed by an injection of tranylcypromine became hyperactive. Judd et al (1975) reported that rats maintained on a diet containing 30 mmol/kg lithium in dry food for 14 days then given tranylcypromine (15 mg/kg, i.p.) became significantly more active than control animals maintained on a lithium free diet. This difference was abolished when the animals were also given a tyrosine hydroxylase inhibitor (α-methyl-p-tyrosine). In their study, lithium treatment alone did not significantly alter whole brain concentrations of DA, NE or 5-HT. Tranylcypromine produced an elevation of all three neurotransmitters in both lithium treated and control groups. The DA concentrations were significantly greater in lithium treated animals than in controls. Treatment with α-methyl-p-tyrosine given before the tranylcypromine eliminated the difference in DA levels as it abolished the differences in activity. In the study by Smith (1975), rats were injected twice daily with LiCl (1.5 meq/kg) for 5 days. The last lithium injection was given approximately 4 hours prior to testing in the open field. Pretreatment of both the lithium and saline treated controls with p-chlorophenylalanine (100 mg/kg/day) for each of the three days prior to testing elevated open field activity and reduced to insignificance the difference between the two groups. Pargyline (100 mg/kg) had a similar effect in elevating activity in both groups. The lithium treated animals were slightly (but not significantly) more active than control groups in response to pargyline.
Matussek and Linsmayer (1968) reported on the ability of lithium to block drug induced hyperactivity in rats. In this study, rats were placed on a 10 cm high cage. Those animals which moved from the top of the cage at least twice and "showed the typical signs of compulsive behavior" were judged to be hyperactive. This state of hyperactivity was produced in 70-100% of the animals treated with desmethyldimipramine (DMI) (20 mg/kg) followed one hour later with the benzoquinolizine Ro 4-1284 (15 mg/kg). Pretreatment of the animals with LiCl (100 mg/kg or 200 mg/kg, i.p.) one hour prior to injections of DMI reduced the incidence of hyperactivity to 30%. Three such injections eight hours apart further reduced the incidence of hyperactivity to 17% and four LiCl injections resulted in the complete elimination of hyperactivity in the ten animals so tested. Interestingly, they found (but did not report quantitatively) that animals receiving four injections of LiCl were not resistant to hyperactivity produced by 6 mg/kg amphetamine. In fact, in their paradigm, amphetamine (0.1-3.0 mg/kg) attenuated the DMI - Ro 4-1284 induced hyperactivity in the same fashion as did lithium. Johnson (1971) reported similar findings for a number of CNS stimulants. Rats tested on an activity wheel were found to increase their level of activity in response of d,l-amphetamine (5.0 mg/kg), methamphetamine (5.0 mg/kg), methylphenidate (10.0 and 20.0 mg/kg) and caffeine (10.0 mg/kg). Animals treated with LiCl (2.4 meq/kg/day) for 4 days showed a potentiation of the stimulant response, actually becoming more active than NaCl treated controls given the same stimulants. Contrary findings were reported by Furukawa et al. (1975) showing that, in mice, LiCl (1.58 or 4.72 meq/kg/day, s.c.) for 1, 7, 14 or 21 days resulted in an antagonism of methamphetamine induced hyperactivity in the open field.
In the study by Segal et al (1975) similar findings obtained. Locomotor activity was measured following either LiCl or NaCl pretreatment and 0.5 mg/kg d-amphetamine. Animals given a single injection of LiCl (1.5 meq/kg) either immediately before or 24 hours before testing and animals given eight daily injections of lithium with the last injection 24 hours prior to testing were all less hyperactive in response to d-amphetamine than were the saline treated controls.

Other behavioral effects of lithium in laboratory animals have also been reported. Delgado and DeFeudis (1969) demonstrated that injections of LiCl into the amygdala-hippocampus region of the rhesus monkey resulted in a decrease in spontaneous restlessness and aggressiveness. Systemic administration of lithium has been shown to reduce foot shock induced aggression in rats (Sheard, 1970; Eichelman and Thoa, 1973). Additionally, lithium has been shown to decrease operant behavior (Ahlenius and Engel, 1973) and intracranial self-stimulation (Edelson et al, 1976).
Lithium Effects on Neurotransmitters: The fact that lithium is distributed throughout the body and shares many physical properties with the endogenous cations sodium and magnesium suggests the potential for effecting a multitude of cellular processes. In fact, lithium has been shown to alter many biological systems including those of neurons.

Early emphasis in lithium studies focused on its effects on catecholaminergic systems. This emphasis was understandable in light of the newly evolving catecholamine hypothesis of affective disorders. In a series of studies from the mid-1960's, Schildkraut and others (Schildkraut et al., 1966; Schildkraut et al., 1969) established that lithium produced alterations in norepinephrine (NE) metabolism in rats. Using intracisternal injections of $^3$H-NE followed by i.p. injections of lithium, they reported a decrease in the amount of $^3$H-NE and $^3$H-normetanephrine associated with an increase in the amount of $^3$H-deaminated metabolites of NE. The observed decrease in $^3$H-NE produced by lithium treatment suggests the possibility of an increase in the turnover rate. This interpretation is supported by the work of Corrodi et al. (1967) which also showed an increase in NE turnover and an increase in the proportion of deaminated metabolites following short term (15 minutes-48 hours) lithium administration. The increased turnover of NE reported by Corrodi et al. following a single injection of 7.5 meq/kg LiCl was measured by the rate of disappearance of NE following inhibition of the synthetic enzyme tyrosine hydroxylase. With this technique NE was found to be lowest 28 hours following lithium administration with some apparent recovery towards normality at 52 hours following lithium. There was no significant alteration in dopamine (DA) or serotonin (5-HT) in this study. Similar results were reported by
Stern et al. (1969) showed an increase in the turnover rate of brain NE with little effect on heart NE when animals were sacrificed within 48 hours following a single injection of lithium (3.75 meq/kg).

Studies examining the effects of longer term lithium administration have shown the importance of treatment duration on the effects on neurotransmitters. Animals given lithium for 5-10 days were shown to have increased rates of NE synthesis (Poitou and Bohuon, 1975; Greenspan et al., 1970). Following longer term lithium administration (15-28 days) the initial effects on NE synthesis are no longer apparent (Poitou and Bohuon, 1975; Ho et al., 1970; and Corrodi et al., 1969). The reports of effects of lithium on DA metabolism have been less consistent. Several researchers have reported no alteration in DA metabolism (Schubert, 1973; Corrodi et al., 1969). Other reports suggest regional and/or treatment duration dependent effects. Poitou and Bohuon (1975) reported a decrease in the level and synthesis of DA following 15 days of lithium treatment. This finding is substantiated by the report of Friedman and Gershon (1973) that treatment with 1 or 2 meq/kg per day LiCl for 14 days resulted in a decrease in DA synthesis of 34 and 62% respectively. In this same study, acute treatment with a single injection of 2 or 4 meq/kg LiCl produced no alteration in DA synthesis nor did lithium added to tissue slices in vitro affect conversion of tyrosine to DA. In the study by Friedman and Gershon, animals were sacrificed and assays performed 1 hour after the last lithium injection. This time factor is suggested by Segal et al. (1975) to account for the differences found in their study in which animals were sacrificed 24 hours after the last of 8 daily injections of LiCl. At that time, tyrosine hydroxylase activity in both the substantia nigra and the caudate-
putamen were significantly increased, a finding not readily integrated with reports of decreased DA synthesis.

