This thesis describes a series of experiments involving the use of the microelectrophoretic technique.

I. The first part of the thesis is concerned with methodological issues.

1. A theoretical model was developed to describe the movement of ions occurring within the tip of a micropipette during the passage of electrophoretic currents. On the basis of this model it was predicted that the kinetics of drug release from a micropipette would be determined by the interaction between the ejection and retaining currents. It was also predicted that drug retention was a time dependent process.

2. A series of experiments was conducted on the release of C\(^{14}\)-labelled noradrenaline from micropipettes in vitro. The results of these experiments were consistent with the predictions generated by the model.

   a. in the absence of an electrophoretic current a constant rate of release was established.

   b. all retaining currents tested gradually reduced spontaneous release to zero.

   c. electrophoretic release was linearly related to the intensity of the ejection current. The transport number of noradrenaline was 0.17.

   d. retaining currents reduced the amount of noradrenaline released during a subsequent ejection period by prolonging the time needed to establish a steady-state rate of release.

3. Studies conducted in vivo indicated that the kinetics of neuronal responses to microelectrophoretically applied drugs were
closely related to the kinetics of drug release.

4. Some implications of these findings for the uses of the microelectrophoretic technique were discussed.

II. The second part of the thesis is concerned with a study of the action of mescaline on cortical neurones.

1. It was found that mescaline could exert both excitatory and depressant actions on cortical neurones. The direction of the response to mescaline was highly correlated with the direction of the response of the same neurone to noradrenaline.

2. On some occasions mescaline could antagonise neuronal responses to noradrenaline and 5-hydroxytryptamine.

3. Responses to mescaline, noradrenaline and 5-hydroxytryptamine but not responses to acetylcholine, could be antagonised by sotalol and methysergide.

4. Responses to mescaline could be antagonised and potentiated by desipramine.

5. It was concluded that mescaline acted similarly to noradrenaline and 5-hydroxytryptamine on cortical neurones.
METHODOLOGICAL STUDIES OF THE MICRO-
ELECTROPHORETIC TECHNIQUE WITH
SPECIAL REFERENCE TO THE
PHARMACOLOGY OF MESCALINE

C. M. BRADSHAW

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SUMMARY

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PREFACE

This thesis describes some experiments carried out using the technique of microelectrophoresis. This technique has been used to study the pharmacological responses of excitable tissue in a wide range of vertebrate and invertebrate species. The technique dates from about twenty years ago (Nastuk, 1953; Castillo & Katz, 1955, 1957), and has become increasingly fashionable in the past ten years (see Curtis & Crawford, 1969), with the almost weekly publication of new papers reporting its use.

The technique involves the use of electrical currents to control the release of drugs from micropipettes, and is usually combined with intra- or extra-cellular recording of the electrical activity of single cells close to the site of drug application. In recent years pharmacological studies of single cells using the technique have become increasingly sophisticated (drug interaction studies have become the rule rather than the exception), and the need for quantification has become especially acute. The technique of microelectrophoresis offers no direct measure of the dose of drug applied. In experiments conducted in the central nervous system, the 'dose' is usually expressed as the intensity of the electrophoretic ejecting current used to facilitate drug release from micropipette, and the 'response' is expressed as some change in neuronal excitability (change in firing rate, change in amplitude of evoked potential, change in membrane potential, change in responsiveness to
some excitant 'test' drug, etc). However, there are still many unresolved controversies about the way in which dose and response should be quantified, and about the conclusions which may legitimately be drawn from the results of microelectrophoretic experiments. The first part of this thesis (I) consists of an attempt to resolve some of these controversies. First some theoretical considerations are discussed (I:1), then some pertinent experiments conducted in vitro (I:2) and in vivo (I:3) are described. In the second part of the thesis (II), I have described some pharmacological studies carried out using the microelectrophoretic technique, hopefully with the benefit of a greater insight into the methodological and interpretative difficulties associated with the technique. These pharmacological studies are concerned with the action of mescaline on single cerebral cortical neurones of the cat.
PART I

THE TECHNIQUE OF MICROELECTROPHORESIS:

METHODOLOGICAL STUDIES
A.1. GENERAL INTRODUCTION: THEORETICAL CONSIDERATIONS

The technique of microelectrophoresis involves the delivery of minute quantities of drugs from fine micropipettes. Glass capillary tubes are heated and drawn out until the diameter of their tips is about 0.5-1.0 μ. These pipettes, frequently arranged in clusters of 5 or 7 are filled with electrolytic drug solutions. (In biological experiments, one pipette usually contains a concentrated solution of NaCl or KCl and acts as an extra- or intra-cellular recording electrode to monitor the electrical activity of the cell.) By applying a potential of appropriate polarity with respect to the external medium (bathing fluid, tissue of the experimental subject) an electrical current can be made to flow through the drug solution. The flow of current entails the outward passage of ions of one polarity from the pipette into the external medium, and the influx of ions of the opposite polarity. The efflux of biologically active ions is referred to as 'drug release', and a current which facilitates drug release is known as an ejecting current. Between ejecting pulses it is usual to pass a current of the opposite direction in order to counteract the spontaneous release of drug: such a current is known as a retaining current.

I:1.1 BASIC PROCESSES UNDERLYING DRUG RELEASE

A. Spontaneous release

Even when no electrophoretic current is passed, there will be some 'spontaneous' release of drug from the micro-
pipette. Spontaneous release can result from two processes.

a. **Diffusion.** A concentration difference for drug molecules exists between the solution contained in the micropipette and the external medium. This may result in the diffusional release of drug molecules into the external medium. The rate of release is dependent on the concentration difference between the two media and upon the diameter of the tip orifice.

b. **Hydrodynamic flow.** The hydrostatic pressure exerted by the column of liquid contained in the micropipette may cause small quantities of the whole drug solution to pass into the external medium (Krnjević et al., 1963b). The rate of hydrodynamic outflow depends on the height of the fluid column, the diameter of the tip orifice and the viscosity of the solution. Obviously, the significance of this hydrodynamic outflow for drug release depends upon the concentration of the solution in the micropipette.

**B. Electrophoretic release**

Electrophoretic drug release resulting from the application of an ejecting current also involves two processes.

a. **Iontophoresis.** It is generally assumed that the rate of iontophoretic ion transport into the external medium can be described by Faraday's Law (Krnjević et al., 1963a; Krnjević et al., 1963b; Curtis, 1964; Herz et al., 1969; Bradley & Candy, 1970; Hoffer et al., 1971a):

\[ R_i = \frac{in}{zF} \]

where \( R_i \) is the rate of iontophoretic release (Moles/sec),
i is the intensity of the ejecting current (amps), z is the valency of the ejected ion, and F is Faraday's constant (coulombs). It is apparent that there should be a linear relationship between the intensity of the ejecting current and the rate of iontophoretic drug release.

b. Electro-osmosis. Electro-osmotic flow of the drug solution into the external medium may give rise to a significant release of drug molecules (Curtis et al., 1960; Krnjević et al., 1963b). The rate of drug release due to electro-osmotic flow depends, of course, upon the concentration of the drug solution; it increases with the square of the radius of the tip orifice, and decreases with the coefficient of viscosity of the solution (Curtis, 1964). The contribution of electro-osmosis to total electrophoretic release is reckoned to be small (Krnjević et al., 1963b; Curtis, 1964), although it may be important in the case of poorly ionised or less soluble compounds (Curtis, 1964).

