J. Pharm. Pharmac., 1973, **25**, 513–520 *Received February* 12, 1973

The reflection of ejecting and retaining currents in the time-course of neuronal responses to microelectrophoretically applied drugs

C. M. BRADSHAW, E. SZABADI AND M. H. T. ROBERTS

Department of Psychiatry, University of Edinburgh, Morningside Park, Edinburgh EH10 5HF, U.K.

The role of ejecting and retaining currents in determining the time-course of neuronal responses to microelectrophoretically applied drugs (acetylcholine, glutamate, noradrenaline, 5-hydroxytryptamine, and mescaline) was investigated. Comparing the parameters of excitatory responses to ejecting currents of successively increasing intensity, the following changes were observed: the response latency became progressively shorter, the plateau became higher, and the recovery time was prolonged. An increase in the intensity or duration of the pre-ejection retaining current resulted in the prolongation of the response latency and the latency to plateau, but did not alter the plateau itself. An increase in the intensity of the post-ejection retaining current reduced the recovery time of the response.

It has been reported that the time-courses of neuronal responses to microelectrophoretically applied drugs are characteristic of the drug applied (see Curtis & Crawford, 1969). Furthermore, antagonists and protagonists can modify the time-courses of responses to agonists (Johnson, Roberts & Straughan, 1970; Hill, Simmonds & Straughan, 1971). This suggests that the time-course of the response could give information about the nature of the drug-receptor interaction (Bradley, 1968; Krnjević & Phillis, 1963a, b; Stone, 1971; Tebēcis, 1970; Yamamoto, 1967). Such conclusions cannot be drawn, however, until it is known to what extent physicochemical processes (release, diffusion) contribute to the time-course of the response.

In an *in vitro* study, using [^{14}C]noradrenaline (Bradshaw, Roberts & Szabadi, submitted for publication), we have shown that ejecting and retaining currents interact in determining the shape of the release curve (rate of release plotted against time) during an ejection period. We have demonstrated that a prior retaining current delays the onset of drug release and prolongs the time necessary to establish a steady-state rate of release. In the present study we have investigated how these distortions in the release curve appear in the time-course of the neuronal response. In the light of our experiments, we conclude that the time-course of the response to a particular drug is largely defined by the parameters of the electrophoretic currents, and reproducible responses cannot be obtained unless these parameters are kept constant throughout the study. We put forward the suggestion that the differences between time-courses of responses to different drugs reflect differences in the physical mobilities of the drug molecules.

METHODS

Cats of either sex, 2·0 to 3·5 kg, were used. Anaesthesia was induced by the intravenous injection of thiopentone and was maintained with halothane (0·6–1·2%)

delivered from a temperature and flow-rate compensated vapouriser (Fluotec Mk III, Cyprane Ltd.). Our technique for the preparation of an area of the anterior or posterior sigmoid gyrus for recording has been described (Bradshaw & Szabadi, 1972).

Five-barrelled glass micropipettes were filled by boiling in distilled water under reduced pressure. The water in the barrels was replaced by the appropriate solutions, using a thin Portex catheter. Two barrels contained 3 M NaCl solution, one for recording action potentials, and the other for use in 'current balancing' (Roberts & Straughan, 1967). The remaining barrels were filled with drug solutions. The drug solutions used were noradrenaline bitartrate (0·2 M, pH 3·0–3·5), 5-hydroxytryptamine bimaleate (0·2 M, pH 3·5), mescaline hydrochloride (0·2 M, pH 3·5–4·5), acetylcholine chloride (0·2 M, pH 4·0), sodium glutamate (0·2 M, pH adjusted to 8·5 by the addition of 0·1 N NaOH). Immediately before use, the tip of each micropipette was broken to give an over-all tip diameter 3–6 μm.

The techniques used for recording action potentials, and for the electrophoretic application of drugs, are described by Roberts & Straughan (1967). A cumulative record of the total number of action potentials was obtained via a Grass UI-1 unit integrator. All the neurons studied were spontaneously active. Only excitatory responses were selected for study in these experiments.