The effects of lithium on serotonin metabolism have been a subject of some interest in recent years. A series of papers from the laboratory of Arnold Mandell (Knapp and Mandell, 1973; Mandell and Knapp, 1975; Knapp and Mandell, 1975) suggests the importance of treatment duration on various parameters of 5-HT metabolism. The overall picture emerging from these studies is that short term (3-5 days) lithium treatment causes an increase in the uptake of tryptophan into striatal nerve endings. At this time, the excess tryptophan uptake is reflected in an increase in the synthesis of 5-HT. This finding is compatible with the previous report by Schildkraut et al (1969) that acute lithium treatment increased the conversion of $^{14}$C-tryptophane to $^{14}$C-5-HT \textit{in vivo}. After 21 days of treatment, however, while the rate of tryptophan uptake by synaptosomes remains elevated above control values, the synthesis of 5-HT returns towards control levels. This effect is associated with a decrease in the $V_{\text{max}}$ of midbrain solubilized tryptophan-5-hydroxylase. \textit{In vitro} studies showed lithium added to a synaptosomal preparation could simulate the observed increase in tryptophan uptake but was without effect on tryptophan-5-hydroxylase activity. This finding is consistent with an effect of lithium, either directly or via a feedback mechanism resulting from increased tryptophan uptake, on the synthesis of the enzyme rather than on alteration of the activity of the molecule. Williams (1973) notes that Group 1A cations typically play a structural rather than a catalytic role in enzyme activity thus altering the $K_{\text{m}}$ of a reaction but not the $V_{\text{max}}$. 
In contrast to the studies on 5-HT synthesis is the evidence that
in vitro lithium decreases stimulated release of 5-HT from striatal brain
slices (Katz and Kopin, 1969; Katz et al, 1968). In light of the previously
mentioned findings of Ho et al (1970) of a decrease in turnover of 5-HT,
in hypothalamus accompanied by an increase in cerebellar 5-HT turnover
the importance of lithium effects which may vary both regionally and as a
function of duration of treatment is emphasized.

While the majority of reports of Li effects on neurotransmitters
concerns the monoamines, there is some evidence for effects on cholinergic
systems as well. The majority of these studies (reviewed by Vizi, 1975)
involve in vitro effects apparent only with very high concentrations
(> 10.0 mM) of lithium. Such effects cannot be entirely ignored since
the concentration of Li⁺ at specific sites of action could well exceed
those in plasma or tissue homogenates. However, in light of the multitude
of changes observed in other neurotransmitter systems with relatively low
concentrations, an action of Li and ACh metabolism does not at present appear
to be among the more profitable lines of investigation.

Both γ-aminobutyric acid (GABA) and glutamic acid have been shown
to be affected by Li. Gottesfeld (1976) treated rats for 5 days with
2 meq/kg LiCl twice daily with the last injection given 1 hour prior to
sacrifice. This treatment resulted in increased concentrations of GABA
in the hypothalamus and increased glutamic acid in the amygdala and
hypothalamus. Neither glutamic acid nor GABA levels in the cortex or
brain stem were affected. An earlier study by Bond (1973) found no effect
of lithium, either fed to animals chronically or added to the incubation
medium, on the uptake of GABA by slices of rat cortex.
SCOPE AND RATIONALE

Lithium shares with many other psychopharmacological agents the property of displaying a latency from the initiation of treatment to the onset of therapeutic action. Such a latency in the case of most drugs could be attributed to a number of factors including: a) time required for distribution to the site(s) of action; b) time required for the agent, once at its site(s) of action, to modify biological systems to the extent required to alter their functioning; and c) time required for the formation of active metabolites to accumulate and distribute to the active sites to exert an effect. In the case of lithium, investigations of this latency effect are somewhat simplified, at least to the extent that the lithium ion undergoes no biotransformation. The study of relevant factors in understanding lithium's effects may then focus on the distribution of the ion and its biological effects.

The pharmacokinetics of lithium were studied with respect to the urinary excretion of a single dose and the plasma, red blood cell (RBC) and tissue concentrations of lithium 24 hours after 1, 6 or 21 days of daily injections of 2.0 meq/kg body weight. The intent of studying the urinary excretion of a single dose of lithium is to provide some information as to its probable biological half life. From this information one can then make some general predictions regarding the probable distribution of the ion at various times following injection. Urinary excretion of most compounds is related to the amount of drug in plasma, the rate at which the drug is filtered through the glomeruli and the extent to which
it is reabsorbed into the circulation. Single dose kinetics usually display a biphasic elimination curve with a fast component, occurring at a time when plasma levels are at a peak, and a slower component reflecting a gradual reduction of plasma levels associated with a redistributed from tissues back to the plasma. Such information reflecting the time course for the variations in lithium concentration in intracellular or other "deep" compartments relative to the concentration in plasma or extracellular fluid may suggest particular aspects of its probable effects at varying times. A drug effect which temporally coincides with the peak plasma levels may be exerting its effects at extracellular sites. Such an action for lithium would not be unreasonable if its mechanism of action involves substitution for endogenous ions in extracellular or membrane processes. The previously discussed behavioral experiments have in fact been largely consistent with such an interpretation since most studies reported behavioral effects only when the animals were tested very shortly following the last administration of lithium.

Other studies have reported a plasma half life for lithium of less than 24 hours and a maximal concentration of lithium in brain occurring approximately 24 hours following administration (Ebadi et al, 1974). These kinetics also suggest the behavioral effects reported for lithium may be related to plasma rather than tissue concentrations of the drug.

One of the major effects of lithium treatment on manic symptoms is a reduction in the patients' motor activity, and this effect requires several days to develop. It therefore seems likely that the suppression of activity reported in most of the studies previously cited may not be mechanistically related to the therapeutic action of lithium. In order
to better define the effects of lithium on motor activity, animals were tested under a variety of circumstances. These studies were designed to assess the effects of lithium on both the "spontaneous" activity of the animals and the increased level of activity stimulated by placing the animal in a novel environment. These tests seem particularly appropriate in light of the fact that one of the symptoms associated with manic behavior is overresponsiveness to changes in the environment. If the effect of lithium is not solely on motor output *per se* but also on sensory or integrative mechanisms mediating the response to the environment one would expect to see a greater effect of lithium on animals exposed to such stimuli as environmental novelty.

To further gauge the effect of lithium on an animal's response to its environment, rats were tested for the magnitude of their response to a stimulus which causes a startle reflex. The test used was the acoustic startle response. In this test, animals are presented with a series of loud noises and the magnitude of the somatic response as reflected by a sudden hind limb flexion is measured. This response has previously been shown to be susceptible to pharmacological manipulation (Overstreet, 1977; Fechter, 1974). Heninger and Davis (1976) showed an effect of lithium in potentiating the startle response in rhesus monkeys occurring at the same time during treatment as did alterations in cortically recorded sensory evoked potentials.

In order to make some judgement of the neurotransmitter systems which might be involved in particular behavioral effects of lithium, animals treated with either NaCl or LiCl were given additional drug treatments shown to have marked effects on particular systems.
Numerous studies have demonstrated a strong influence of catecholaminergic systems on locomotor activity. Activity has been shown to be increased by agents which augment noradrenergic and dopaminergic activity (Randrup and Munkvad, 1966; Hughes and Grieg, 1976; Jacobs et al, 1975; Benkert et al, 1973). For this reason some animals were treated with the precursor to dopamine and noradrenaline, L-dopa. Other animals were administered the monoamine oxidase inhibitor, pargyline, which increases both catecholamine and 5-hydroxytryptamine brain levels. Amphetamine, which at the doses used has been shown to stimulate the release of noradrenaline and dopamine, was used with animals tested in the startle response. If the locomotor effects of lithium treatment are associated with a marked alteration in catecholaminergic systems, a significant decrease in the functional status of such systems would be expected to obscure the lithium effects, resulting in little difference between lithium treated subjects and controls. To test this possibility, the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine was administered to animals following various durations of lithium treatment. The same rationale is applied to the use of p-chlorophenylalanine which is known to block the synthesis of 5-hydroxytryptamine.

A further question addressed in this study is the relationship between lithium levels in various brain regions following different durations of lithium treatment. If the lithium concentration varies from region to region and the effects of lithium are dependent upon its concentration at its site(s) of action such a differential distribution might be expected to correlate with observed behavioral effects.
METHODS AND PROCEDURES

**Subjects:** All animals used in these experiments were Long-Evans Hooded rats from Blue Spruce Farms, Inc. of Altamont, New York. Animals weighed between 225 and 250 g at time of arrival at the vivarium and between 250 and 300 g at time of testing. Immediately upon arrival at the vivarium animals were housed either individually (cage dimensions: 42 x 20 x 15 cm in height) or in groups of either 3 or 4 animals per cage (dimensions 54 x 25 x 16 cm in height). All animals were maintained in the vivarium for a period of at least 5 days before beginning experimental procedures. During this time lighting was by overhead fluorescent lamps with lights on between 5:00 A.M. and 5:00 P.M. Ambient temperature was maintained at 23 ± 1 °C. Animals were given free access to food (Purina Rat Chow) and water.

