REFERENCES

BALLANTYNE, B. (1970). Autopsy findings following death by intramuscular hydrogen cyanide: an experimental study. *Med. Sci. Law*, in the Press.

BALLANTYNE, B., BRIGHT, J. & WILLIAMS, P. (1970). Levels of cyanide in whole blood and serum following lethal intramuscular injections to experimental mammals. *Med. Sci. Law*, in the Press.

EPSTEIN, J. (1947). Estimation of microquantities of cyanide. *Anal. Chem.*, **19**, 272–274.

## Jumping after naloxone precipitated withdrawal of chronic morphine in the rat

D. L. FRANCIS and C. SCHNEIDER, *Research Department, Miles Laboratories Limited, Stoke Court, Stoke Poges, Buckinghamshire*

There have been several reports (Maggiolo & Huidobro, 1961; Way, Loh & Shen, 1968; Marshall & Weinstock, 1969) that mice can be made physically dependent on morphine-like drugs and that one of the withdrawal effects after challenge with a morphine antagonist is jumping. Although Buckett (1964) and Lorenzetti & Sancilio (1970) have described morphine withdrawal effects in rats, jumping was not reported.

We have been able to produce and quantitate morphine withdrawal jumping in the rat similar to the effect in mice recently demonstrated to the Society by Marshall (1970).

Male Wistar albino rats weighing 100–120 g were given subcutaneous injections of morphine sulphate at 0.900 h, 12.00 and 16.00 h for 4 days and at 0.900 h on the fifth day. The dose of morphine was raised gradually from an initial 10·0 mg base/kg to a final 33·6 mg base/kg. Naloxone hydrobromide at 0·25 mg base/kg was given subcutaneously 5 h later to precipitate withdrawal effects.

Immediately after naloxone challenge, each rat was placed in a plastic bucket 25 cm high, covered by a perforated transparent lid. The rats showed typical morphine withdrawal effects such as diarrhoea, irritability, occasional head twitches and paw tremors; in addition, for about 20 min after challenge, most of the animals attempted many times to leap out of the container in a co-ordinated manner.

Jumping was rarely seen in the following control animals: rats given subcutaneous injections of saline and challenged with saline or naloxone; rats chronically treated

TABLE 1. *Effect of naloxone in rats pretreated with morphine*

| Pretreatment | | | | Challenge | | Responses within 15 min of challenge | | | |
|---|---|---|---|---|---|---|---|---|---|
| Drug | Dose range mg/kg | Days | No. of doses | Drug | Dose mg/kg | % of rats having diarrhoea | % of rats jumping | Mean no. of jumps/rat | No. of rats |
| M | 10 | 1 | 1 | N | 0·25 | 8 | 8 | 0·31 | 13 |
| M | 10–14 | 2 | 4 | N | 0·25 | 80* | 0 | 0 | 10 |
| M | 10–18 | 3 | 7 | N | 0·25 | 90* | 20 | 1·70 | 10 |
| M | 10–25 | 4 | 10 | N | 0·25 | 93* | 43* | 0·86* | 14 |
| M | 10–34 | 5 | 13 | N | 0·13 | 100* | 60* | 7·10* | 10 |
| M | 10–34 | 5 | 13 | N | 0·25 | 85* | 70* | 6·47* | 47 |
| M | 10–34 | 5 | 13 | N | 0·50 | 90* | 60* | 3·00* | 10 |
| M | 10–34 | 5 | 13 | N | 1·00 | 100* | 70* | 4·90* | 10 |
| M | 10–34 | 5 | 14 | N | 0·25 | 0 | 27 | 1·82 | 11 |
| Last dose 30 min before challenge | | | | | | | | | |
| M | 10–34 | 5 | 13 | S | — | 0 | 9 | 0·09 | 11 |
| S | — | 1 | 1 | S | — | 0 | 10 | 0·10 | 10 |
| S | — | 5 | 13 | N | 0·13–1·00 | 0 | 0 | 0 | 25 |

Morphine sulphate (M), naloxone HBr (N), or saline (S) given subcutaneously. Pretreatment given at 09.00, 12.00 and 16.00 h. Dose volume 10 ml. Challenge usually given 5 h after final pretreatment. Responses observed in plastic buckets. * Significantly different from control level ($P \leq 0.01$).

with morphine and challenged with saline; or rats given a single large dose of morphine and challenged with naloxone.