In the following pages a model is developed in an attempt to explain the kinetics of drug release from micropipettes. Some predictions generated by this model are examined in the experiments described in section I:2. The model deals only with diffusional and iontophoretic drug release. Hydrodynamic outflow has been neglected since its contribution to spontaneous release is likely to be very small, especially when dilute drug solutions are used as was the case in most of our in vitro experiments (see below, I:2.4.1.). In our experiments electrophoretic release resulting from electro-osmosis could not be differentiated from that resulting from iontophoresis,
and thus, for the sake of simplicity electrophoretic release was regarded as resulting entirely from iontophoresis.

I:1.2. THE MODEL

It is proposed that the orifice at the tip of the micropipette can be regarded as a hypothetical boundary separating two fluid compartments, the drug solution contained within the micropipette and the external medium. Drug release thus can be defined as the passage of drug molecules across this boundary into the external medium.

a. Ion transport in the absence of an electrophoretic current

Let us assume that the drug solution in the micropipette is an infinitely large diffusional source, of uniform concentration $c$, and that the external medium is an infinitely large diffusional sink. Thus, when the tip of a micropipette is immersed in the external medium, a steady-state rate of diffusional release will be established after an initial period of gradually declining rate of release (Fig. 1, I). This initial period reflects the gradual development of an 'interphase layer' between the bulk of the drug solution in the micropipette and the external medium. The establishment of a steady-state rate of release implies the existence of an interphase layer of unchanging thickness. Fick's first law of diffusion is applicable to this condition:

$$ R_D = D \frac{dc}{dx} \pi r^2 $$

where $R_D$ is the rate of diffusional release, $D$ is the
FIG. 1. Hypothetical concentration changes in the tip of a micropipette. Hatched area: bulk of the solution; stippled area: interphase layer; open area: fluid depleted of drug ions. a: boundary of the bulk of the solution; b: lower boundary of the interphase layer; c: tip boundary. \( \delta \): thickness of the interphase layer; \( \delta_c \): critical thickness of the interphase layer. Long arrows indicate the direction of iontophoretic transport of drug ions; short arrows indicate drug release.
I  IN THE ABSENCE OF AN ELECTROPHORETIC CURRENT

(1)  
(2)  

II  DURING THE APPLICATION OF A RETAINING CURRENT

(1)  
(2)  
(3)  

III  DURING THE APPLICATION OF AN EJECTING CURRENT

(1)  
(2)  
(3)  

a  
b  
c
diffusion coefficient, \( \frac{dc}{dx} \) is the concentration gradient across the tip boundary, and \( r \) is the radius of the tip. For the sake of simplicity the concentration gradient may be regarded as being linear across the whole of the interphase layer. This assumption of linearity is also made in the derivation of the Planck-Henderson equation for the diffusional potential across an interphase region. The assumption is necessary, since without it any mathematical treatment of ion transport in the interphase layer becomes unmanageable (Bockris & Reddy, 1970; pp 417-420). On the basis of this approximation, \( R_D \) can be expressed as

\[
R_D = D \frac{c - c_0}{\bar{s}} \pi r^2
\]

(2)

where \( c \) is the concentration of the drug ions in the bulk of the solution, \( c_0 \) is the concentration of drug just outside the tip, and \( \bar{s} \) is the thickness of the interphase layer. Since \( c_0 \ll c \), equation (2) can be simplified:

\[
R_D = \frac{Dc \pi r^2}{\bar{s}}
\]

(3)

b. Ion transport during the application of a retaining current

Ions can be transported in a solution either under the influence of a concentration gradient (diffusion), or under the influence of an electrostatic potential gradient (iontophoresis). When both forces are acting, the net ion flux (rate of ion transport) will reflect the algebraic sum of the two forces (Bockris & Reddy, 1970). When a retaining potential is applied to the drug solution within the micropipette,
these forces act in opposite directions; when an ejecting potential is applied, both forces act in the same direction.

When a retaining potential is applied, ion transport in the bulk of the solution and in the interphase layer will be affected differently. In the bulk of the solution (where there is no concentration gradient) there will be only one ion flux, the iontophoretic flux \( R_i \). In the interphase layer the net ion flux \( R_T \) will be the algebraic sum of the inward iontophoretic flux \( R_i \) and the outward diffusional flux \( R_D \): 
\[
R_T = R_D \pm R_i.
\]
The diffusional ion transport across the tip boundary (diffusional release) in the presence of a retaining current will reflect the relationship between the ion fluxes. If \( R_i \ll R_D \), there will be some diffusional release, if \( R_i \gg R_D \), there will be no outward ion transport across the tip boundary.

If a relatively weak retaining current is applied at time \( t = 0 \) it will not initially counteract diffusional leakage, i.e. \( R_i \ll R_D \) (Fig. 1, II/1). However, an upopposed ionic drift \( R_i \) will start in the bulk of the solution instantaneously. Thus the boundary of the bulk will drift inwards, away from the tip, and the thickness of the interphase layer will increase. Since the rate of diffusional flux in the interphase layer is inversely related to the thickness of the interphase layer (see equation (3)), the rate of diffusional release will gradually decrease, until at time \( t = t_{\text{min}} \), \( R_i = R_D \) (Fig. 1, II/2). From this time on, there will be no diffusional release. Thus a retaining current, which initially is not adequate, can after some time ('minimal effective retention
time', \( t_{\text{min}} \), become effective. If the application of a retaining current is continued for longer than \( t_{\text{min}} \) the ionic drift in the bulk of the solution will further increase the thickness of the interphase layer, and this will further decrease the diffusional flux in the interphase layer \((R_i > R_D)\). Under these circumstances, the lower boundary of the interphase layer will move away from the tip boundary, and the terminal part of the micropipette will become devoid of drug ions (Fig. 1, II/3). (In the terminal region, the drug ions removed from the depleted region will be replaced by other ions of the same polarity drawn in from the external medium.) Three 'concentration layers' can be distinguished at this stage: the bulk of the solution, the interphase layer, and the terminal depleted layer. The thickness of these layers is determined by both parameters (intensity and duration) of the retaining current pulse. The distance travelled by the boundary of the bulk during the retention period depends upon the velocity of the ionic drift in the bulk (determined by the intensity of the retaining current and the transport number of the drug ions) and on the retention time.

The minimal effective retention time can be calculated; let us assume that the terminal part of the micropipette has a cylindrical shape of radius \( r \), and the thickness of the interphase layer is \( \delta_c \) at time \( t = 0 \), and \( \delta_c' \) at \( t = t_{\text{min}} \). At \( t = t_{\text{min}} \) the boundary between the bulk of the solution and the interphase layer will have moved through a distance \( \delta_c' - \delta_o \).
Hence,

\[ t_{\text{min}} = \frac{\delta_l - \delta_0}{v} \quad (4) \]

where \( v \) is the drift velocity in the bulk of the solution.