**Drug Administration:** Sodium chloride (NaCl) and lithium chloride (LiCl) were administered by intraperitoneal (i.p.) injection. Sodium chloride was administered as a solution of either 0.9% (1.54 meq/ml) or 1.0 meq/ml. Lithium chloride was administered as a solution of 1.0 meq/ml.

**l-dopa:** One hour prior to testing animals were injected with \( l-3,4-\) dihydroxyphenylalanine (l-dopa) (Calbiochem). The doses were 100 mg/kg, i.p. in a suspension of 25 mg/ml.

**Pargyline:** The monoamine oxidase inhibitor pargyline HCl (Sigma) was injected at a dose of 75.0 mg/kg i.p. as a solution of 15.0 mg/ml of distilled water 5 hours before testing.
α-methyl-p-tyrosine methyl ester: On the day of testing animals were injected at 12 hours, 8 hours and 4 hours prior to testing with α-methyl-p-tyrosine methyl ester (Sigma) in dosages of 75.0 mg/kg at 12 hours and 50 mg/kg at 8 and 4 hours pre-test. All injections were given i.p. in a solution of 37.5 mg/ml distilled water.

p-chlorophenylalanine methyl ester: Thirty-three hours prior to testing animals were given a single injection of 316.0 mg/kg p-chlorophenylalanine methyl ester (Sigma) dissolved in distilled water to a concentration of 105.0 mg/ml.

d-amphetamine sulfate: Five minutes prior to testing in the acoustic startle response apparatus animals were injected with either 0.9% saline or 2.5 mg/kg d-amphetamine sulfate (Sigma). These injections were given i.p.

Testing of Locomotor Activity: Three to five days prior to initiating any drug or saline injection schedule animals were removed from the vivarium and kept in the same room in which they were to be tested. The lighting schedule and ambient temperature were identical to those in the vivarium. Food and water were provided on an ad lib basis. Following the initial 3 to 5 day adaptation period, injection schedules were begun as described below.

At the time of activity testing animals were removed from their home cages and placed in freshly cleaned cages identical to those used for individually housed animals as previously described. Food and the water bottle were removed from the cage top during 30 minute activity tests. These test cages were positioned on activity counting platforms which sense movements of a mass above the platform by the perturbations created
in the electromagnetic field produced by the activity. These perturbations are transduced to pulses transmitted to Quartec electro-mechanical counters which accumulate the pulses as activity counts. The sensitivity of the activity monitoring devices is adjusted by a control on the counter itself. Before testing the 3 activity monitors were calibrated by moving a metal shelf back and forth approximately 2 inches above all 3 platforms simultaneously for a period of one minute. Sensitivity was adjusted to provide 100 counts per minute when the shelf was moved at a frequency of 1 passage across the platforms per second.

In those cases where activity was monitored hourly for 24 hour periods the pulses from the platforms were accumulated on Model PC-Z double channel printing counters from Columbus Instruments. This allowed the collection of printed hourly activity scores without necessitating the presence of the experimenter to record the data. During these tests the animals were continued on an ad lib schedule for food and water.

**Acoustic Startle Response:** Animals were housed in groups of 3 per cage and treated for 6 days with either LiCl or NaCl (2.0 meq/kg/day, i.p.) as previously described. On the seventh day, 5 minutes before the start of the test session, each subject received an injection of either 2.5 mg/kg d-amphetamine sulfate or an equal volume of 0.9% NaCl.

Testing of the animals took place in a sound attenuated chamber. Animals were tested individually for a period of 50 minutes for the magnitude of their sensory motor response to a series of 50 presentations of a 105 db, 2500 hz tone given at 60 second intervals.

The test apparatus was a modification, described by Beattie (1977), of the stabilimeter of Hoffman and Fleshler (1964). Animals were placed
in an enclosure measuring 10 x 15 x 15 cm. The animal enclosure was sus­
pended from a sheet of 0.64 mm thick plexiglass attached to a rigid
aluminum frame. This suspension system allowed high frequency movement
of the animal (i.e. hindlimb flexion startle response) to be partially
absorbed by the movement of the animal enclosure itself. Attached to the
bottom of the enclosure was a metal rod extending downward to juxteposition
with a small audio speaker. Vertical movements of the cage were then
transduced by the magnetic speaker to voltages delivered to an AC pream­
plifier on a Grass model 7 polygraph. Pen deflections recorded the
occurrence of the acoustic stimulus on one channel and the magnitude of
the startle response on another.

Analysis of Lithium: Lithium concentrations in body tissues and
fluids were analyzed with the use of a Varian Techtron AA 6 atomic absorp­
tion spectrophotometer. The lithium lamp current was set at 4.0 mA and
total absorbance read at a setting of 670.1 nm. Slit width was set at
8.0 nm and the spectral band pass at 0.35nm. Samples were aspirated using
the flame mode with air, acetyling at a flow ratio of 3:3. The burner
height was adjusted to 14 mm.

Preparation of Samples:

Urine: Urine samples collected from animals maintained in metabolic
cages were diluted with double distilled water to a dilution of 0.2 ml
urine per 10.0 ml.

Blood: Immediately following activity testing animals were sacrificed
by decapitation and blood from the cervical wound was collected in 50 ml
beakers containing approximately 0.02 ml sodium heparin (Lipo-hepin, 1000
U.S.P. units per ml). Blood samples were then transfered to glass culture
tubes and centrifuged for 10 minutes at 3500 rpm in an International Equipment Company Model HN-S centrifuge. Following centrifugation, plasma was transferred to 12 x 75 mm polypropylene tubes with caps and stored at -10°C until time of assay. The remaining plasma and "buffy coat" were aspirated and discarded and the sedimented red blood cells stored in the original tubes at -10°C until assayed.

Plasma samples were prepared for lithium assay by diluting 0.5 ml of plasma with 4.5 ml double distilled water. Standards were prepared by adding known concentrations of lithium chloride to diluted plasma from NaCl treated animals.

The sedimented red blood cells were lysed and prepared for analysis by diluting the samples 1:10 (weight:volume) with double distilled water. The diluted samples were mixed thoroughly with a Vortex-Genie tube agitator and allowed to stand at room temperature for 12 to 24 hours. Immediately prior to assay, samples were centrifuged for 10 minutes at 3500 rpm to sediment particulate matter. The supernatant was assayed as described above.

Tissues: Following decapitation of the animals the brain was removed and placed in a petri dish on filter paper saturated with 0.9% NaCl. The dura mater and superficial blood vessels were removed with forceps. The brain was then dissected by the method of Glownski and Iversen (1966) with the following minor modifications. The hypothalamus was dissected with a circular cut just posterior to the optic chiasm. This resulted in somewhat smaller hypothalamic samples than reported in the original procedure. The "cortical sample" consisted of the cortical regions posterior to the vertical cut made near the optic chiasma and the anterior cortical sections remaining
after removal of the sub-cortical tissue. A schematic diagram of this dissection procedure as described by Glowinski and Iversen is shown in Figure 1 along with the average weights of the sections obtained. Each section was touched lightly to filter paper to remove excess moisture. Sample weights were obtained by directly weighing each section with a Roller-Smith balance (capacity: 500 mg) or by weighing pre-tared polypropylene tubes after the addition of the tissues to the tubes using a Mettler H•20•T analytical balance. Samples were stored in (12 x 75 mm) polypropylene tubes at -10°C until being prepared for assay.

Additional tissue samples were taken from the ventricles of the heart, the lower (posterior) tips of the lung, liver and kidney and perirenal fat for some animals. These samples were weighed and stored in the same manner as the brain tissues.

