Table IV. Supression of the conditioned responses by phenethylamine in the rat (i.p.)

Dose (mg/kg)	Blocking effects (%)	Probits	$ED_{50} \pm SE$
Animals without pretreatment			
25	0	0	38 $\pm$ 1.64
35	30	4.48	
40	65	5.4	
50	90	6.25	
Animals pretreated during 10 days with 1 daily dose of 35 mg/kg of phenethylamine			
25	0	0	36.5 $\pm$ 2
35	27	4.4	
40	69	5	
50	87	6.1	

electrical shock to the feet and conditioned to do the same also after hearing the sound of a buzzer.

According to unpublished observations, phenethylamine and amphetamine are amongst the substances that block the conditioned responses. We did not differentiate between specific and unspecific blocking since we thought this of no importance from our point of view. We considered simply as blocking when the animals did not react to the buzzer's sound. The effective dose 50 (ED_{50}) was calculated by taking into account the percentage of positive answers, according to MILLER and TAINTER[16]. The tests were repeated after 10 days of treatment of the rats with N-methylamphetamine (3 mg/kg) and phenethylamine (35 mg/kg), respectively. Control tests were performed giving 25 and 50 mg/kg of phenethylamine to non-tolerance conditioned rats 24 h before test. The injections were always administered by the i.p.

Results. The results obtained in assays of lethal effects are shown on Tables I and II. It can be seen on Table I that the LD_{50} of N-methylamphetamine is raised from 115 to 156 mg/kg after a pretreatment with 40 mg/kg of the same substance for 10 days. The difference is significant ($p < 0.005$). Table II shows that the lethal dose of phenethylamine remains practically unaltered after a pretreatment with 100 mg/kg of this substance daily for 10 days. The results obtained in assays on conditioned responses are shown on Tables III and IV. Table III shows that the ED_{50} of N-methylamphetamine is raised from 5.5 mg/kg to 8.5 mg/kg after a daily pretreatment with 3 mg/kg of this substance for 10 days. The difference is significant ($p < 0.005$). Table IV shows the results on control tests according to which 25 and 50 mg/kg of phenethylamine do not produce tolerance to the conditioned responses in rats.

Discussion. Our supposition that amphetamine tolerance is produced by phenethylamine depletion would have been invalidated if phenethylamine itself had produced a tolerance. This did not happen. Taking into consideration these circumstances and the fact that chronic administration of N-methylamphetamine produce a depletion of cerebral phenethylamine[14], we think that our observations are at least consistent with the supposition that amphetamine acts in the tests employed by us mainly by liberating phenethylamine.

[16] L. C. MILLER and M. L. TAINTER, Proc. Soc. exp. Biol. Med. *57*, 261 (1974).

Lithium Effects Upon Components of Activity in Rats

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Summary. When lithium chloride was administered to rats several changes occurred in a number of components of activity, some of which were sex-related. There was a reduction in both ambulatory and rearing activity and in behaviour associated particularly with the mouth and nose. The findings are related to a suggestion that lithium effects on behaviour may be more subtle than hitherto thought.

Almost alone amongst psychoactive agents presently finding widespread usage in clinical medicine, lithium has still not been subjected to thorough psychopharmacological analysis leading to any consistent picture of the behavioural effects of its salts in animal subjects. A number of recent studies have produced widely varying results[1]. Some investigators have indeed reported a lack of effect, except upon animals previously treated with combinations of other psychoactive agents[2].

It may be that lithium modifies behaviour subtly, and that the screening tests used to date either impose too rigid a control upon the actions of experimental animals, or direct the experimenter's attention to too narrow a band of activities, effectively masking what may be important lithium effects[3].

To overcome this problem the present study was designed to observe rats under the influence of lithium chloride in a test situation which allowed the animals relatively free movement and in which a number of behavioural components could be simultaneously observed.