If, however, the bulk of the solution starts migrating from the tip boundary (as can be the case after the application of an ejecting current, see below p14), \( \delta_0 = 0 \). In this case, equation (4) simplifies to

\[ t_{\text{min}} = \frac{\delta_c}{v} \quad (5) \]

It is possible to derive expressions for both \( \delta_c \) and \( v \).

At \( t = t_{\text{min}} \), \( R_i = R_D \).

Thus,

\[ R_i = \frac{Dc_T r^2}{\delta_c}, \quad \text{or} \quad \delta_c = \frac{Dc_T r^2}{R_i}. \quad (6) \]

Now,

\[ v = \frac{R_i}{c \pi r^2}, \quad \text{(Bockris & Reddy, 1970).} \quad (7) \]

Substituting \( \delta_c \) from equation (6), and \( v \) from equation (7), equation (5) becomes:

\[ t_{\text{min}} = \frac{Dc^2 T r^2 r_4}{R_i^2} \quad (8) \]

From Faraday's Law,

\[ R_i = \frac{i_n}{zF} \quad (9) \]

where \( i \) is the intensity of the iontophoretic current, \( n \) is
the transport number of the drug, \( z \) is the valency, and \( F \) is Faraday's constant.

Substituting \( R_1 \) from equation (9), equation (8) becomes:

\[
t_{\text{min}} = \frac{D c^2 z^2 F^2 n^2 r^4}{i^2 n^2}
\]  

(10)

In the above derivation it was assumed that the transport number of a given ionic species is the same in the interphase layer and in the bulk of the solution. When a (negative) retaining current is applied, positive ions from the external medium (mainly sodium ions) will migrate into the fluid compartment within the micropipette. In the presence of these other ions, the transport number of the drug ions will depend upon the relative concentrations of all ionic species present. Any reduction in the transport number of the drug ions due to the presence of other ions will be greater in the interphase layer than in the bulk of the solution, because of the lower concentration of drug ions within the interphase layer. Thus a more precise version of equation (10) is

\[
t_{\text{min}} = \frac{D c^2 z^2 F^2 n^2 r^4}{i^2 n_1 n_2}
\]

(11)

where \( n_1 \) is the transport number of the drug ions in the bulk of the solution, and \( n_2 \) is the average transport number of the drug ions in the interphase layer. Since \( n_2 < n_1 \), equation (11) yields a greater value for \( t_{\text{min}} \) than equation (10).

In deriving equation (10), it was assumed that \( r \) was
constant in the terminal part of the micropipette. In reality, however, the terminal part usually has a conical shape, and thus r increases gradually with the distance from the tip boundary. This would prolong $t_{\text{min}}$ partly because the drift velocity of the boundary of the bulk will gradually decrease as the boundary migrates away from the tip, and partly because there will be a higher diffusional efflux from the bulk as the surface area of this boundary gradually increases.

$t_{\text{min}}$ is an expression of the efficacy of a retaining current of given intensity. Equation (10) shows that $t_{\text{min}}$ increases linearly with the square of the equivalent concentration \((c,z)\) of the drug solution. $t_{\text{min}}$ also increases linearly with the square of the transport number. As both the diffusion coefficient and the transport number depend upon the absolute mobility of the ions, it is apparent that a given retaining current will be more effective in counteracting the diffusional efflux of more mobile ions. Since $t_{\text{min}}$ increases linearly with the fourth power of the radius, it is obvious that a small increase in the size of the tip orifice will greatly reduce the effectiveness of a given retaining current.

So far, we have only considered retaining currents which are not adequate to counteract diffusional release at the beginning of their application. However, it is apparent from equation (10) that $t_{\text{min}}$ decreases as \(i\) increases. Thus it is predicted that the higher the retaining current the more quickly will it abolish spontaneous release. In the presence of high retaining currents, where $R_{i} \ll R_{D}$, spontaneous
release should be stopped instantaneously (i.e. $t_{\text{min}} = 0$).

As $t_{\text{min}}$ reflects the distance through which the boundary of the bulk of the solution has to migrate before a particular retaining current becomes effective, $t_{\text{min}}$ will be longer after an ejection period (Fig. 1, III/3) than after a period of steady-state diffusional release (Fig. 1, I/2).

c. Ion transport during the application of an ejecting current

When an ejecting potential is applied, drug ions in the bulk of the solution drift at a steady rate towards the tip. The rate of iontophoretic transport across the tip boundary (i.e., rate of iontophoretic release), however, will change in time until the boundary of the bulk arrives at the tip boundary (Fig. 1, III). After the boundary of the bulk has reached the tip boundary, the concentration of drug ions in the pipette tip should remain constant and therefore there will be an undamping rate of release. The rate of release at any time is the sum of the iontophoretic and diffusional fluxes across the tip boundary:

$$R_T = R_i + R_D.$$  

I: 1.3. PREDICTIONS FOR THE SHAPE OF THE RELEASE CURVE

The release curve describes how the rate of release changes in time in response to the application of an ejecting current pulse. The total release curve is obtained by the
summation of the iontophoretic and diffusional curves (Fig. 2).

a. Iontophoretic release curve:

The hypothetical shape of the iontophoretic release curve is shown in Fig. 2/B. When an ejecting potential is applied after a prior retaining current (a), the current flow starts immediately, but drug release will not start until the interphase layer reaches the tip boundary (b). There will be a further delay before the bulk of the solution reaches the tip boundary and thus a steady-state rate of release is established (c). The rate of drug release continues at this steady level until the end of the ejection period. The height of the plateau of the iontophoretic release curve is entirely determined by the intensity of the ejecting current and the transport number of the drug ions (Faraday's Law). When the application of the ejecting current is terminated (d), iontophoretic drug release stops instantaneously.

The gradual rise in the rate of release between b and c reflects a gradual increase in the transport number of drug ions in the interphase layer. (This gradual increase in transport number is due to the gradual increase in concentration of drug molecules in the interphase layer towards the bulk of the solution, since the transport number of any ion depends upon the concentration if several ionic species are present. This is inevitably the case in the tip of a micro-pipette, which freely communicates with the external medium).

Since the shape of the release curve reflects the
FIG. 2. Hypothetical relationship between ejecting current pulse (A), iontophoretic release curve (B), diffusional release curve (C), and total release curve (D). The positions of the concentration layers are indicated at the top of the figure (cf. Fig. 1). a: onset of ejecting current pulse; b: onset of drug release; c: establishment of steady-state rate of release; d: termination of ejecting pulse; e: offset of diffusional release.
CURRENT INTENSITY

RATE OF RELEASE

TOTAL

DIFFUSIONAL

ONTOPHORETIC
position and thickness of the 'concentration layers' in the terminal part of the micropipette, it can be predicted that a higher retaining current or a longer retention time will increase the release latency (a - b) and prolong the rising phase of the release curve (b - c).

b. Diffusional release curve

The hypothetical (simplified) shape of the diffusional release curve is shown in Fig. 2/C. The rate of diffusional release depends upon the concentration gradient across the tip boundary. Thus as the bulk of the solution moves towards the tip boundary during the ejection period (b - c), so diffusional release will gradually increase. When the bulk reaches the tip boundary, a constant rate of diffusional release will be established. When the application of the ejecting current is terminated, diffusional release will not stop instantaneously, but will continue at a gradually decreasing rate, as the thickness of the interphase layer is progressively increased in the presence of the post-ejection retaining current (Fig. 2,e).

