DISPUTANDUM

Lithium Effects on Vertical Activity in Rats: A Reply to D. F. Smith

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Summary. Arguments presented against a hypothesis of the mechanisms underlying the behavioural effects of lithium ions are examined and found to be inadequate in a number of important respects, including some of a logical nature.

In an interesting paper by SMITH[1] our interpretation of the differential effects of lithium chloride on components of motor activity in rats[2-4] has been questioned on a number of grounds.

The central issue concerns the possibility that noxious effects may follow i.p. injection of doses of 6 mEq LiCl/kg administered in volumes of 0.1 ml/100 g subject body weight. The behavioural signs indicative of tissue damage noted by SMITH were not observed in our own studies, though it is true that a mild post-injection syndrome has been described previously[5] and in one of our early reports we recorded skin ulceration resulting from s.c. administration of LiCl[2]. The discrepant results may in part be accounted for by differences in the strains of animals used: whilst SMITH employed albino Wistar rats our studies have typically involved either rats of the Roman Control (RCA) strain[2,3] or F_1 hybrids of the Roman High and Low Avoidance strains[6,7].

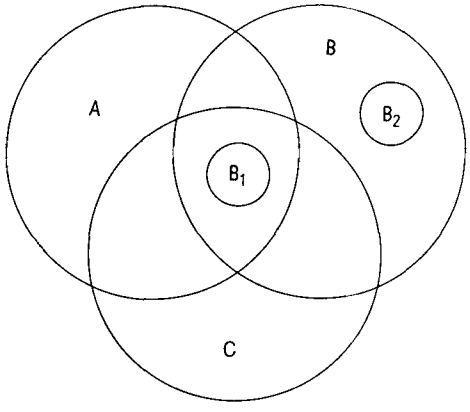

Fig. 1. Venn diagrams to illustrate the logic of the conclusions drawn by JOHNSON[8]. A) the class of behaviours which are stimulus controlled; B) the class of behaviours which occur in the test apparatus; C) the class of behaviours affected by LiCl; B_1) rearing activity; B_2) horizontal activity. A conclusion of the form 'lithium affects vertical rearing activity which belongs to a class of behaviours which are stimulus controlled' is not only fully justified by the evidence, but is also not incompatible with the additional possibilities that lithium affects other types of behaviour too, including some which are stimulus-independent and fails to affect still others, some of which may be stimulus-dependent.

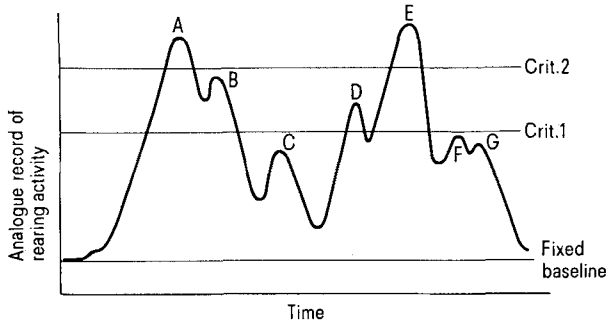

Fig. 2. A comparison of the techniques used by SMITH[1] and JOHNSON[3] to record vertical rearing activity. Using an analogue print-out curve and the definition of a rearing response given in the text, JOHNSON would designate all the peaks A to F as rearing responses; SMITH, on the other hand, using a fixed criterion would record only peaks A and E as rearing responses (using, for example, Criterion 2), or only A, D and E (using Criterion 1). No fixed criterion value would lead to all the peaks being designated as rearing responses.

SMITH concludes that the behavioural effects produced by lithium in our animals resulted from nonspecific adverse actions of LiCl, and that they could not have been due to a drug action on behavioural mechanisms alone. It could, however, be argued that if a drug produces a behavioural effect then whatever mechanism is involved is *ipso facto* a behavioural control mechanism, and I have suggested elsewhere[8] that the distinction which is frequently drawn between toxic and non-toxic drug effects, particularly in animal studies, is to a large extent an arbitrary one and may, indeed, be irrelevant to the process of hypothesis construction which follows the completion of an empirical drug study.

It should, in any case, be noted that SMITH investigated only one dose level of LiCl. In several studies[6,7] we have reported similar behavioural effects of LiCl even when employing much lower dose levels and considerably less concentrated solutions. Thus, in an investigation into the effects of LiCl on learned responses[7], a dose of 3 mEq LiCl/kg in volumes of 0.1 ml/100 g body weight was used, whilst in a comparative study of the effects of various alkali metal chlorides on vertical rearing[6] a dose of only 1 mEq LiCl/kg was delivered in volumes of 0.5 ml/100 g body weight, i.e., 30 times less concentrated than the solutions investigated by SMITH.

Since vertical and horizontal components of activity have been shown to be differentially suppressed by LiCl the behavioural effects which this substance produces are unlikely to depend upon nothing more than diffuse tissue damage which would be expected to produce impairments across a broad spectrum of activities.

In the first report to appear of the action of LiCl in suppressing vertical rearing activity in rats[2], doses of 2 mEq/kg produced this effect even though the injections were performed not i.p. but s.c. It is difficult to see how the results could then have been an artifact of damage occurring at intraperitoneal sites.