Typical results from an experiment using 181 animals are shown in Table 1. In the group of rats withdrawn from chronic morphine the incidence of jumping and diarrhoea was statistically significant from controls ($P<0{\cdot}01$).

We thank Sankyo Laboratories, Japan, for naloxone.

REFERENCES

BUCKETT, W. R. (1964). A new test for morphine-like physical dependence (addiction liability in rats). *Psychopharmacologia*, **6**, 410–416.

LORENZETTI, O. J. & SANCILIO, L. F. (1970). Morphine dependent rats as a model for evaluating potential addiction liability of analgesic compounds. *Archs int. Pharmacodyn. Ther.*, **183**, 391–402.

MAGGIOLO, C. & HUIDOBRO, F. (1961). Administration of pellets of morphine to mice; abstinence syndrome. *Acta physiol. Latinoam.*, **11**, 70–78.

MARSHALL, I. (1970). Laboratory experiments suitable for practical classes in psychopharmacology. *Br. J. Pharmac.*, **39**, 246–247 *P*.

MARSHALL, I. & WEINSTOCK, M. (1969). A quantitative method for the assessment of physical dependence on narcotic analgesics in mice. *Br. J. Pharmac.*, **37**, 505–506 *P*.

WAY, E. L., LOH, H. H. & SHEN, F. H. (1968). Quantitative assessment of morphine tolerance and physical dependence. *Pharmacologist*, **10**, 188.

## Some multivariate statistical techniques applied to pharmacological research

NORMA CHERRINGTON and J. V. SMART (introduced by P. TURNER), *Department of Pharmacology, St. Bartholomew's Hospital Medical College, London, EC*1 *and Smith Kline and French Laboratories Ltd., Welwyn Garden City, Herts*

When the effects of a drug are statistically evaluated, it is possible to measure several responses (such as heart rate and blood pressure) at the same time, or the same response at several times after treatment. In these circumstances the multiple responses can be correlated with one another, and the methods used in statistical analysis of the results should take such correlation into account.

Smart, Sneddon & Turner (1967) described the application of a multivariate technique to psychopharmacological data. The method has now been modified using Hotelling's $T^2$ statistic (Hotelling, 1931; Anderson, 1958), to test the significance of differences between treatments and of individual regressor variables.

If there are $p$ regressor variables $x_1 \ldots . x_p$ and $q$ response variables $z_1 \ldots . z_q$ the error, sums of squares and products (SSP) matrix $\mathbf{E}$ [based on $n$ degrees of freedom (DF)] can be written as a partitioned matrix

$$\left[\begin{array}{c|c} \mathbf{X} & \mathbf{Y} \\ \hline \mathbf{Y}' & \mathbf{Z} \end{array}\right]$$

where $\mathbf{X}$ is the $p \times p$ matrix of SSP for the regressors, $\mathbf{Y}$ is the $p \times q$ matrix of sums of products of the regressors with the response variables, $\mathbf{Y}'$ is the transpose of $\mathbf{Y}$, and $\mathbf{Z}$ is the $q \times q$ SSP matrix for the response variables. Then the matrix of partial regression coefficients of the response variables on the regressors is defined as

$$\mathbf{B} = \mathbf{Y}' \mathbf{X}^{-1}$$

where $\mathbf{X}^{-1}$ is the inverse of $\mathbf{X}$. The matrix of SSP for regression is $\mathbf{R}=\mathbf{BY}$, and we define the adjusted variance-covariance matrix of the response variables as