I: 1.4. CONCENTRATION CHANGES AT RECEPTOR SITES

a. The diffusional theory

There are basically two stages in the application of drugs by microelectrophoresis: 1. the release of the drug from the micropipette tip, 2. the build-up of a certain concentration of drug molecules close to the receptor sites some distance away from the pipette tip. So far, we have considered only the first of these stages. We now turn to the second
stage.

Castillo & Katz (1955, 1957) and Curtis et al. (1960) have applied the equations of Carslaw & Jaegar (1959) to the diffusional phenomena which give rise to the changes in drug concentration at receptor sites. When very short ejecting pulses are used, the micropipette may be regarded as an 'instantaneous point source' from which a known quantity of drug is released. The rise and fall of the drug concentration \( c(r) \) at a fixed point in the external medium \( d \) cm from the tip orifice is described by the following equation (Castillo & Katz, 1955):

\[
C_r = \frac{Q}{8(\pi D t)^{1.5}} \exp\left(-\frac{r^2}{4Dt}\right) \quad (12)
\]

where \( Q \) is the quantity of drug released in an 'instantaneous' pulse at \( t = 0 \), \( D \) is the diffusion coefficient of the drug, and \( t \) is time after the ejecting pulse. (It should be noted that if retaining currents are applied between pulses, the value of \( Q \) will not be calculable simply in terms of the parameters of the ejecting pulse and the transport number of the drug ions.)

In microelectrophoretic studies in the central nervous system, drugs are not usually applied in very brief pulses, but in longer pulses of many seconds' or minutes' duration. The neuronal response is usually measured during the drug application. Under these circumstances equation (12) is not applicable, since the micropipette can no longer be regarded as an instantaneous point source. However, if the drug is released from the pipette at a constant rate, the tip of the
micropipette may be regarded as a 'continuous point source', and the rise in concentration at a fixed point in the external medium during the period of drug release may be described thus (Curtis et al. 1960):

\[
C_r = \frac{R_i}{D_4 \pi d} \operatorname{erfc} \frac{d}{4 \sqrt{D t}} \quad (13)
\]

where \( R_i \) is the rate of electrophoretic release from the micropipette. If the ejecting pulse is sufficiently long (i.e. \( t \to \infty \)), equation (13) simplifies to

\[
C_r = \frac{R_i}{D_4 \pi d} \quad (13a)
\]

This equation indicates that during the prolonged passage of an ejecting current, a steady-state concentration should eventually be attained at any given distance from the pipette tip. This steady-state concentration would reflect a balanced relationship between arrival of drug molecules at the fixed point in the external medium and diffusion away from the point. Provided that Faraday's Law is applicable to describe the release of drug from the micropipette, we now have a linear relationship between the intensity of the ejecting current and the rate of drug release, and between the rate of drug release and the concentration achieved at a fixed point in the external medium:

\[
i = k_1 R_i = k_2 C_r \quad (14)
\]

c. The 'concentration curve'

The 'concentration curve' describes how the concentration changes in time at a given fixed point in the external medium.
during the application of an ejecting current. Equations (13a) and (14) indicate that under steady-state conditions there is a linear relationship between the rate of drug release and the concentration at the fixed point. However, it is clear from equation (13) that even if drug release started at an equilibrium level, it would take some time before an equilibrium concentration were attained at a distance (d) from the pipette tip. After this time the concentration would remain constant. When drug release is terminated the concentration would decline at a rate which would depend on diffusion.

If the rate of drug release does not rise instantaneously to a plateau level at the beginning of the ejection period (Fig. 3/B), the concentration curve will reflect the distortions in the release curve: it will take a longer time to establish the equilibrium concentration, and the declining phase will be prolonged due to continuing diffusional release after the termination of the ejecting current pulse. These hypothetical changes in the concentration curve are shown in Fig. 3/C.

When the same drug is applied with ejecting currents of successively increasing intensities, the concentration curve is likely to be altered in the following way: the latency should become shorter (reflecting the shortening of the release latency), the plateau higher and the decay time longer (as it would take a longer time for diffusion to reduce the concentration from a higher level to zero).

The shape of the concentration curve will reflect the
FIG. 3. Hypothetical relationship between ejecting current pulse (A), total release curve (B), and concentration curve (C). Horizontal broken line in C indicates level of threshold concentration.
absolute mobility of the drug ions. Let us compare the hypothetical concentration curves for a more mobile drug (A) and a less mobile drug (B), released under identical conditions (the same molarity of the drug solution, the same ejecting current, the same condition of the 'concentration layer' within the micropipette before the start of the ejecting pulse). The release curve of drug A will have a shorter latency, a steeper rise, and a higher plateau, due to the higher transport number of A. All these features will be reflected in the concentration curve of drug A, which in addition will have a faster declining phase, due to the higher diffusion coefficient of A. Thus the 'fast' time course (short latency, steep rise, high plateau, fast decline) of the concentration curve of drug A contrasts with the 'slow' time course of the concentration curve of drug B (longer latency, less steep rise, lower plateau, long decline).

It must be emphasized at this point that this discussion of the concentration curve has been concerned only with the contribution of physical factors. In vivo, the concentration curve may be modified by biological factors such as diffusional barriers and pathways, active transport mechanisms and so on.

I: 1.5. THE 'RESPONSE CURVE'

The dependent variable in most pharmacological studies using the microelectrophoretic technique is the electrical excitability of the cell under investigation. Various measures are available. If intracellular recording is used it is
possible to observe the graded membrane response. However, when extracellular recording is used (as in the present experiments), one can only observe the unitary action potential, and the most commonly used index of cellular excitability is the frequency of action potential production. Central neurones studied in vivo are often spontaneously active due to their afferent synaptic input. Thus the response of a single neurone to the presence of drug molecules can either be an increase (excitation) or a decrease (depression) in its firing rate. The time-course of this change is described by the response curve (firing rate plotted against time, Fig. 4/D). The response curve consists of three main parts: 1. rising phase (including the response latency), 2. plateau, 3. declining phase.

It has been known for a long time that the shape of the response curve is characteristic of the drug applied (Curtis & Crawford, 1969). This has been taken as suggestive that the time-course of responses could yield information about the nature of the drug-receptor interaction and possibly about the physiological role of the drug (e.g. Yamamoto, 1967; Bradley, 1968; Stone, 1972b).