In preparing tissues for lithium analysis, 9 volumes of a 0.5% solution of Triton X-100 tissue solubilizer was added to the tissue tubes after allowing the tubes to reach room temperature. Smaller sections weighing less than approximately 70 mg were diluted with 19 volumes of solution. The tubes with solubilizer added were then vigorously mixed and transferred to acid washed, ground glass tissue homogenized (pyrex corning #7725, 13 x 100 mm). Brain sections were homogenized with 20-30 passes of the homogenizer. Other tissues, e.g. liver, lung and heart, required 40-60 passes to eliminate visible particles from the homogenate. Following homogenization, the homogenate was transferred back to polypropylene tubes and allowed to stand at room temperature for 24-48 hours. Tubes were then centrifuged for 10 minutes at 3500 rpm to reduce the viscosity of the supernatant which was then assayed for lithium as described above.
Figure 1. Diagrammatic representation of the dissection procedure used. Dotted lines indicate positions of initial sections. (from Glowinski and Iversen, 1966). Actual dissection was modified as described in the text.
Standards were prepared by adding known concentrations of lithium chloride to tissue homogenates from NaCl treated animals. Data generated from standard curves were entered in the linear regression program (ST1-08) available for the Texas Instrument SR-52 programmable calculator. Tissue and blood lithium levels were then calculated by entering the absorbance in the program and using calculated lithium concentrations.
RESULTS

Acute Lithium Toxicity: Animals injected with a single dose of LiCl (10.0 meq/kg, i.p.) were closely observed during the following 24 hours. Observations were recorded at 10 minute intervals for the first 2 hours and at 30 minute intervals during the following 4 hours. Abnormal physiological and behavioral effects were noted. All four animals exhibited abnormal head movements within the first two hours. These consisted of a side to side movement of the head and a chewing motion of the jaws not unlike those seen in the advanced stages of amphetamine stereotypy. Other features of the toxic reaction included ataxia and a peculiar pushing motion of the forepaws against the bottom of the cage. By four hours following the injection there was a watery discharge from around the eyes of all four animals. By the end of the 24 hour observation period, all four subjects appeared relatively unimpaired with the exception of a slightly decreased level of activity and reactivity to mild stimuli such as touching the animals back. All four animals also had a high water content in their feces. In the experiments which followed these initial observations, attention was given to the appearance of any of the noted effects as indices of possible toxicity.

Effects of Lithium on Locomotor Activity: Initial observations of the effects of lithium on locomotor activity were conducted by monitoring total activity per day prior to and during lithium administration. Animals were housed individually in cages positioned on top of the activity counters. As shown in Figure 2, daily lithium chloride injections
Figure 2. 24 hour activity (represented as thousands of counts per day) for each of 3 different animals. Days 1 through 4 represent baseline activity prior to any injections. At the end of the 4th day each animal was given the 1st of 6 daily injections of either 0.9% NaCl or 4.0 meq/kg LiCl as indicated.
Figure 2
(4 meq/kg, i.p.) had effects which were not easily interpretable due to the wide variation in the amount of activity from one day to the next. Using only three animals it appeared that the activity of both the lithium treated subjects and that of the animal receiving saline injections was reduced by initiating the injection procedures. However, the trend for the control animal and the two lithium treated animals was inconsistent, fluctuating greatly. Such a measurement would be likely to obscure any lithium effects within the large variation in activity from day to day both within and between individual subjects.

In order to establish the optimal time for testing activity, three animals were placed on the activity monitoring platforms and counts were accumulated and printed out hourly for each subject. After a three day period of adaption, the three animals were monitored for hourly activity for an additional three day test period. The activity scores for each animal for each hour were expressed as a percentage of the total activity during that twenty-four hour period. The hourly percentage for each animal were then averaged over the three day test period. These mean hourly activity scores for twenty-four hours are plotted for each of the three animals in Figure 3. These results indicate that approximately 20% of the animals' total daily activity occurred between the hours of 8:00 and 10:00 P.M. Therefore all subsequent testing was done during this time frame in order to maximize the baseline activity from which any inhibitory effects of lithium could more easily be observed.

Effects of Lithium Treatment on Activity of Animals Placed in a Novel Environment: Rats housed in groups of 4 per cage for a minimum of 2 weeks following arrival at the vivarium were then given a series
Figure 3. Diurnal variations in activity of 3 untreated animals represented as the percent of total daily activity occurring in each hour. Each point represents the mean for 3 consecutive days for an individual animal.
of 6 daily injections of either 2.0 or 4.0 meq/kg LiCl or an equal volume of 0.9% NaCl. Twenty-four hours after the last injection (between 8:00 and 10:00 P.M.) animals were removed from their home cages and placed in the smaller activity testing cages. Activity counts, recorded at 5 minutes intervals for a total of 30 minutes, are shown in Figure .

Both levels of lithium treatment produced a significant reduction in activity during the thirty minute test period. While the rats receiving the lower dose of lithium (2 meq/kg) appeared in all other ways to be "normal", several of the animals receiving the higher dosage of 4 meq/kg showed some signs of toxicity including water feces and, in one instance, the appearance of a watery discharge around the eyes.

Comparison of Individually Housed and Group Housed Animals: Immediately upon arrival at the vivarium all animals were placed in their respective pre-test housing, either in groups of 3 per cage or individually. Animals were maintained under these conditions for a period of at least one week for adaption to the housing conditions prior to the initiation of the injection schedule.

The subjects were injected daily for six days prior to testing with either NaCl (2 meq/kg) or LiCl (2.0 or 4.0 meq/kg). Injections were given i.p. between the hours of 7:00 and 9:00 P.M. with the last NaCl or LiCl injection given 24 ± 1 hours prior to testing.

On the seventh day following the initiation of the treatment schedule and twenty-four hours following the last injection animals were removed from their home cages and placed in activity monitoring cages which were identical to those of the individually housed animals. The cumulative activity counts were recorded at five minute intervals for a period of
Figure 4. Cumulative locomotor activity counts for rats treated with 0.9% NaCl (●●●●●) N:8, 2 meq/kg LiCl (●—●) N:8 or 4 meq/kg LiCl (●—●—●) N:6 for 6 days prior to testing. Each point represents the mean (+ std. error of the mean for 30 minute activity). Groups differed significantly from one another (p<.01).
Cumulative Activity Counts

Figure 4
thirty minutes.

As shown in Figure 5, NaCl treated animals which were housed in groups of 3 per cage prior to testing were considerably more active during the 30 minute test session than were individually housed NaCl treated animals. Administration of lithium for 6 days considerably reduced the activity of group housed animals without having an equivalent effect on individually housed animals when compared to their NaCl treated controls. The activity of these four groups is summarized below in Table 3.

Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-test Housing</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Individually</td>
<td>Grouped</td>
<td></td>
</tr>
<tr>
<td>LiCl (2 meq/kg/day, 6 days)</td>
<td>1441.4 ± 246.1 (6)</td>
<td>1856.8 ± 156.8 (11)</td>
<td></td>
</tr>
<tr>
<td>NaCl (2 meq/kg/day, 6 days)</td>
<td>1657.7 ± 195.3 (8)</td>
<td>2743.8 ± 187.8 (9)</td>
<td></td>
</tr>
</tbody>
</table>

These results were analyzed by 2 factorial analysis of variance designed to show any statistical significance of the effects of pre-test housing, lithium vs. NaCl treatment and the interactions between these two variables. Table 4 is the summary of that analysis of variance.
Figure 5. Cumulative locomotor activity counts for animals housed either individually or in groups of 3 per cage treated with 2.0 meq/kg LiCl or NaCl for 6 days prior to testing. Group housed NaCl treated (■—■), group housed LiCl treated (□—□), individually housed NaCl treated (■—■■■), individually housed LiCl treated (□—□□□).
Table 4

SUMMARY OF ANOVA

Summary of Analysis of Variance for Activity of Animals
Housed Individually or In Groups of 3
and treated with LiCl or NaCl for 6 days

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Housing</td>
<td>1</td>
<td>4,987,860.8</td>
<td>15.726</td>
<td>p = .0004</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>2,699,894.9</td>
<td>8.490</td>
<td>p = .0065</td>
</tr>
<tr>
<td>Interaction</td>
<td>31</td>
<td>995,103.9</td>
<td>3.137</td>
<td>p = .0861</td>
</tr>
<tr>
<td>Within Cell</td>
<td>32</td>
<td>317,171.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As shown in this summary, both pre-test housing conditions and lithium treatment have significant effects on the animals' activity. The interaction effect is also apparent though the F value fails to achieve significance at p.<.05. Further analysis of the simple effects of lithium treatment compared to NaCl shows that lithium treatment has no significant effect on the activity of individually housed animals, (F (1.32) = 0.653). However, as one would expect from the graphed data in Figure 5, lithium has a significant suppressive effect (p<.005) on the activity of animals housed in groups of 3 prior to testing.