The following parameters were measured in order to evaluate the effects of the drugs upon neuronal activity (Fig. 1):

(*a*) The spontaneous (baseline) firing rate (F_1).

(*b*) The latency to onset of excitation, measured from the onset of the ejecting pulse (response latency) (T_0–T_1).

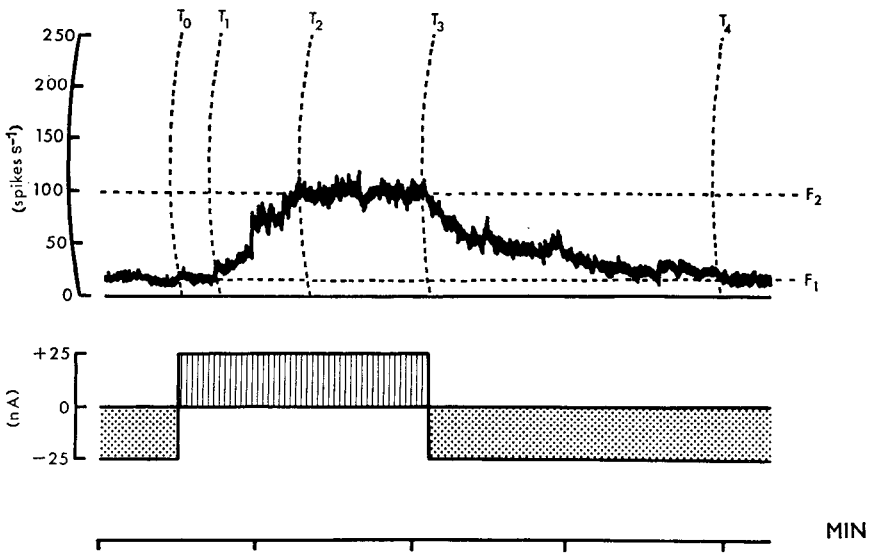

FIG. 1. Parameters of an excitatory response to ACh. Lower trace: electrophoretic current passed through the solution of ACh chloride (0·2 M) contained in one barrel of the micropipette (ordinate: current intensity, positive upwards; abscissa: running time). Stippled area: retaining current; hatched area: ejecting current. Upper trace: record of the firing rate of a single cortical neuron (ordinate: firing rate, abscissa: running time, the same scale as in lower trace). T_0: onset of ejecting pulse; T_1: onset of the neuronal response; T_2: attainment of the plateau firing rate; T_3: termination of the ejecting pulse; T_4: termination of the excitatory response. F_1: spontaneous baseline firing rate; F_2: plateau firing rate.

(*c*) The maximum (plateau) firing rate obtained by the cell during the ejection period (F_2). The maximum firing rate was measured under equilibrium conditions, i.e. when the prolongation of the ejection period led to no further change in the firing rate. The baseline firing rate was subtracted from the maximum (plateau) firing rate in order to obtain the maximum change in the firing rate (F_2–F_1).

(*d*) The time taken to achieve the plateau firing rate (latency to plateau) (T_0–T_2).

(*e*) The time taken for the recovery of the spontaneous firing rate after the termination of the ejecting pulse (recovery time) (T_3–T_4).

(*f*) The total number of action potentials generated in response to the ejecting pulse (total spike number). This measure was used as an index of the magnitude of the excitatory response.

The response latency, the latency to plateau, and the recovery time were estimated by determining the point in time at which the angle of slope of the write-out of the cumulative spike counter stopped changing. This method was found to yield more reliable values than could be obtained by direct measurement of the ratemeter tracing. The total spike number was either measured directly, using the cumulative spike counter, or was estimated by measurement of the area under the ratemeter tracing, after subtraction of the mean rate of spontaneous firing.