However, the biological significance of the time-course of neuronal responses cannot be evaluated until it is known to what extent physical factors are involved. In terms of parsimony, the assumption made by Cuthbert & Dunment (1970) and others that cells respond virtually instantaneously to the presence of a given concentration of drug molecules at their receptor sites, has considerable advantages. If this
assumption is valid then the shape of the response curve should reflect only the shape of the concentration curve. A hypothetical relationship between the release curve, the concentration curve and the response curve is illustrated in Fig. 4. The proposal put forward here is that the time-course of the response closely follows the concentration curve. The onset of the response (Fig. 4, b) indicates the presence of a threshold concentration of drug molecules which elicits a detectable change in firing rate. As the concentration rises the firing rate gradually increases (in the case of an excitatory response) (Fig. 4, b-c), and the establishment of a steady concentration will be reflected in the attainment of the plateau firing rate (Fig. 4, c). The declining phase of the concentration curve is reflected in the declining phase of the response curve (Fig. 4, d-e), and when the concentration falls to the threshold level the baseline firing rate recovers (Fig. 4, e).

According to this account of the response curve, drugs which raise the threshold concentration (antagonists) would be expected to increase the response latency and reduce the recovery time of response as well as reducing the plateau response. On the other hand, drugs which lower the threshold (protagonists) should shorten the latency and prolong the recovery time of responses as well as raising the plateau response.

The present interpretation of the response curve is based on a consideration of physical factors alone. It is not my intention to deny the involvement of biological factors;
FIG. 4. Hypothetical relationship between ejecting current pulse (A), total release curve (B), concentration curve (C), (as in Fig. 3), and response curve for an excitatory response (D). Horizontal broken line in C indicates level of threshold concentration; arrows indicate the direction of the shift of the threshold in the presence of an antagonist (upward) or a protagonist (downward). Small letters indicate response parameters: a, onset of ejecting pulse; b, onset of response; c, establishment of plateau firing rate; d, termination of ejecting pulse; e, offset of response; f, baseline firing rate; f2, plateau firing rate.
however it would seem to be unparsimonious to involve unknown biological mechanisms if the response curve can be satisfactorily explained in terms of physical variables. Some experiments which are relevant to this physical interpretation of the kinetics of neuronal responses are described in I: 3.3.1.
1:2. THE RELEASE OF DRUGS FROM MICROPIPETTES

1:2.1. INTRODUCTION

In view of the number and complexity of the processes contributing to drug release, it is essential that drug release is examined experimentally. Such experiments, conducted in vitro, may yield information about the relationship between the electrophoretic currents applied in in vivo experiments and the kinetics of drug release. This information is obviously of importance if quantitative pharmacological studies are to be conducted using the microelectrophoretic technique.

There are a number of papers in the literature in which the transport numbers of various drugs released from micropipettes have been reported. Earlier investigations used biological (Krnjević et al. 1963b) and fluorimetric (Krnjević et al. 1963a) techniques to assay the released drugs. More recent studies, however, have used the liquid scintillation technique (Bradley & Candy, 1970; Hoffer et al. 1971a) for the assay of $^3$H-labelled drugs. The latter technique is more sensitive, but less specific than the fluorimetric method. For example, Offerman & Merrills (1968) found that in some samples of commercially obtained noradrenaline-$^3$H up to 50% of the total radioactivity was not carried by authentic noradrenaline. In confirmation of this finding, E. S. Johnson and E. Szabadi (unpublished observations) observed that considerable amounts of radioactivity were released from an $^3$H-noradrenaline solution when high retaining currents were passed; subsequent chromatographic analysis showed that this radioactivity was not
associated with noradrenaline. In the present study we attempted to overcome this difficulty by using a C^{14}-labelled drug, since C^{14}-labelled compounds have a much greater radiochemical stability than H^{3}-labelled compounds.

In these experiments we used noradrenaline (NA) as a model drug for the study of drug release. This drug has been used extensively in our in vivo experiments (see Part II) and unlike mescaline, was readily available as a C^{14}-labelled compound. The release of NA from micropipettes has been investigated by three other groups of workers. Krnjević et al., (1963a) using a fluorimetric assay technique, obtained values for the transport number of NA of 0.34 and 0.37. Lower values have been obtained in more recent studies using H^{3}-NA. Bradley & Candy (1970) found the transport number of NA to be 0.09 or 0.19 depending on the strength of the drug solution, and Hoffer et al., (1971a) obtained values between 0.05 and 0.30.

Studies of the kinetics of drug release seemed to be especially relevant in the present study. Accordingly, in the following experiments considerable emphasis has been placed on measurements of the time-course of drug release under various experimental conditions.

### 2.2. METHODS

Five barrelled micropipettes were constructed from Pyrex glass tubing of external diameter 1.5 mm and internal diameter 1.0 mm (Herz et al., 1965). Air was removed from the barrels by boiling the pipettes in glass distilled water
under reduced pressure at 65°C. The water was then removed from each of the barrels down to the level of the shank, and was replaced by the appropriate solution using a thin Portex catheter. After filling, the pipettes were stored in the dark at 4°C in nitrogen atmosphere for at least 36 hours before use. Immediately before use, the tip of the micropipette was broken in order to obtain an over-all diameter of 4.0 - 6.5 μ.

One barrel of the micropipette was always filled with 3 M NaCl solution. After the tip had been broken, the resistance of this barrel, measured in 0.165 M NaCl solution, was between 2 and 5 megohms. The remaining four barrels of each micropipette were filled with radioactive noradrenaline (NA) solution (0.02 M, specific activity 5 mCi/m mole, or 0.2 M, specific activity 0.5 mCi/m mole).

Because of its greater radiochemical stability, we decided to use C\textsuperscript{14}-NA, rather than the H\textsuperscript{3}-labelled compound, in our experiments. The lower specific activity of the C\textsuperscript{14}-NA, however, made it necessary to dilute the radioactivity as little as possible when preparing the final solution. Accordingly in most of these experiments we used a 0.02 M solution, rather than the 0.2 M solution which we used in the in vivo experiments (I:3, II:3). In order to increase the resolution of the method when very small outputs were measured, as in the presence of retaining currents, four barrels of each micropipette were filled with the radioactive NA solution, and the electrophoretic currents were applied to all of them simultaneously. Errors were further reduced by the use of
long sampling periods. (4-126 min).

Freeze-dried D,L-noradrenaline (carbinol C\(^{14}\))
D,L-bitartrate (specific activity 22 and 54 mCi/m mole)
was obtained from the Radiochemical Centre, Amersham,
bitartrate was prepared by mixing the required amount of
radioactive and non-radioactive NA to give the final solution
to be used in the micropipettes. The pH of this solution
was 3.0 - 3.5.

An additional series of experiments was conducted
using 0.2 M NA. In these experiments it was intended to
examine the effect of the addition of other ionic species
into the drug solution. Six-barrelled pipettes were used.
Three of the barrels contained D,L-noradrenaline (carbinol-
C\(^{14}\))D,L-bitartrate (0.2 M, pH 3.5, specific activity 0.5 mCi/m
mole). Since one of the purposes of these experiments was
to determine whether adjustment of the pH of the drug solution
had an appreciable effect on drug release, the other three
barrels contained a similar solution whose pH had been
adjusted to 5.0 by the addition of 1 N NaOH. The eventual
concentration of Na\(^{+}\) in the NA solution at pH 5.0 was 0.1 M.