Effects of Duration of Lithium Treatment on Locomotor Activity of Group Housed Animals: Following the previous observation of the suppressive effects of 6 days of lithium treatment in group housed (GH) animals, the time course of the onset of this effect was studied. The animals used in these groups were identical to those previously described. Two additional groups were studied. One group of animals (designated GHLixl) received 5 days of injections with NaCl (2.0 meq/kg) followed by a single injection of LiCl (2.0 meq/kg) on the 6th day 24 hours prior to testing. The
second group (GH Li x 21) were given daily injections of LiCl (2.0 meq/kg) for 21 days prior to testing on day 22. Both groups were housed in groups of 3 animals per cage during treatment. Procedures for testing were as previously described. The results of these treatments are shown below in Table 5 expressed as the mean number of activity counts ± the standard error during the 30 minute test period.

Table 5

Locomotor Activity for Animals Treated for 1 or 21 days with 2.0 meq/kg LiCl

<table>
<thead>
<tr>
<th>GROUP</th>
<th>N</th>
<th>ACTIVITY (MEAN ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH Li x 1</td>
<td>6</td>
<td>2740.8 ± 209.35</td>
</tr>
<tr>
<td>GH Li x 21</td>
<td>9</td>
<td>2390.3 ± 145.01</td>
</tr>
</tbody>
</table>

Figure 6 shows the 30 minute activity scores as a function of the duration of lithium treatment for animals receiving 0 (GH Na x 6), 1 (GH Li x 1), 6 (GH Li x 6) or 21 (GH Li x 21) daily injections of lithium. The apparent suppressive effects of lithium treatment for 6 days but not for 1 or 21 days was analyzed by one-way analysis of variance a summary of which is shown below in Table 6.

Table 6

Summary of Analysis of Variance for Activity of Animals Treated with LiCl (2.0 meq/kg) for 0, 1, 6 or 21 Days

<table>
<thead>
<tr>
<th>SOURCE OF VARIATION</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment duration</td>
<td>3</td>
<td>1660888.05</td>
<td>6.379</td>
<td>.0018</td>
</tr>
<tr>
<td>Within Cell (error)</td>
<td>31</td>
<td>260412.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 6. Locomotor activity scores for 30 minute test session for animals given LiCl (2 meq/kg/day) for 0 (N=9), 1 (N=6), 6(N=11) or 21 (N=9) days. Each point represents the mean ± standard error of the mean for that particular group.
Figure 6

Locomotor Activity

Days of Lithium Treatment
The finding of a significant difference among groups band on duration of lithium treatment allows further comparisons of individual groups with one another. For this purpose, a Scheffe test (Winer, 1962) was used to arrive at a critical value of $F$ appropriate for all individual comparisons within a series. This test has the advantage of being extremely conservative and assuring that the level of significance of individual comparisons will never be less than $a$, thus reducing the possibility of falsely rejecting the null hypothesis of no difference between individual groups. The summary of the individual comparisons is shown in Table 7.

Summary of Results - Effects of Lithium on Locomotor Activity:
These results indicate that treatment with doses of lithium producing no apparent signs of toxicity (2.0 meq/kg/day) suppresses locomotor activity in rats. The suppressive effects are shown to be dependent on at least two factors: the duration of treatment and the nature of the testing environment. Lithium treatment for 6 days has significant suppressive effects on the locomotor activity of animals exposed to a novel environment but no significant effect on the activity of animals tested in a more familiar environment. In this case the change in environment consisted of two components: a) separation of the group housed animals from their cage mates during the period of testing; and b) a change in the dimensions of the housing environment. The larger cages used for pre-test housing of the group housed animals have floor dimensions of 25 cm x 54 cm providing a total floor surface area of 1350 cm$^2$, or 450 cm$^2$ per animal. The smaller cages used for pre-test housing of the individually housed animals and for testing of all animals have floor dimensions of 21 cm x 43 cm,
### TABLE 7

EFFECTS OF DURATION OF LITHIUM TREATMENT ON LOCOMOTOR ACTIVITY

**INDIVIDUAL COMPARISONS**

<table>
<thead>
<tr>
<th></th>
<th>GH Li × 1</th>
<th>GH Li × 6</th>
<th>GH Li × 21</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GH Na × 6</strong></td>
<td>F = .0001</td>
<td>F = 13.367</td>
<td>F = 2.253</td>
</tr>
<tr>
<td></td>
<td>p. N.S.</td>
<td>p. .05</td>
<td>p. N.S.</td>
</tr>
<tr>
<td>df1 = 1; df2 = 13</td>
<td></td>
<td>df1 = 1; df2 = 18</td>
<td>df1 = 1; df2 = 16</td>
</tr>
<tr>
<td><strong>GH Li × 1</strong></td>
<td>F = 11.320</td>
<td></td>
<td>F = 2.093</td>
</tr>
<tr>
<td></td>
<td>p. .05</td>
<td></td>
<td>p. N.S.</td>
</tr>
<tr>
<td>df1 = 1; df2 = 15</td>
<td></td>
<td></td>
<td>df1 = 1; df2 = 13</td>
</tr>
<tr>
<td><strong>GH Li × 6</strong></td>
<td>--</td>
<td>--</td>
<td>F = 6.012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p. N.S.</td>
</tr>
<tr>
<td>df1 = 1; df2 = 18</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scheffe Critical Value of $F_{.95} = 7.23$; $F_{.99} = 13.47$
or a total floor surface area of 903 cm². The relative contribution of these two components of novelty to the change in activity cannot be assessed within the design of this experiment.

The duration of lithium treatment on the animals' response to the novel environment was assessed. It was found that a single injection of lithium 24 hours prior to testing had no effect on the activity response to novelty. The fact that this suppressive response was apparent following 6 days of lithium treatment suggested that some critical level of lithium within particular body compartments must be achieved to demonstrate the suppressive action. However, animals treated with lithium for 21 days did not show further suppression of activity, but actually displayed recovery from the suppressive effects seen at 6 days of treatment. These 21 day lithium treated animals were significantly more active than the 6 day treatment group and did not differ significantly from the NaCl treated control group.
Supplemental Neuropharmacological Manipulation: In order to demonstrate the extent to which particular neurotransmitter systems may contribute to the suppression of activity in animals following 6 days of lithium treatment and the change in these systems associated with recovery from the suppressive effects of lithium following 21 days of treatment, animals were maintained on the 6 or 21 day lithium schedule as previously described. These animals were then compared to 6 day NaCl treated controls in the locomotor activity test following the additional treatment with neuropharmacological agents known to alter with some selectivity particular neurotransmitter systems. The agents used were: 1-dopa (100 mg/kg, given 1 hour prior to testing); pargyline (75 mg/kg, given 5 hours prior to testing); α-methyl-p-tyrosine methyl ester (α-mpt) (75 mg/kg, 50 mg/kg and 50 mg/kg given successively at 12, 8 and 4 hours respectively prior to testing) or p-chlorophenylalanine methyl ester (PCPA), (316 mg/kg, given 33 hours prior to testing). The results of these various manipulations are summarized in Table 8 seen below. The interaction of lithium treatment with these other drug effects may be seen in Figure 7 which shows total activity during the 30 minute test period. For each of the additional drug treatments (e.g. pargyline or α-mpt) the resulting change in locomotor activity at the various durations of lithium treatment was analyzed by multi-factorial analyses of variance. The summaries of these analyses are shown in Tables 9 through 12.