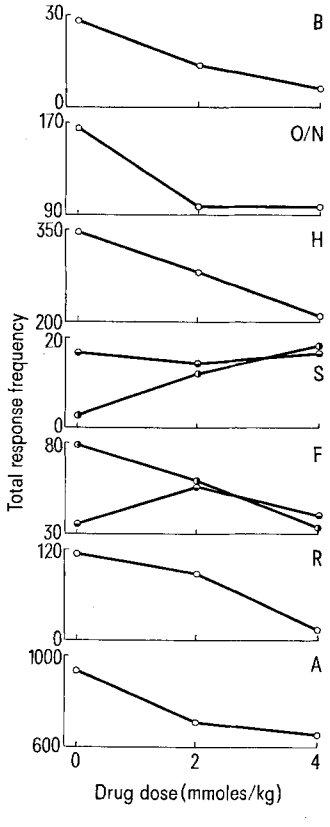
Methods. The animals were placed singly into a box having a square floor area 30.4 × 30.4 cm, and 32.6 deep. The interior of the box was painted white and the floor was marked into 4 equally sized squares, 15.2 × 15.2 cm. Fluorescent room lighting, directly above the apparatus, was used throughout the experiment. The experimenter noted the occurrence of particular kinds of behaviour by speaking into the microphone of a tape recorder, for later analysis.

The taped records were used to assess the frequency of occurence of a number of behavioural components, namely: ambulation (moving all 4 feet from one floor square to another); rearing (standing on the hind legs, either against the walls of the box or more centrally in the box); freezing (a sudden cessation of all movement); crouching (lowering

[1] F. N. JOHNSON, in *Lithium Research and Therapy* (Ed. F. N. JOHNSON; Academic Press, London 1975), p. 315.
[2] H. STEINBERG, Biochem. Soc. Trans. *1*, 38 (1973).
[3] L. A. SYME and G. J. SYME, Psychopharmacologia *29*, 85 (1973).

of either the whole body or just the anterior portion); jumping; twitching; shaking; trembling; body arching; stretching; scratching (grooming with hind legs); head grooming (with the fore legs); piloerection; head movements (raising, lowering, or making lateral movements of the head relative to the rest of the body); sniffing (at various parts of the box); biting (the box walls); grooming with the teeth; licking the genitalia; vocalizing; and sneezing.

In all, 12 female and 12 male Wistar strain rats were used, each being about 100 days old at the start of experiment. Each rat was injected with drug or placebo 30 min prior to being observed for a 2 min period in the test apparatus, previous studies[4] having demonstrated this to be about the time of maximum behavioural effect of lithium chloride injections in rats of the age and weight used in this experiment. 3 test sessions were given to each rat, separated by intervals of 1 week, a different drug being used on each occasion. Two dose levels of lithium chloride were used, 2.0 and 4.0 mmoles/kg, made up in water for Injection BP, which was also used as placebo; drug injections were made i.p., 0.1 ml being administered per 100 g body weight. The 2 drug doses and the placebo injection could be given in 6 different sequences and 2 male and 2 female rats were allocated at random to each sequence. Drug administration was carried out blind, the solutions being coded until the experiment had been completed. The full experimental design was thus a 3 (drug doses + placebo) $\times$ 2 (sexes) $\times$ 6 (sequences of administration) factorial, with repeated measures on the first factor and 2 subjects in each condition.



Lithium effects on various components of activity. Effects of lithium on biting (B), oral/nasal activity (O/N), head movements (H), scratching (S), freezing (F), rearing (R) and ambulating (A) in rats. Symbols: ○ indicates combined totals for male and female subjects; ◒ indicates females only; ◐ indicates males only.

Results. When the data were subjected to analysis of variance, lithium chloride was found to have little effect upon many of the behaviours which had been recorded, at least under the conditions of this experiment (Table). Several categories of behaviour did, however, show some change with lithium as compared with the placebo treatment condition (Figure).