RESULTS

The effect of ejecting currents

In these experiments, the parameters of the retaining pulse (intensity and duration) were kept constant. As the intensity of the ejecting current was increased with successive applications, the following changes in the excitatory responses were observed (Fig. 2): (A) The response latency became progressively shorter. (B) The

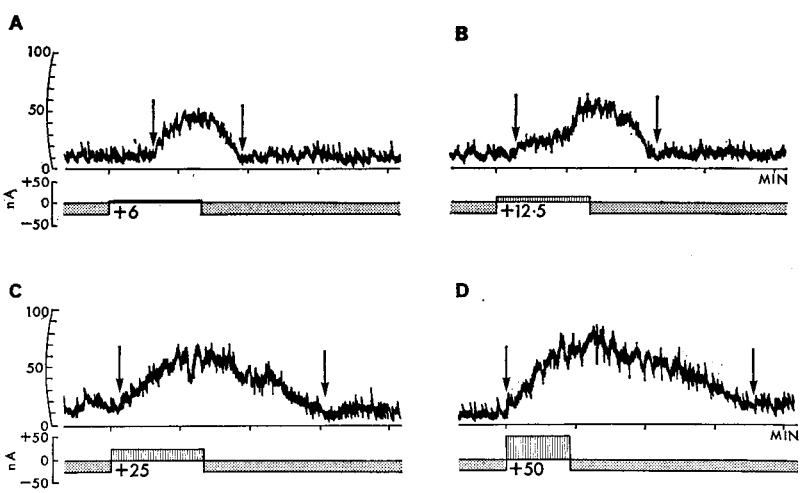

FIG. 2. The excitatory responses of a single cortical neuron to 5-HT applied microelectrophoretically with different ejecting currents. Lower traces: electrophoretic currents (ordinates: current intensity, positive upwards; abscissae: running time). Stippled area: retaining current; hatched area: ejecting current. Upper traces: excerpts from the recording of the firing rate of the neuron (ordinates: firing rate (spikes s^{-1}), abscissae: running time, the same scale as the lower trace). Arrows above the trace indicate the onset of the response and the recovery of the baseline firing rate. Note that the application of +50 nA was terminated prematurely because the cell became over-excited and the spike amplitude was reduced.

maximum firing rate attained by the cell increased. (C) The recovery time was prolonged. (D) The total spike number increased (when a standard ejection time was used).

In this experiment responses to 5-HT were used: similar observations were made with responses to acetylcholine, noradrenaline, and mescaline. Responses to