The results of these analyses show that 1-dopa, which was given to animals treated with either NaCl or LiCl for 6 days, resulted in a significant reduction of the activity of both groups. The interaction effect showed an F value of 3.288 which failed to reach significance at
Figure 7. Effects of l-dopa (—□), pargyline (○), α-methyl-p-tyrosine (●) and p-chlorophenylalanine (■) on locomotor activity following various durations of treatment with LiCl. Activity of animals treated only with LiCl are shown for comparison (■). Each point represents the mean activity counts for a specific group as described in the text. The standard error (shown in Table 8) have been omitted for the sake of clarity.
### Table 8
Locomotor Activity (mean $\pm$ S.E.M.)

<table>
<thead>
<tr>
<th>Additional Drug Treatment</th>
<th>Days of Lithium Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (N)</td>
</tr>
<tr>
<td>None</td>
<td>$2743.8 + 187.8$ (9)</td>
</tr>
<tr>
<td>1-dopa</td>
<td>$1494.2 + 167.9$ (6)</td>
</tr>
<tr>
<td>Pargyline</td>
<td>$774.9 + 154.8$ (9)</td>
</tr>
<tr>
<td>$\alpha$-mpt</td>
<td>$1919.7 + 176.7$ (6)</td>
</tr>
<tr>
<td>PCPA</td>
<td>$2110.7 + 374.0$ (6)</td>
</tr>
</tbody>
</table>
Table 9

Summary of Analysis of Variance for Animals Treated for 6 Days with 2 meg/kg of NaCl or LiCl followed by L-Dopa

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-dopa treatment</td>
<td>1</td>
<td>5,876,087</td>
<td>19.644</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>NaCl or LiCl treatment</td>
<td>1</td>
<td>2,052,807</td>
<td>6.862</td>
<td>p &lt; .05</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>983,548</td>
<td>3.288</td>
<td></td>
</tr>
<tr>
<td>Within Cell</td>
<td>28</td>
<td>299,135</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 10

Summary of Analysis of Variance for Animals treated for 6 days with NaCl (= 0 days LiCl), 6 or 21 days LiCl followed by Pargyline

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pargyline treatment</td>
<td>1</td>
<td>15,880,265</td>
<td>58.56</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Days of LiCl treatment</td>
<td>2</td>
<td>1,010,460</td>
<td>3.726</td>
<td>N.S.*</td>
</tr>
<tr>
<td>Interaction</td>
<td>2</td>
<td>2,358,175</td>
<td>8.696</td>
<td>p &lt; .005</td>
</tr>
<tr>
<td>Within Cell</td>
<td>47</td>
<td>271,165</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The apparent significant differences due to the duration of LiCl treatment is obscured as a result of the high level of the interaction effect.
Table 11

Summary of analysis of variance for animals treated for 6 days with NaCl (=0 days LiCl), 6 or 21 days LiCl followed by α-methyl-p-tyrosine (α-mpt)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Mean Squares</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-mpt Treatment</td>
<td>1</td>
<td>4,475,226</td>
<td>14.848</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Days of LiCl Treatment</td>
<td>2</td>
<td>1,999,343</td>
<td>6.633</td>
<td>p &lt; .005</td>
</tr>
<tr>
<td>Interaction</td>
<td>2</td>
<td>106,467</td>
<td>0.353</td>
<td>N.S.</td>
</tr>
<tr>
<td>Within Cell</td>
<td>41</td>
<td>301,409</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 12

Summary of analysis of variance for animals treated for 6 days with NaCl (=0 days LiCl), 6 or 21 days LiCl followed by p-chlorophenylalanine (PCPA)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Mean Squares</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCPA Treatment</td>
<td>1</td>
<td>7,575,950</td>
<td>16.335</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Days of LiCl Treatment</td>
<td>2</td>
<td>3,893,828</td>
<td>8.396</td>
<td>p &lt; .005</td>
</tr>
<tr>
<td>Interaction</td>
<td>2</td>
<td>118,483</td>
<td>0.255</td>
<td>N.S.</td>
</tr>
<tr>
<td>Within Cell</td>
<td>40</td>
<td>463,780</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the .05 level. All other drugs were tested in animals receiving lithium for 6 and 21 days in addition to the NaCl treated controls. Pargyline also significantly reduced the activity of all groups compared to their non-pargyline controls. However, the interaction effect (significant at p<.005) demonstrates a differential effect of pargyline in lithium treated subjects compared to NaCl treated controls. As shown in Figure 7, the degree of pargyline-induced suppression is reduced in direct proportion to the duration of lithium treatment. The tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine methyl ester, reduces the activity of all groups to an equivalent extent regardless of the duration of lithium treatment. Inhibition of tryptophan hydroxylase by p-chlorophenylalanine methyl ester also has effects which appear to be independent of the duration of lithium treatment. However, observation of individual subjects within the PCPA treated group pretreated with lithium for 6 days indicates a large degree of variability in the locomotor response produced by this combination of drugs. Of the 6 animals treated in this manner, one died between the time of PCPA injection and the time of testing. Two others appeared extremely lethargic and had activity scores reflecting this while another two subjects were extremely active in the locomotor activity test. These variations in response to this particular treatment combination may be seen reflected in the high standard error of the mean for the group.
Acoustic Startle Response: Rats treated with LiCl or NaCl for 6 days were then given an injection of either d-amphetamine sulfate or an equal volume of 0.9% saline 5 minutes before being tested in the acoustic startle response (ASR) apparatus. Figure shows the effects of these treatments on the magnitude of the motor component of the ASR. Lithium treatment alone failed to alter significantly either the absolute magnitude of the response or the amount of habituation to the stimulus when compared to control animals given only NaCl. It may be noted that although lithium did not significantly alter response magnitude there was a tendency for this parameter to be somewhat greater in lithium treated animals than in controls.

When the NaCl pre-treated rats were injected with d-amphetamine before testing there was a significant increase in the response magnitude over the duration of the test period. Pre-treatment with LiCl for 6 days significantly attenuated this amphetamine induced potentiation.

Statistical Analysis was by Kruskal-Wallis H test followed by individual comparisons using Mann-Whitney U tests. Data was analyzed by averaging the response magnitude for each subject for each 5 blocks of 10 successive trials per block. The data in Figure 8 represents response magnitude for each of the 5 10-trial blocks as a percent of response magnitude for block 1.
Figure 8. Effects of lithium administration (2.0 meq/kg per day for 6 days) on the magnitude of the acoustic startle response and potentiation of startle response by 2.5 mg/kg d-amphetamine sulfate. LiCl treatment alone (O--O)N=6, NaCl treatment alone (□--□), LiCl + amphetamine (■—■)N=7, NaCl + amphetamine (●—●). Response magnitude for each subject was averaged for each 10 successive trials constituting a total of 5 10-trial blocks. The magnitude of the response is expressed as the percent of mean response for block 1 for each group.
Figure 8
Urinary Excretion of a Single Dose of Lithium: Male Long-Evans hooded rats identical to those used in the behavioral experiments were placed individually in glass metabolic housings designed to allow the animal to have free access to food and water and to allow collection of urine and feces. Following a period for adaption to the housing, each subject was injected with a single dose of LiCl (2.0 or 4.0 meq/kg, i.p.). Urine was collected every 8 hours up to 96 hours following the injection.

Figure 9 shows the percentage of the original dose remaining in the body at 8 hour intervals assuming all lithium was excreted in the urine. As this graph shows, there is an apparent 2 component rate of excretion with the fast elimination phase occurring between 0 and 16 hours and the slow phase apparent after 16 hours.