The microelectrophoretic circuit used in these exper-
iments is described by Roberts & Straughan (1967) (see also
I:3.2). Changes in current flow associated with changes in
the resistance of the drug barrels were minimised by the use
of a 1,000 megohm source resistor as the final output of the
circuit. Occasionally, however, current 'blocking' did occur.
If the degree of blocking was small (less than 5%), it was compensated for by increasing the potential applied to the drug barrel. If the degree of blocking exceeded 5%, the micropipette was rejected. The electrophoretic current was continuously monitored using a Pye Scalamp Galvanometer.

The micropipette was held vertically in a micromanipulator and the tip was lowered into a small glass vial containing 1.0 ml 0.165 M NaCl solution. A silver wire, immersed into the saline solution, served as an earth electrode. Electrophoretic currents of identical intensities were applied to each of the NA-containing barrels. (Throughout this section (I:2) values of current intensity will always refer to the intensity of the electrophoretic current applied to each of the NA-containing barrels). The outer surface of the micropipette was washed with distilled water before the collection of each sample.

At the end of each period of collection, the contents of the vial were transferred into a glass scintillation vial. The collecting vial was washed repeatedly with the scintillator and the washings were added to the contents of the scintillation vial. A total of 10 ml of scintillator was added to each sample. The scintillator contained 0.267% P.P.O. (2,5-diphenyloxazole), 0.0067% P.O.P.O.P. (1,2-bis-(5-phenyloxazol-2-yl)-benzene) in toluene with 33u Triton X-100.

Disintegration rate for each sample was counted for 10 min in a Packard Tricarb liquid scintillation spectrometer. Radioactive counts for each sample were corrected by the subtraction of background activity. Counting efficiency was
determined by the channels ratio method. D.p.m. (disintegrations per minute) values were determined for each sample, and these were converted into p mole values on the basis of the specific activity of the NA solution contained in the micropipettes. Output per barrel was calculated from the total output from the micropipette measured in each collection period. The background count rate was approximately equivalent to 2.5 - 3.0 p mole NA in our experiments.

I:2.3. RESULTS

Drug release was investigated systematically using five-barreled pipettes, four barrels of which contained NA (0.02 M, specific activity 5 mCi/m mole). This work is described in I:2.3.1., I:2.3.2. and I:2.3.3. Sections I:2.3.4. and I:2.3.5. are concerned with the effect of concentration and the effect of other ionic species present in the drug solution. A more concentrated solution was used in these latter experiments (0.2 M), but since this entailed a ten-fold reduction in the specific activity (0.5 mCi/m mole) very small outputs could not be measured reliably in these experiments.

I:2.3.1. Release in the absence of an electrophoretic current

a. Release before any current had been applied

In fourteen micropipettes (nos. 1-14, see Table 1) the spontaneous release of C\textsuperscript{14}-NA was measured before any electrophoretic current had been applied to the micropipette. Before the first sample was collected, the tip of the micro-
TABLE 1. Rate of steady-state spontaneous release and transport number of NA obtained from each of the micropipettes (1-14).

Transport numbers were calculated individually for each of the samples collected from each micropipette. Four barrels of each micropipette contained C\textsuperscript{14}-NA (0.02M, specific activity 5 Ci/m mole).

<table>
<thead>
<tr>
<th>Micro-pipette number</th>
<th>Tip diameter ((\mu))</th>
<th>Rate of spontaneous release (p mole/barrel/min)</th>
<th>Transport number (mean ± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>0.09</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0.34</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0.35</td>
<td>0.03 ± 0.00</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0.12</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0.16</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>0.22</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>0.75</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>0.32</td>
<td>0.29 ± 0.06</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>0.04</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>10</td>
<td>6.5</td>
<td>0.82</td>
<td>0.17 ± 0.00</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>0.20</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>0.48</td>
<td>0.33 ± 0.05</td>
</tr>
<tr>
<td>13</td>
<td>5.5</td>
<td>0.64</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>0.51</td>
<td>not measured</td>
</tr>
</tbody>
</table>

The mean value of the transport number obtained from 13 micropipettes was 0.17.
pipette was broken in order to obtain the desired tip diameter (4.0 - 6.5 μ). Then, samples were collected during successively increasing periods of time (8-128 min). With the majority of the micropipettes (9 out of 14) the rate of spontaneous release fell from an initially high level, until a steady-state rate of release was attained 8-64 min after the start of the experiment (Fig. 5). The steady-state rate of release is reflected in a linear relationship between the output of NA and time (Fig. 6).

The mean rate of spontaneous release from four NA-containing barrels, measured under steady-state conditions, was 0.38 p mole/barrel/min. There was a considerable variation between micropipettes, however (see Table 1); the highest rate of release was recorded from the pipette with the greatest tip diameter.

b. Release after a retaining pulse

The effect of a prior application of a retaining current upon spontaneous release was investigated using two micropipettes (nos. 10 and 14). The retaining currents tested in these experiments were -5, -10, -15 and -20 nA. Following a 16 min application of a retaining current, the spontaneous output of NA during the subsequent 16 min period was less than the spontaneous output measured under steady-state conditions. This effect was greater after higher retaining currents. In the next 16 min period, however, the original level of spontaneous release recovered. An example of this observation is illustrated in Fig. 7.
FIG. 5. The rate of spontaneous release of NA from two micropipettes. Ordinates: rate of release of NA (p mole/barrel/min). Abscissae: running time (min). The times at which samples were collected are indicated by arrows below the time base.

The rate of spontaneous release, measured before any current had been applied to the pipette, declined from an initially high level, until a steady-state rate was attained. A: micropipette no. 9; B: micropipette no. 4, (see Table 1).

FIG. 6. The relationship between the spontaneous release of NA and time, for one micropipette. Micropipette no. 4 (see Table 1). Ordinate: amount of NA released (p mole/barrel). Abscissa: time (min). The spontaneous release of NA was linearly related to time. See also Fig. 5.
RATE OF RELEASE OF NA

AMOUNT OF NA RELEASED
FIG. 7. The effect of the application of a retaining current upon the subsequent spontaneous release of NA from one micropipette. Micropipette no. 10 (see Table 1). Lower graph: electrophoretic current applied to each of the four NA-containing barrels (ordinate: current intensity, nA, negative downwards; abscissa: running time, min). Stippled area: retaining current. Upper graph: output of NA during sequential 16 min periods of sample collection (ordinate: output of NA, p mole/barrel; abscissa: running time, the same scale as in the lower graph).

The application of a retaining current of -15 nA was accompanied by a marked reduction in the spontaneous release of NA. The spontaneous release was still reduced during a subsequent 16 min period when no current was applied. During a further 16 min period the spontaneous release recovered to its original level.
c. Release after an ejecting pulse

The effect of a prior application of an ejecting current upon spontaneous release was investigated using one micropipette (no. 12). Following a period of steady-state electrophoretic release (+50 nA) the application of the ejecting current was terminated, and the rate of spontaneous release was measured during successive 5 min periods. The results obtained from this micropipette are shown in Fig. 8. Following the passage of ejecting current, the rate of spontaneous release declined during successive sample collection periods, until a steady-state rate of release was attained.