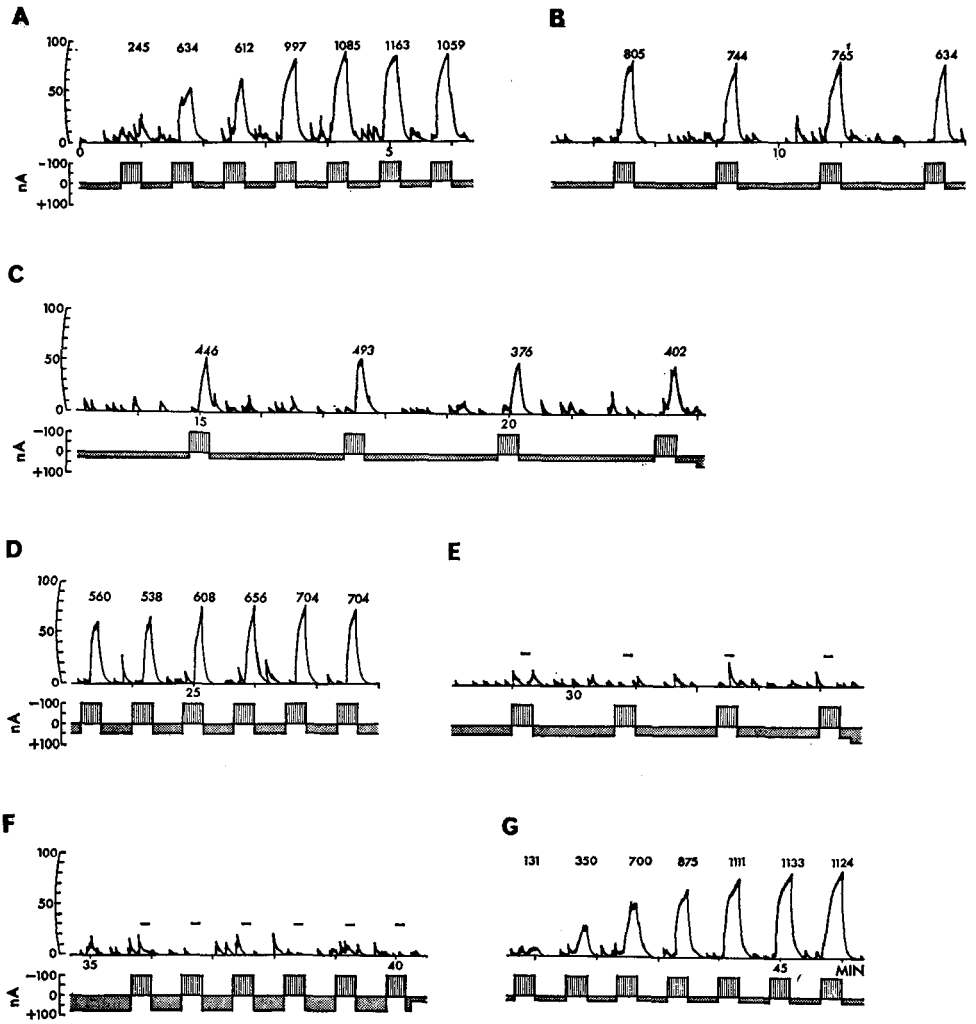

FIG. 3. The effect of changes in the parameters (intensity and duration) of the retaining pulse upon responses to glutamate. Lower traces: electrophoretic current; upper traces: continuous recording of the firing rate of a single cortical neuron (as in Fig. 2). Figures above the traces represent the total number of spikes generated in response to each ejecting pulse. Before the study started, the retaining current (+25 nA) had been applied for 3 min. The parameters (intensity and duration) of the ejecting pulse were kept constant throughout the study, whereas the parameters of the retaining pulse were systematically varied. When either parameter of the retaining pulse was increased, there was a reduction in the total spike number and an increase in the response latency. A: retaining pulse: +25 nA, 30 s; responses progressively increase, until a standard response is established. B: retaining pulse: +25 nA, 80 s; responses are reduced. C: retaining pulse: +25 nA, 130 s; responses are further reduced. D: retaining pulse: +50 nA, 30 s; responses increase, but are still smaller than in A. E: retaining pulse: +50 nA, 80 s; responses are abolished. F: retaining pulse: +75 nA, 30 s; no responses. G; retaining pulse: +25 nA, 30 s; original responses (Compare A) recover.

glutamate were similar, but it was not possible to measure the recovery time due to the fast time-course of these responses.

The effect of retaining currents

Effect of retaining currents upon subsequent responses. An increase in either parameter (intensity or duration) of the retaining current resulted in a decrease in the total spike number and in an increase in the response latency of the response to a subsequent ejecting pulse. This observation is illustrated in Fig. 3. In this study responses to glutamate were used; similar observations were made with other agonists.

In the study shown in Fig. 3 ejecting pulses of standard duration were used. Thus, as the response latency increased following a higher retaining current or a longer retention time, it was not always possible to achieve the plateau firing rate. To obtain information about the effect of retaining currents upon other response parameters (plateau firing rate, latency to plateau and recovery time), it was necessary to vary the ejection time according to the response. Therefore, following each retention period, an ejecting current of standard intensity was applied continuously for a sufficient length of time to enable the firing rate of the cell to reach an equilibrium value. Such an experiment is illustrated in Fig. 4. When either parameter of the retaining current was increased, the response latency and the latency to plateau increased (Figs 4 and 5), but there was no change in the height of the plateau or in the recovery time in the presence of a retaining current of standard intensity (Fig. 4). In the study shown in these figures the retention time was increased; similar observations were made when the intensity of the retaining current was increased.

Post-ejection retaining currents. When the intensity of the post-ejection retaining current was increased, a reduction in the recovery time was observed, although it was not possible to reduce the recovery time to zero, even when very high retaining currents were applied (see Fig. 6).