The urinary elimination of lithium for the dose used in the behavioral experiments (2.0 meq/kg) was analyzed to provide an estimate of the kinetics. The inflection point apparent at 16 hours was taken as the time at which elimination became dependent upon redistribution of lithium from tissues to the plasma for renal excretion. Linear regression analysis of the percent of drug remaining at each 8 hour interval from 0 to 16 hours showed a correlation coefficient of .980, a slope of -.294 and an intercept at 28.89 hours for total elimination. The elimination t1/2 for the fast phase was 14.20 hours. Similar analysis of the slower phase from 16 to 40 hours showed a correlation coefficient of .929, a slope of -2.606 and an intercept at 139.83 hours for total elimination.
Figure 9. Urinary elimination of lithium following a single injection of 2.0 meq/kg LiCl (●-----●) or 4.0 meq/kg LiCl (□-----□) N=4. Data is expressed as percentage of dose remaining in the body.
Figure 9
Distribution of Lithium: Tissues and blood samples as described in the "Methods" section were analyzed for lithium concentration following various durations of treatment. Figure 10 shows the standard curves used for calculation of lithium concentrations in those various samples. For all standards and unknowns, duplicate readings were made with the exception of hypothalamic tissue sections which were too small to allow duplicate determinations under the conditions of the assay used.

Plasma lithium levels were determined for a number of treatment groups as shown in Table 13. The results of the plasma lithium assays were statistically analyzed for possible differences due to duration of treatment, supplemental drug treatment and correlations with activity scores. One way analysis of variance due to duration of treatment showed no statistically significant increase in mean plasma levels of lithium as a function of duration of treatment. Neither was there any significant correlation between plasma lithium levels and locomotor activity within any of the groups receiving lithium alone. Analysis of the groups receiving lithium for 6 days followed by either α-methyl-p-tyrosine (α-mpt), p-chlorophenylalanine (PCPA) or no additional drug showed there was a significant difference (p<.001) in plasma lithium as a function of additional drug treatment. Comparisons of differences in the means between any two groups showed the animals receiving PCPA had significantly higher lithium levels (p<.001) than either animals receiving only lithium or those given α-mpt plus lithium. Similar results are seen with animals treated for 21 days with lithium. The group given PCPA had significantly higher plasma lithium levels than either of the other two groups (p<.001).
Figure 10: Standard Curves for lithium assays. Plasma standards (★— ★) showed a linear regression correlation coefficient of .9999 with intercept at -.00027. Tissue standards (C—●— ●) displayed a correlation coefficient of .99962 and an intercept at -.0004. Each point represents 2 determinations of the sample at that concentration. In no case did absorbance values vary by more than .001 units between the two determinations.
TABLE 13

PLASMA LITHIUM CONCENTRATIONS

(in ueq / ml)

( mean ± S.E.M. )

<table>
<thead>
<tr>
<th>Additional Drug Treatment</th>
<th>1 day</th>
<th>6 days</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>0.049 ± .008</td>
<td>0.054 ± .007</td>
<td>0.065 ± .006</td>
</tr>
<tr>
<td>pargyline</td>
<td></td>
<td>* 0.086 ± .022</td>
<td></td>
</tr>
<tr>
<td>α-mpt</td>
<td></td>
<td>0.048 ± .008</td>
<td>0.073 ± .007</td>
</tr>
<tr>
<td>PCPA</td>
<td></td>
<td>0.701 ± .273</td>
<td>0.169 ± .038</td>
</tr>
</tbody>
</table>

* Plasma from pargyline treated subjects were taken from animals housed individually prior to testing. All other subjects in this group were group housed animals previously described.
The only significant correlations of plasma lithium concentration with locomotor activity was seen in the animals treated with PCPA following 6 days of lithium treatment. The high degree of variance in both activity and plasma lithium in this group precluded the use of parametric statistics. Using a Spearman rank correlation test showed a negative correlation significant at p<.01.

Analyses of tissue concentrations of lithium for the various brain regions studied reflect a large variation in the brain concentrations within any given treatment group. These results are shown in Table 14. This large variation between subjects within a treatment group precluded the use of parametric statistics in analyzing differences within specific brain regions. For this reason the relative concentrations of lithium in the various regions were analyzed by non-parametric methods. The apparent high proportion of lithium localized in the hypothalamus and cortex following 6 days of treatment were confirmed by these analyses. Using both a Kendall's coefficient of correlation and a Friedman two-way analysis of variance, 6 day treated animals were shown to have significant differences (p<.01) in the relative concentrations of lithium in brain regions. At this time, the hypothalamus contains the highest lithium concentration, followed closely by the cortex. After 21 days of lithium treatment, there was no longer any significant difference in the various brain regions of the samples assayed. However, cortex still appeared to be highest with approximately equal distribution among the other regions.
<table>
<thead>
<tr>
<th>TISSUE</th>
<th>1 (n)</th>
<th>6 (n)</th>
<th>21 (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Brain</td>
<td>0.0730 (2)</td>
<td>0.1448 ± .0373 (5)</td>
<td>0.1393 (1)</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>---</td>
<td>0.0745 ± .0204 (4)</td>
<td>0.1024 ± .0286 (5)</td>
</tr>
<tr>
<td>Medulla Oblongata</td>
<td>---</td>
<td>0.0676 ± .0233 (4)</td>
<td>0.1093 ± .0399 (5)</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>---</td>
<td>0.1079 ± .0234 (4)</td>
<td>0.0902 ± .0213 (5)</td>
</tr>
<tr>
<td>Striatum</td>
<td>---</td>
<td>0.0812 ± .0281 (4)</td>
<td>0.0942 ± .0164 (5)</td>
</tr>
<tr>
<td>Midbrain</td>
<td>---</td>
<td>0.0693 ± .0182 (4)</td>
<td>0.0915 ± .0143 (5)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>---</td>
<td>0.0881 ± .0226 (4)</td>
<td>0.0820 ± .0171 (5)</td>
</tr>
<tr>
<td>Cortex</td>
<td>---</td>
<td>0.1188 ± .0372 (4)</td>
<td>0.1202 ± .0181 (5)</td>
</tr>
<tr>
<td>Ventricle of Heart</td>
<td>---</td>
<td>0.0333 (2)</td>
<td>0.0437 (1)</td>
</tr>
<tr>
<td>Liver</td>
<td>---</td>
<td>0.265 (2)</td>
<td>0.0300 (1)</td>
</tr>
<tr>
<td>Lung</td>
<td>---</td>
<td>0.0505 (2)</td>
<td>0.0300 (1)</td>
</tr>
<tr>
<td>Kidney</td>
<td>---</td>
<td>0.0607 (2)</td>
<td>0.0710 (1)</td>
</tr>
</tbody>
</table>
Summary of Results - Lithium Interactions with Neurotransmitter Systems: Interactions of lithium treatment with other neuropharmacological agents suggest some possibilities for the neurochemical effects of lithium which may be associated with locomotor suppression. Increasing plasma levels of L-dopa, the substrate for both dopamine and norepinephrine results in a reduction of locomotor activity in animals treated with NaCl. Pretreatment with LiCl for 6 days produces a degree (albeit not statistically significant) of protection against the L-dopa induced suppression.

Similar results occur to a greater extent in animals treated with pargyline. In the case of pargyline-induced suppression of locomotor activity, the ability of lithium to reverse that effect is increased with the duration of lithium treatment. This effect of lithium is significant after 6 days of lithium treatment and even more pronounced following 21 days of treatment. Pargyline inhibits both types A and B monoamine oxidase, increasing brain catecholamines and serotonin and a decreasing hepatic deamination of benzydamines. The fact that this lithium-pargyline interaction increases with the duration of lithium treatment is temporally compatible with an action of lithium which persists beyond the locomotor suppressive effects occuring at 6 days. This interaction may be related to the mechanism of the therapeutic action of lithium.

Inhibition of the synthesis of catecholamines with α-methyl-p-tyrosine resulted in a decrease in locomotor activity. There was no significant interaction between α-mpt and lithium treatment in this suppression. At both 6 and 21 days of lithium administration the effects of α-mpt were additive with the effects of lithium.
Inhibition of tryptophane hydroxylase with p-chlorophenylalanine did not result in significant interactive effects in lithium treated animals as seen in the mean locomotor activity scores. However, following 6 days of lithium administration the PCPA treatment resulted in large variations in both locomotor activity and plasma lithium levels. There was also a significant inverse correlation between plasma lithium levels and locomotor activity in this group. These animals with high plasma lithium concentrations showed a marked reduction in locomotion and appeared otherwise to be toxic. Animals with plasma lithium levels approximating those of subjects given lithium alone, however, had activity scores above the mean for the lithium treated group. When rats were pretreated for 21 days with lithium prior to PCPA administration the effects were less marked. However, there was still a wide variation in activity in this group with scores ranging from about 300 to over 3,000 for the 30 minute test. Plasma lithium levels for this group were somewhat higher than for the group given lithium alone but were not significantly correlated with locomotor activity. Some of these animals which had low activity scores appeared nonetheless very reactive to handling.