Increasing doses of lithium chloride reduced biting ($F = 7.41$; df 2/24; $p < 0.001$), head movement ($F = 5.40$; df 2/24; $p < 0.001$), ambulating ($F = 3.72$; df 2/24; $p < 0.05$) and rearing ($F = 9.23$; df 2/24; $p < 0.001$). Freezing behaviour was reduced in female rats but, since activity tended not to occur to any great degree in the male animals even under placebo treatment, there was a resultant sex $\times$ doses interaction ($F = 4.60$; df 2/24; $p < 0.05$). A similar sex effect was noted in scratching behaviour, the males again showing no variation with drug dosage, but the females increasing their scratching as the lithium chloride dose was raised, producing a significant overall dose effect ($F = 4.04$; df 2/24; $p < 0.001$). When all activities involving the mouth and nose (sniffing, grooming with the teeth, licking the genitalia, vocalizing, and sneezing) were pooled, it was found that lithium chloride significantly reduced the combined score ($F = 9.56$; df 2/24; $p < 0.001$). Biting, which had been found to be affected by lithium, was omitted from the pooled oral/nasal activity score.

Discussion. Decreases in rearing and general ambulation scores have been noted previously[3,4] and have been related to a reduced responsiveness of the lithium treated animal to environmental stimulation[5]. A similar explanation might be extended to the present findings from some other forms of activity. Biting may, for example, be considered as an environment-directed response, and freezing as it is defined here probably occurs as a defensive reaction to slight but sudden changes in surroundings. Head movements are certainly related to olfactory and

[4] F. N. Johnson and S. Wormington, Nature New Biol. *235*, 159 (1972).
[5] F. N. Johnson, Dis. nerv. Syst. *33*, 235 (1972).

Statistically non-significant effects of lithium on the frequency of various forms of activity

Behaviour component	Treatment		
	Placebo	Lithium (mmoles/kg i.p.) 2.0	4.0
Crouching	28	33	32
Jumping	2	4	0
Twitching	0	0	1
Shaking	0	1	0
Trembling	0	4	0
Body arching	2	2	2
Stretching	16	16	8
Head grooming	2	5	9
Oral/nasal activities			
Sniffing	116	63	75
Grooming with teeth	23	20	11
Licking genitalia	12	4	3
Vocalizing	5	0	3
Sneezing	5	7	6

Results for both sexes and all 6 test sequences have been pooled.

visual searching. In rodents, the nose and mouth are importantly involved in investigating the immediate environment; all these activities may, therefore, be expected to decrease in frequency of occurence if lithium does in fact suppress the performance of behaviour associated with awareness of the environment.

The increase in frequency of scratching amongst the lithium-treated female rats cannot readily be explained in terms of reduced environmental awareness. It may be that this behaviour compensates for an observed slight, but statistically non-significant, decrease in grooming with the teeth, but it is also possible that it represents a response to a local irritation caused by the drug injection: its non-occurrence in males, however, remains puzzling.

Lithium salts are toxic and are known to produce a condition in rats which has been referred to as 'lithium sickness'[6]; it is not easy to see how this could have produced the pattern of findings noted in the present study, though the possibility must still be borne in mind that some of the effects may represent toxic reactions.

Our understanding of the actions of lithium in the treatment of recurrent mood disorders[7], and of the mechanisms underlying those disorders themselves, will be enhanced by the closer investigation of the drug's action upon animal behaviour, but such studies must involve the use of appropriate test situations which are likely to prove sensitive to the slight behaviour modifications which lithium can produce. Such subtle effects may eventually be found to be crucial in defining the psychopharmacological profile of lithium.

[6] M. NACHMAN, J. comp. physiol. Psychol. *56*, 343 (1963).
[7] M. SCHOU and K. THOMSEN, in *Lithium Research and Therapy* (Ed. F. N. JOHNSON; Academic Press, London 1975), p. 63.