DISCUSSION

When an ejecting current of higher intensity is applied, the response latency becomes shorter, the maximum change in firing rate increases, and the recovery time becomes longer (Fig. 2). These changes can be interpreted in terms of an increase in the rate of release: a threshold concentration at the receptor sites will be achieved sooner, a steady concentration will be established at a higher level, and it will take a longer time for diffusion to reduce the concentration from this higher level to the threshold concentration for the neuron after the termination of the ejecting pulse.

Previously (Bradshaw & others, submitted for publication) we described how a prior retaining current distorts the rising phase of the release curve. The present results indicate that these distortions are reflected in neuronal responses. Moreover, our results show that both parameters of the retaining current (intensity and duration) are equally important in causing this effect. It is apparent from Fig. 3 that an increase in the interval between consecutive ejecting pulses can result in the complete abolition of neuronal responses to glutamate. It is obvious, therefore, that if quantitatively reliable responses are to be achieved in a microelectrophoresis experiment, it is essential that each drug being tested is applied at regular intervals throughout the study.

The decrease in the overall size of the response after an increase in either parameter of the pre-ejection retaining current is due to an increase in the response latency and

in the latency to plateau, and not to a decrease in the height of the plateau (Figs 4, 5). This is in agreement with our observation that the pre-ejection retaining current delays the onset of drug release and prolongs the rising phase of the release curve, but does not influence the steady-state rate of release (Bradshaw & others, submitted for publication).

It has been suggested that the effect of the pre-ejection retaining current on the time-course of the neuronal response may be avoided by the selection of a 'just adequate' retaining current, which counteracts spontaneous release without interfering with subsequent electrophoretic release (Curtis, 1964). However, it has been demonstrated that a retaining current cannot be just adequate: as soon as a retaining current becomes effective in counteracting spontaneous release, it necessarily starts to interfere with electrophoretic release during a subsequent ejection period (Bradshaw, Roberts & Szabadi, and submitted for publication).