I:2,3.2. Release in the presence of a retaining current

a. When the application of a retaining current is not preceded by an ejecting pulse

The effectiveness of various retaining currents in counteracting spontaneous release was examined using two micropipettes (nos. 10 and 14). The spontaneous output of NA during a 16 min period during which no current was applied was compared with the output measured during a 16 min period when a retaining current was applied. The retaining currents used were -5, -10, -15, -20 and -25 nA. Each application of a retaining current was preceded by 32 min during which no current was applied. In the case of both micropipettes a retaining current of -5 nA reduced the output to about 25% of the spontaneous release measured during an identical time period when no current was applied. A further reduction in the spontaneous release was observed when higher retaining currents were used. When a retaining current of -25 nA was
FIG. 8. Spontaneous release following the application of an ejecting current. Micropipette no. 12 (see Table 1). Lower graph: electrophoretic current applied to each of the four Na-containing barrels (ordinate: current intensity, nA, positive upwards; abscissa, running time, min). Hatched area: ejecting current. Upper graph: output of Na during sequential 5 min periods of sample collection (ordinate: output of Na, p mole/barrel/5 min); abscissa: running time, the same scale as in the lower graph).

Following the application of an ejecting current, the rate of spontaneous release declined until a steady-state rate of release was attained.
applied, no spontaneous release could be detected. The results obtained from micropipette no 10 are displayed in Fig. 9.

b. When the application of a retaining current is preceded by an ejecting pulse

The efficacy of various retaining currents in countering spontaneous release immediately after the application of an ejecting current was determined using three micropipettes (nos. 8, 9 and 10).

Under these circumstances a higher output was measured during the application of the retaining current than was the case when the application of the same retaining current was not preceded by an ejecting pulse (see previous section). The results obtained from micropipette no. 10 are shown in Fig. 9. Similar observations were made using micropipettes no. 8 and 9, with which retaining currents of up to -100 nA were tested. The output decreased with higher retaining currents.

The time-course of the action of retaining currents was measured by applying a retaining current of given intensity continuously and measuring the output at regular intervals of 5 min. Every retaining current tested (-5 to -100 nA) invariably became effective if it was applied for a long enough time. This was true even of weak retaining currents which failed to abolish spontaneous release during the first collection period (Fig. 12/c).
FIG. 9. The relationship between the intensity of the retaining current and the release of NA from one micropipette. Micropipette no. 10 (see Table 1). Ordinate: amount of NA released during each 16 min period (p mole/barrel). Abscissa: intensity of retaining current applied to each of the four NA-containing barrels (nA). Closed circle: release in the presence of a retaining current following a period (32 min) when no current was applied. Closed triangle: release in the presence of a retaining current following an ejecting pulse (+100 nA, 16 min). The release of NA declined as higher retaining currents were applied. A retaining current was less effective following an ejecting pulse.
I: 2.3.3. Release during the application of an ejecting current

a. When the application of an ejecting current is not preceded by a retaining pulse

Thirteen micropipettes (nos. 1-13) were used in these experiments. The electrophoretic release of C\textsuperscript{14}-NA was measured using a wide range of ejecting current intensities (+25 - +200 nA) and a wide range of collection times (4-128 min). Since the prior application of a retaining current can influence subsequent drug release (see below I: 2.3.3.b.), it was important to ensure that measurements of the rate of electrophoretic release were not subject to this distortion. Therefore retention periods or periods of spontaneous release were not interspersed between ejection periods.

In all the micropipettes tested (nos. 1-13) the rate of release of NA was linearly related to the intensity of the ejecting current. The results obtained from micropipettes nos. 2, 7 and 10 are shown in Fig. 10.

The transport number of NA was calculated individually for each sample collected from each micropipette, by substitution in the following equation: \( n = \frac{R_i z F}{i} \), where \( n \) is the transport number, \( z \) is the valency (in the case of NA, \( z = 1 \)), \( F \) is Faraday's constant (coulombs), \( i \) (amps) is the total ejecting current applied to all four barrels, and \( R_i \) (moles/sec) is the rate of electrophoretic release of NA from all four barrels (total rate of release minus steady-state rate of spontaneous release). Mean values of the transport numbers
FIG. 10. The relationship between the intensity of the ejecting current and the rate of release of NA from three micropipettes. Ordinates: rate of release of NA (p mole/barrel/min). Abscissae: intensity of ejecting currents applied to each of the four NA-containing barrels (nA).

The rate of release was linearly related to current intensity over a wide range of ejecting current intensities (12.5 – 200 nA).

A: micropipette no. 10 (see Table 1); calculated regression line: \[ y = 1.623 + 0.414 x \]

B: micropipette no. 7; calculated regression line: \[ y = 3.690 + 0.308 x \]

C: micropipette no. 2; calculated regression line: \[ y = 0.832 + 0.331 x \]
RATE OF RELEASE OF NA

A

\[
\begin{align*}
\text{Current (nA)} & \quad 0 & 25 & 50 & 100 \\
\text{p.mole/barrel/min} & \quad 0 & 5 & 10 & 15
\end{align*}
\]

B

\[
\begin{align*}
\text{Current (nA)} & \quad 0 & 25 & 50 & 100 \\
\text{p.mole/barrel/min} & \quad 0 & 5 & 10 & 15
\end{align*}
\]

C

\[
\begin{align*}
\text{Current (nA)} & \quad 0 & 25 & 50 & 100 & 200 \\
\text{p.mole/barrel/min} & \quad 0 & 5 & 10 & 15
\end{align*}
\]
obtained from each of the micropipettes are shown in Table 1; the average value for the 13 micropipettes was 0.17. It can be seen that, apart from three micropipettes (nos. 3, 8 and 12), the transport number of NA measured in each individual micropipette was between 0.10 and 0.25. Application of the product moment correlation test revealed no significant correlation between the value of the transport number obtained in an individual pipette and the tip diameter or the steady-state rate of spontaneous release.

b. When the application of an ejecting current is preceded by a retaining pulse

In microelectrophoresis experiments the application of an ejecting current is normally preceded by the application of a retaining current (pre-ejection retaining current). We therefore examined the release of NA during ejection periods following retaining pulses of various intensities and durations using five micropipettes (nos. 8, 9, 12, 13 and 14).

When standard ejecting pulses were used, an increase in the intensity of the retaining current reduced the amount of NA released during the subsequent ejecting pulses. The results obtained from micropipette no. 8 are shown in Fig. 11. Following the prolonged application of the retaining current of -25 nA (B), the first application of the standard ejecting current gave rise to a small output of Na. The output of Na measured during the standard ejection period increased with successive ejecting pulses, even though the intensity of the retaining current applied between ejecting pulses was kept constant (-25 nA).
The effect of various retaining currents upon the electrophoretic release of $\text{Na}^+$ from one micropipette. Micropipette no. 8 (see Table 1).

Upper graph: release of $\text{Na}^+$, ordinate: current intensity, $\text{Na}^+$, positive upwards; abscissa: running time, min.