[1] D. F. SMITH, Experientia *32*, 1320 (1976).
[2] F. N. JOHNSON and S. WORMINGTON, Nature, Lond. *235*, 159 (1972).
[3] F. N. JOHNSON, Experientia *28*, 533 (1972).
[4] F. N. JOHNSON, Dis. nerv. Syst. *33*, 235 (1972).
[5] S. H. JOHNSON, Ph. D. thesis, Univ. of Tennessee, 1971.
[6] F. N. JOHNSON and G. J. BARKER, Dis. nerv. Syst. *33*, 664 (1972).
[7] F. N. JOHNSON, Nature, Lond. *238*, 333 (1972).
[8] F. N. JOHNSON, Comprehens. Psychiat., in press.

SMITH describes as a *non sequitur*, and therefore fallacious, my conclusion that (to quote SMITH) 'environmental stimuli affected control rats and LiCl-treated rats differently'[1]: it might well have been had I in fact drawn that conclusion. What I actually said was that 'lithium chloride... acted to suppress selectively activity which was stimulus controlled'[3]. My statement was carefully chosen to conform with the logic of the experiment and the rationale can be illustrated most directly by the use of Venn diagrams (Figure 1).

Of course, the form of analysis applied in Figure 1 to the behavioural effects of LiCl does not apply if LiCl either fails to affect vertical activity or fails to do so differentially as compared to measures of horizontal activity. SMITH made no test of the latter possibility, but he did confirm that LiCl reduced rearing significantly when compared with NaCl-treated control rats, albeit only in the first 5 min of the test period. However, he failed to confirm that vertical movements fall into the class of behaviours which are stimulus controlled. The explanation for this may lie in the methods used to record rearing activity. SMITH employed a fixed criterion of rearing height (9 cm above the floor of the apparatus) and a hand-operated recorder. In our studies we recorded vertical movements in an analogue form on a moving pen recorder, defining a rear as a 'peak on the activity record which was preceded by a rise of at least 1 cm from the previous low point and followed by a drop of at least 1 cm to the next low point'[2]. This system, which does not depend on a fixed criterion of rearing height arbitrarily imposed by the experimenter (Figure 2), was chosen because we had found, in previous work using other drugs[9], that it enabled quite subtle drug effects to be detected in a way not possible by simple observational techniques. In later studies[6] we further refined our recording method to provide greater sensitivity, by using the integrated area under the analogue curve as our index of vertical activity.

Using our recording technique we were able to establish the stimulus-dependence of rearing behaviour, confirming the finding of previous workers[10].

Many of the behavioural effects of lithium may be very subtle[11] and subject to the influence of factors still to be elucidated: the apparently contradictory findings which are so characteristic of studies involving lithium[12] may well stem from differences in behavioural recording techniques. It has, I think, to be remembered that failure to replicate an experimental finding can have many causes, all of which need to be carefully explored before firm conclusions may be drawn. It is a common error to confuse a failure to replicate an experimental finding with the refutation of an hypothesis based upon that finding, and SMITH's data, whilst interesting in themselves, do not warrant his conclusion about the falsity of our hypothesis concerning the mode of action of lithium salts in modifying behaviour.

[9] A. KEENAN and F. N. JOHNSON, Experientia *28*, 428 (1972).
[10] H. C. HOLLAND, B. D. GUPTA and E. WELDON, Activ. nerv. Super. *8*, 140 (1966).
[11] F. N. JOHNSON, Experientia *32*, 212 (1976).
[12] F. N. JOHNSON, in *Lithium Research and Therapy* (Ed. F. N. JOHNSON; Academic Press, London 1975), p. 315.

PRO EXPERIMENTIS

A Method for the Detection of Chemotaxis in Mammalian Tissue Cells

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Summary. A new method is described of culture of mammalian tissue cells beneath a solid medium, permitting the assessment of growth-promoting, growth-inhibiting and chemotactic substances.

Chemotaxis in mammalian leucocytes has been greatly clarified since the introduction of the BOYDEN chamber[2], but the equally important problem of detecting chemotaxis in tissue cells such as fibroblasts and smooth muscle cells has not yet been solved.

One approach would be to culture cells in a solid medium, in which diffusion gradients can be established; the medium must however lack a fibrillar structure, because cultured cells tend to grow along fibres. Accordingly the use of culture media containing agar was investigated.

Commercial preparations of agar were found to have varying properties; the lowest concentration to produce a solid (not sloppy) medium at room temperature was achieved by melting 17 ml of 3% agar (Oxoid) and adding 83 ml of culture medium when it had cooled to approx. 54°C. The medium used was Dulbecco and Vogt's modification of minimal Eagle's medium containing 10% foetal calf serum (Flow Laboratories, Scotland). The mixture was made under sterile conditions and introduced into 60 mm. Falcon plastic tissue culture dishes (4 ml in each).

Mouse fibroblasts of the 3T6 line were concentrated in suspension in fluid medium following trypsinization and a drop of suspension was inoculated on to the surface of the solid medium at room temperature. The dishes were incubated at 37°C in sealed Filde's jars, after gassing with 20% O_2, 5% CO_2 and 75% N_2 in order to buffer the bicarbonate-containing medium.

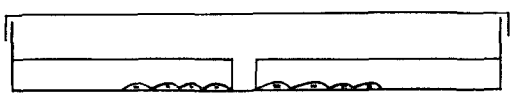

Fig. 1. Diagram of culture system.

[1] Our thanks are due to Mr. D. S. LEAKE, who provided the smooth muscle cell suspensions; and to Mr. J. F. STEVENSON, for expertise in preparing the medium. This work is supported by the British Heart Foundation, Grant No. 528.
[2] S. V. BOYDEN, J. exp. Med. *115*, 453 (1962).