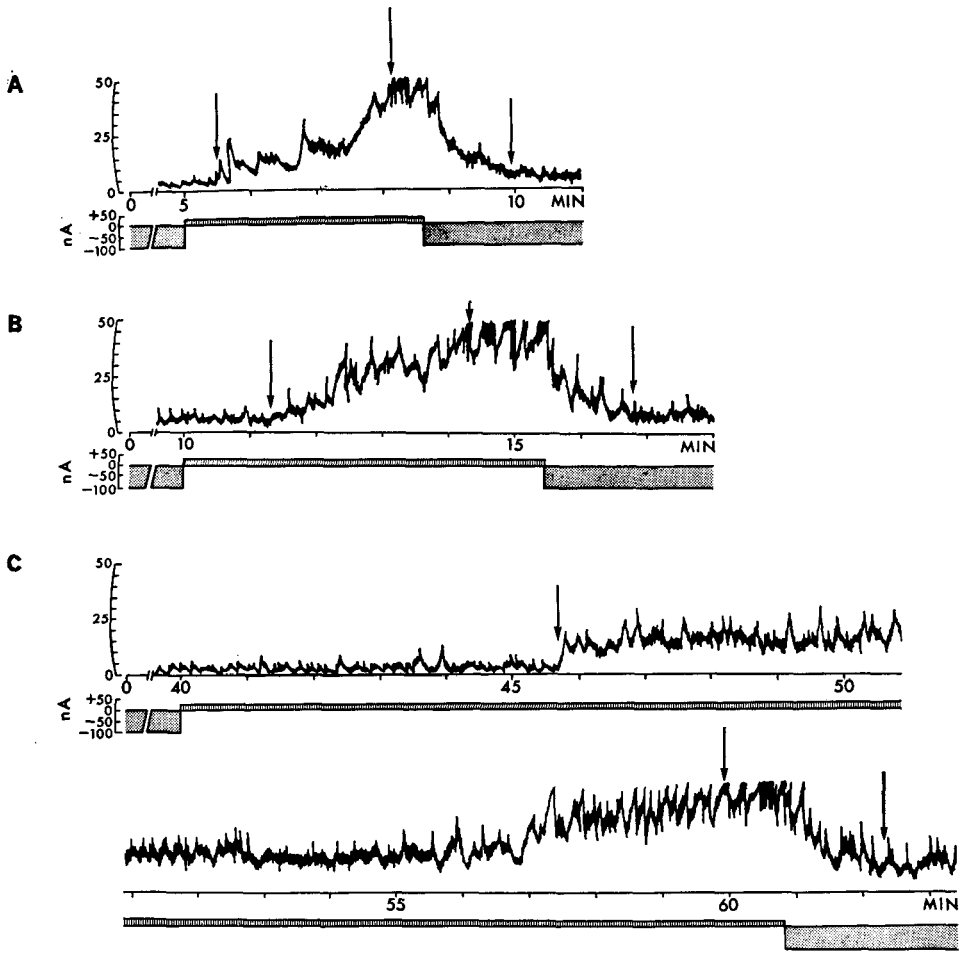

FIG. 4. The effect of different retention times upon responses to ACh. Lower trace: electrophoretic current: upper traces: excerpts from the ratemeter record (see Fig. 2). Arrows above the traces indicate the onset of the response, the attainment of the plateau firing rate, and the recovery of the baseline firing rate. The intensity of the retaining and ejecting currents was kept constant throughout the study, but the retention time was varied (5, 10, 40 min). An increase in the retention time resulted in an increase in the response latency and latency to plateau. See also Fig. 5.

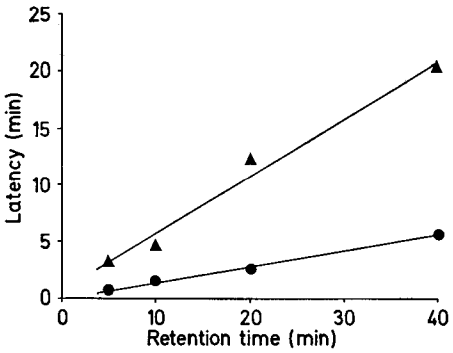

FIG. 5. The effect of the retention time upon response latency (●—●) and latency to plateau (▲—▲) of responses to ACh. The intensity of the retaining current was —100 nA, and the intensity of the ejecting current was +25 nA.

The post-ejection retaining current can modify the recovery time of the response (Fig. 6). The fact that —25 nA was practically as effective as —100 nA suggests that —25 nA almost instantaneously abolished spontaneous release, and that the recovery time observed in the presence of these currents reflects the reduction in concentration of the drug at receptor sites when there was no diffusional leakage from the micropipette. The finding that even in the presence of —5 nA the original baseline firing rate completely recovered is supported by the finding that very weak retaining currents, which are not capable of abolishing diffusional release instantaneously, eventually become completely effective (Bradshaw & others, submitted for publication).

It has long been known that there are differences between the time-courses of neuronal responses to different drugs (Curtis & Crawford, 1969), and many workers have assumed that these differences may yield information about the physiological role of these drugs. For intance, it has been held that the rapid onset and decay of responses to glutamate support the suggestion that this compound is a neurotransmitter. On the other hand, it has also been suggested that the characteristically slow responses to the monoamines may indicate that synaptic responses mediated by these compounds have a slow time-course (Bradley, 1968). The different time-courses of neuronal

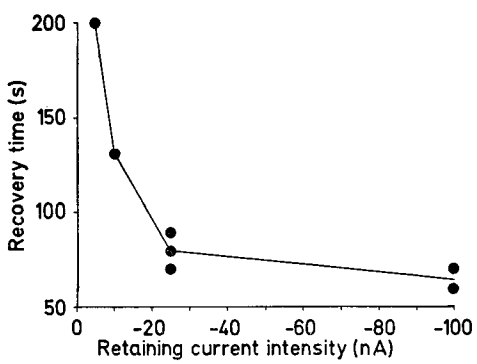

FIG. 6. The effect of the intensity of the post-ejection retaining current upon the recovery time of responses to ACh. Recovery times were measured in the presence of retaining currents of various intensities following plateau responses to ACh (ejecting current, +100 nA). The recovery time was reduced when higher post-ejection retaining currents were used.

responses have also been thought to indicate different modes of action of the exogenously applied drugs. For example, it has been suggested that the relatively slow and prolonged responses to acetylcholine and the monoamines may reflect an indirect mode of action (Krnjević & Phillis, 1963a; Stone, 1971, 1972), or a prolonged binding of the drug molecules to receptor sites (Krnjević & Phillis, 1963b).