The Action of Substance P on Mesencephalic Reticular and Substantia Nigral Neurones of the Rat

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Summary. Extracellular recordings were made from neurones in the mesencephalic reticular formation and substantia nigra of the rat which was anaesthetized with urethane 1.5–2 g/kg i.p. Out of 44 cells tested 42 were excited by Substance P applied iontophoretically and in some cases this excitation was rapid. Evidence is presented for Substance P as a putative excitatory transmitter onto reticular and nigral neurones possibly released from primary sensory afferents.

Substance P is found distributed widely in the central nervous system[2] and there are particularly high levels in the substantia nigra[3]. Recently NILSSON, HÖKFELT and PERNOW[4] have investigated the distribution of Substance P in the rat central nervous system using immunohistochemistry. They found evidence for its location in nerve endings but not in cell bodies. Particularly high levels occurred in the substantia gelatinosa, nucleus parasolitarius, dorsal and ventral lateral geniculate body, medial and central amygdaloid nuclei and the substantia nigra. They suggested these nerve endings could arise from primary sensory afferents. Substance P has been shown to have an effect on cells in several areas of the brain and cord[5–7]. For example, KRNJEVIC and MORRIS[6] found that it was excitatory on cuneate neurones but while the excitation was strong it was slow in onset, the onset occurring after a delay of 10–30 sec and reaching a peak after 30 sec. Following termination of ejection the response decreased gradually over a 60 sec or longer period. These authors concluded that Substance P was unlikely to be the quick acting transmitter released from primary afferent terminals. In the present study we have investigated the effect of Substance P on reticular and nigral cells and compared its action with that of acetylcholine.

Materials and methods. Experiments were performed on 18 female Wistar Albino rats weighing 150 g and anaesthetized with urethane 1.5–2 g/kg i.p. Extracellular recordings were made from single neurones in the mesencephalic reticular formation and substantia nigra using parallel multibarrel glass microelectrodes[8]. Cell position was located by ejection of Pontamine Sky Blue from the recording barrel[9] and then preparing frozen sections. Substance P was synthesized by a method fully described by YAJIMA, KITAGAWA and SEGAWA[10], and ejected iontophoretically from a 0.003 *M* solution of dilute tartaric acid, pH 5.5–6.0. Both cathodal and anodal current were used to release Substance P. Positive results were obtained only with an anodal current. As previous workers[6] reported release of Substance P by a cathodal current, the release of Substance P was measured from electrodes which had produced results in vivo. Release of Substance P was measured by placing the tip of the electrode in 0.2 ml Krebs solution and applying 50 nA of either a cathodal or anodal current for 20 min. The amount of Substance P in the solutions was measured using a bio-assay technique[2]. Acetylcholine and γ-aminobutyric acid (GABA) were ejected iontophoretically as cations from 0.2 *M* solution in distilled water, pH 4.5. Current balancing was used during the ejection of all drugs[11].

[1] Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan.
[2] A. H. AMIN, T. B. B. CRAWFORD and J. H. GADDUM, J. Physiol., Lond. *126*, 596 (1954).
[3] G. ZETLER, in *Handbook of Neuroch...*, (Ed. A. LAJTHA; Plenum, New York 1970), vol. 4.
[4] G. NILSSON, T. HÖKFELT and B. PERNOW, Med. Biol. *52*, 424 (1974).
[5] F. LEMBECK, Arch. exp. Path. Pharmak. *219*, 197 (1953).
[6] K. KRNJEVIC and M. E. MORRIS, Can. J. Physiol. Pharmac. *52*, 736 (1974).
[7] J. W. PHILLIS and J. J. LIMACHER, Brain Res. *69*, 158 (1974).
[8] A. R. CROSSMAN, R. J. WALKER and G. N. WOODRUFF, Neuropharmacology *13*, 547 (1974).
[9] J. M. GODFRAIND, J. Physiol., Paris *61*, Suppl. *2*, 436 (1969).
[10] H. YAJIMA, K. KITAGAWA and T. SEGAWA, Chem. Pharm. Bull. *21*, 2500 (1973).
[11] G. C. SALMOIRACHI and F. WEIGHT, Anaesthesiology *28*, 54 (1967).