Locomotor activity is a frequently used behavioral measure in assessing drug responses. The amount of activity observed in response to a stimulus does not follow a linear relationship. Environmental stimuli as well as CNS stimulant drugs (e.g. amphetamine) may increase locomotor activity when the intensity of the stimulus is low only to result in decreased locomotion (e.g. high intensity amphetamine stereotypy or the "freezing response" to high intensity environmental stimuli) as the magnitude of the stimulus is increased. The inability of α-mpt to alter the animals
locomotor response to lithium treatment suggests that brain catecholamines may not play a significant role in lithium's action in these tests. The fact that both pargyline and PCPA resulted in alterations in the animals responsiveness to lithium pretreatment suggests that serotonergic mechanisms may play a more important role than catecholamines in the test. The response of the animals to multiple drug treatments is interposed on normal variations in the response of a group of animals to environmental novelty and may be interacting in such a way as to increase the response to novelty past the point at which that response is manifest as increased locomotion. Such an interpretation would be compatible with the large variations seen in animals given lithium plus PCPA.
DISCUSSION

As is the case with many, if not most drugs used in the treatment of major psychiatric disorders, lithium requires a latency period between the initiation of treatment and the onset of the therapeutic response. For this reason, Hemmelhock (1976) is one of many who suggest that treatment of highly active manic patients should employ both lithium and haloperidol in the initial phase of therapy. The antipsychotic dopamine receptor blocker haloperidol is used to effect a more immediate response on the activity of the patient. As Schou (1973) describes the situation, "the neuroleptic is given initially for the sake of the other patients and the nurses; lithium is then permitted to take over for the sake of the patient himself". This being the case, it seems unlikely that the mechanism by which lithium suppresses locomotor activity in acute animal studies is identical to the mechanism by which lithium reduces the activity of manic patients. The method described in this report seems particularly sensitive as a measure of the suppressive effects of lithium as it alters environmentally induced hyperactivity without significantly affecting the activity of animals tested in a more familiar environment. Since the animals were tested 24 hours following the last dose of lithium, it is reasonable to conclude that changes in plasma or extracellular fluid concentrations of lithium reflect the redistribution of lithium from cellular sites back into the circulation. This conclusion is supported by the data presented here on urinary excretion of a single dose of lithium and by previous studies.
of the pharmacokinetics of the ion (Mukherjee et al., 1976; Ebadi et al., 1974). Using the methods described here, the time course of the onset of action of lithium's effects is within the general range of the time course of lithium's therapeutic effects. As shown in Figure 11, the behavioral suppression seen with lithium has little relationship to plasma lithium levels. Furthermore, the recovery of lithium treated animals from such effects seen following 21 days of treatment is associated with slightly higher plasma lithium levels than exist at 6 days. The regional brain distribution of lithium at the two treatment durations differs in a manner which is significant even with the relatively small number of animals used for that portion of the study. At both durations of lithium treatment, there is significant variation among animals in the same group in the amount of lithium in all tissues studied and in plasma. However, if the lithium concentration for each brain region are ranked in order of the amount of lithium present per gram of tissue, fairly consistent patterns of distribution may be observed. Following 6 days of lithium treatment, the hypothalamus is likely to have the highest concentration followed closely by cortical regions. After 21 days of treatment, although there are no significant differences in this measure, cortical tissue is likely to have the highest concentration with the hypothalamus being approximately equal to the other regions. These results are in general agreement in that sense with those of Mukherjee et al., (1976; 1977) using a much shorter time course they reported highest brain concentrations of lithium at 8 hours following a single injection. At that time, the hypothalamus had the highest lithium concentration of the brain regions studied and the animals showed the greatest degree of locomotor suppression.
The results of Bond et al. (1975) show that following 14 days of maintaining animals on a lithium diet the hypothalamus, the cerebellum and the pons-medulla region had the lowest lithium levels of any areas studied with cortex, corpus striatum and hippocampus having relatively high values. These findings are compatible with the conclusion that there is a relationship between regional brain distribution of lithium and its ability to suppress locomotor activity. This relationship could obviously be a coincidental and spurious one however the possibility of a casual relationship remains interesting. Numerous studies have shown that the activity of an animal can be dramatically altered through intervention in hypothalamic functioning (Reviewed by Myers, 1975).

The finding that lithium attenuates the amphetamine potentiation of the acoustic startle response is not incompatible with the previously mentioned study by Heninger (1976) which showed an increased magnitude in both the acoustic startle response and in sensory evoked potentials in the rhesus monkey. The slight increase in absolute magnitude of the response reported here did not, however, reach levels of significance. Given that the observed increase in sensory evoked potentials reported by Heninger was previously reported to occur in manic depressive patients receiving lithium, this would be an interesting phenomenon to pursue in animals receiving lithium for 21 days.

The effects of the other neuropharmacological agents on saline and lithium treated animals suggests the likelihood that neither catecholaminergic nor serotonergic systems alone are responsible for the behavioral effects. The dose of α-methyl-p-tyrosine used has been shown to significantly decrease levels of brain dopamine by approximately 80% at times corresponding
to those used in these studies. Brain levels of norepinephrine were also reduced though not to the extent of the dopamine depletion (Moore and Dominic, 1971). At the time of this depletion, there was also observed a decrease in locomotor activity consistent with the results of this study. It is interesting that chronic administration of α-methyl-p-tyrosine in the diet resulted in a long lasting depletion of both norepinephrine and dopamine in whole brain. The suppression of locomotor activity resulting from such treatment was significant after the 1st day but showed recovery towards control levels, the difference becoming insignificant by 10 days of treatment and approximately control activity by 14 days.

The dose of p-chlorophenylalanine (PCPA) used has been shown to reduce whole brain tryptophan hydroxylase activity by approximately 90% at a time consistent with the time of testing of locomotor activity. The work of Knapp and Mandell (1973) showed that lithium treatment alone results in a significant decrease in midbrain tryptophan hydroxylase activity and an increased conversion of striatal synaptosomal conversion of tryptophan to serotonin following 5 days of lithium treatment. After 21 days of lithium administration the midbrain tryptophan hydroxylase activity remains decreased but striatal conversion of tryptophan to serotonin returns to control levels. This is of interest in light of the toxicity produced by PCPA in animals treated for 6 days with lithium and the apparent recovery of that interactive effect after 21 days.

Pargyline is known to inhibit both type A and type B monoamine oxidase thus effecting the metabolism of both catecholamines and serotonin. Ho et al, (1970) reported that 28 days of lithium treatment (2.0 meq/kg/day) produced a significant alteration in serotonin turnover in the cerebellum.
and hypothalamus. While cerebellar turnover was increased by 37%, hypothalamic turnover was decreased by 51.5%. Other brain regions were not significantly effected. These measures were based on administration of 75 mg/kg pargyline which resulted no significant change in steady state levels of norepinephrine or dopamine.

There are several avenues of further investigation suggested by the studies reported here. Both the regional brain distribution and the published reports of alterations in neurotransmitter systems suggest lithium may have a potent effect on hypothalamic functioning. The relationship of the particular functions of the hypothalamic nuclei follow various durations of lithium treatment could well shed light on the nature of lithiums actions and the nature of the manic depressive disorder. The fact that lithium produces marked alterations in serotonin metabolism and the observations of increased toxicity of lithium following PCPA may be related to the phenomenon of increased tolerance of patients to lithium while the patient is in the manic phase but not after the manic phase "breaks". Some of these observations will be pursued in future research.
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