However, the biological significance of the time-courses of neuronal responses cannot be evaluated until it is known to what extent physical factors are involved. The present findings indicate that the time-course of a response to any particular drug is largely defined by the interaction between the ejecting and retaining currents. Since the efficacy of these currents depends upon the transport number (and thus upon the absolute mobility) of the drug ion, it is possible that differences between the time-courses of neuronal responses to different drugs are mostly due to differences between the mobilities of the drug molecules. A more mobile drug ion will be released at a higher rate, and will diffuse faster in the tissue than a less mobile ion. This could be reflected in a shorter latency, a steeper rising phase, and a shorter recovery time of the neuronal response. Such a fast time-course is characteristic of responses to glutamate (Fig. 3), while slower time-courses are characteristic of responses to the monoamines (Fig. 2). Indeed, glutamate has a higher transport number than the monoamines. [The transport number of glutamate is about 0·40, estimated from the data of Zieglgänsberger, Herz & Teschemacher (1969), whereas the transport number of 5-HT has been reported to be 0·14 (Krnjević, Laverty & Sharman, 1963) and 0·18 or 0·31 (Bradley & Candy, 1970), and the transport number of noradrenaline was found to be 0·17 (Bradshaw & others, submitted for publication) and 0·09 or 0·19 (Bradley & Candy, 1970.]

Acknowledgements. This work was supported in part by the Scottish Home & Health Department and the Mental Health Research Fund. M. H. T. Roberts is the Rothschild Fellow of the Schizophrenia Research Fund.

REFERENCES

BRADLEY, P. B. (1968). *The Pharmacology of Synapses in the Central Nervous System.* Ch. 12 in Recent Advances in Pharmacology (4th edn). Editors: Robson, J. M. & Stacey, R. S. London: Churchill.

BRADLEY, P. B. & CANDY, J. M. (1970). *Br. J. Pharmac.*, **40**, 194–201.

BRADSHAW, C. M., ROBERTS, M. H. T. & SZABADI, E. (1973). *Ibid.*, **47**, 657–658P.

BRADSHAW, C. M. & SZABADI, E. (1972). *Br. J. Pharmac.*, **45**, 185–186P.

CURTIS, D. R. (1964). *Microelectrophoresis.* Ch. 4 in *Physical Techniques in Biological Research.* Vol. 5A. Editor: Nastuk, W. L. New York: Academic Press.

CURTIS, D. R. & CRAWFORD, J. M. (1969). *A. Rev. Pharmac.*, **9**, 209–240.

HILL, R. G., SIMMONDS, M. A. & STRAUGHAN, D. W. (1971). *Br. J. Pharmac.*, **42**, 339–340P.

JOHNSON, E. S., ROBERTS, M. H. T. & STRAUGHAN, D. W. (1970). *Ibid.*, **38**, 359–366.

KRNJEVIĆ, K., LAVERTY, R. & SHARMAN, D. E. (1963). *Ibid.*, **20**, 491–496.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963a). *J. Physiol., Lond.*, **166**, 296–327.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963b). *Ibid.*, **166**, 328–350.

ROBERTS, M. H. T. & STRAUGHAN, D. W. (1967). *Ibid.*, **193**, 269–294.

STONE, T. W. (1971). *Nature, Lond.*, **234**, 145–146.

STONE, T. W. (1972). *J. Physiol., Lond.*, **225**, 485–499.

TEBĒCIS, A. K. (1970). *Neuropharmac.*, **9**, 381–390.

YAMAMOTO, C. (1967). *J. Pharmac. exp. Ther.*, **156**, 39–47.

ZIEGLGÄNSBERGER, W., HERZ, A. & TESCHEMACHER, H. (1969). *Brain res.*, **15**, 298–300.