Lower graph: electrophoretic current applied to each of the four $\text{Na}$-containing barrels (ordinate: current intensity, $\text{Na}^+$, positive upwards; abscissa: running time, min). Stippled area: retaining current; hatched area: ejecting current; parameters of the applied currents were kept constant throughout the study. Changes in the parameters (intensity and duration) of the ejecting pulses are indicated by capital letters under the time base.

Increases in the intensity and duration of the ejecting current (A - B, C - D, E - F) evoked progressively greater outputs. Restoration of the original retaining pulse (B - C) evoked progressively greater outputs. After a prolonged application of a retaining current (A - B), successive ejecting pulses (B - C) and retaining current (E) were applied alternately; the same scale as in the lower graph was used for the scale of the ordinate.

Increases in the intensity of the retaining current reduced the amount of $\text{Na}^+$ released by the ejecting pulse (C - D, D - E). Restoration of the retaining pulse (E) was followed by progressively increasing outputs. Increases in the intensity and duration of the ejecting pulse (A - B, C - D, E - F) evoked progressively greater outputs. After a prolonged application of a retaining current (A - B), successive ejecting pulses (B - C) and retaining current (E) were applied alternately; the same scale as in the lower graph was used for the scale of the ordinate.

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Increases in the intensity of the retaining current reduced the amount of $\text{Na}^+$ released by the ejecting pulse (C - D, D - E). Restoration of the original retaining pulse (B - C) evoked progressively greater outputs. After a prolonged application of a retaining current (A - B), successive ejecting pulses (B - C) and retaining current (E) were applied alternately; the same scale as in the lower graph was used for the scale of the ordinate.

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Increases in the intensity of the retaining current reduced the amount of $\text{Na}^+$ released by the ejecting pulse (C - D, D - E). Restoration of the original retaining pulse (B - C) evoked progressively greater outputs. After a prolonged application of a retaining current (A - B), successive ejecting pulses (B - C) and retaining current (E) were applied alternately; the same scale as in the lower graph was used for the scale of the ordinate.
When the intensity of the retaining current was increased to -50 nA (C), the amount of NA released during the standard ejection period was markedly reduced. When the intensity of the retaining current was further increased to -100 nA, there was virtually no drug release during the ejection periods. Finally, when the retaining current was reduced to -25 nA again (E), the amount of NA released increased during successive ejection periods.

We then examined whether the reduction in output brought about by a prior retaining current was due to a change in the time-course of drug release. In these experiments the ejecting current was applied for a prolonged period and samples were collected at regular intervals of 5 min (Fig. 12). When no retaining current had been applied previously, the rate of release of NA rose to its plateau level during the first collection period, in other words the 'release curve' (rate of release plotted against time) was rectangular in shape. The effect of the pre-ejection retaining current was to prolong the rising phase of the release curve without affecting the plateau. Increases in either the intensity (Fig. 12/B: micropipette no. 11) or the duration (Fig. 12/A: micropipette no. 12) of the retaining current magnified this distortion of the release curve. Even weak retaining currents when applied for prolonged periods could distort the rising phase of the release curve. In the experiment shown in Fig. 12/C (micropipette no. 12) a retaining current of -5 nA was not effective for the initial 20 min of its application, but after it had been applied for a further 60 min it caused a marked distortion.
FIG. 12. The effect of retaining currents on the kinetics of electrophoretic release of NA. Lower graph: electrophoretic currents applied (see Fig. 14). Upper graph: release curves for NA (ordinate: rate of release of NA p mole/barrel/5 min; abscissa: running time, min). A: micropipette no. 11 (see Table 1): increases in the retention time prolonged the rising phase of the release curve. B: micropipette no. 13: increases in the intensity of the retaining current prolonged the rising phase of the release curve. C: micropipette no. 12: a weak retaining current (-5 nA) did not abolish spontaneous release immediately, but prolonged the rising phase of a subsequent release curve.
RATE OF RELEASE OF NA

A

B

C

PMOLE/BARREL/5MIN

0

100

200

300

min

nA

0

50

25

0

+50

-50

0

100

200

min

nA

0

50

25

0

+50

-50

0

100

min

nA

0

50

25

0

+50

-50

0

100 min
of the release curve during the next ejection period.

I:2.3.4. Effect of concentration

Since in our in vivo experiments 0.2 M NA solutions were used, it was of interest to study NA release from pipettes containing this more concentrated solution. Five six-barrelled pipettes were used in these experiments (nos. 15 - 19, see Table 2). Three barrels of the pipettes contained C\textsuperscript{14}-NA (0.2 M pH 3.5, specific activity 0.5 mCi/m mole). The remaining three barrels contained C\textsuperscript{14}-NA (0.2 M, pH 5.0, specific activity 0.5 mCi/m mole). These latter three barrels were used for evaluating the effects of the addition of other ions (I:2.3.5.). When the release from barrels containing one solution was being studied, spontaneous release from the other three barrels was either taken into account as a constant factor to be subtracted from the total release measured, or was eliminated by the passage of a high retaining current (-300 nA) which had proved instantaneously effective. 10 min sample collection periods were used.

a. Spontaneous release

There was a considerable variation between micropipettes with respect to the rate of spontaneous release (see Table 2) The mean rate of release of NA from the solution at pH 3.5 (i.e. when no other ions had been added) was 8.8 p mole/barrel/min. Micropipette no. 15, which had a tip diameter of 8 \(\mu\) showed a very high rate of release (24.1 p mole/barrel/min). If this micropipette is excluded the mean rate of spontaneous
Transport numbers were calculated individually for each of the samples collected from each micropipette. Three barrels of each micropipette contained C\textsubscript{14}-NA (0.2 M, pH 3.5, specific activity 0.5 mCi/m mole); the other three barrels contained C\textsubscript{14}-NA (0.2 M, pH adjusted to 5.0 with NaOH, specific activity 0.5 mCi/m mole).

<table>
<thead>
<tr>
<th>MICROPIPETTE NUMBER</th>
<th>TIP DIAMETER (µ)</th>
<th>ELECTRICAL RESISTANCE OF EACH DRUG BARREL (M~)</th>
<th>RATE OF SPONTANEOUS RELEASE (pmole/barrel/min; mean ± S.E.)</th>
<th>TRANSPORT NUMBER (mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NA (pH 3.5)</td>
<td>NA (pH 5.0)</td>
<td>NA (pH 3.5)</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>16</td>
<td>4</td>
<td>75</td>
<td>75</td>
<td>100</td>
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<tr>
<td>17</td>
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<td>19</td>
<td>5</td>
<td>85</td>
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<td>85</td>
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</tbody>
</table>

The mean values of the transport number obtained from 5 micropipettes were 0.29 (pH 3.5 solution) and 0.11 (pH 5.0 solution).
release becomes 4.9 p mole/barrel/min. This value is somewhat greater than 10 times the mean rate of spontaneous release from the 0.02 M solution (0.36 p mole/barrel/min, see I:2,3,1.).

There was a significant correlation between the tip diameter and the rate of spontaneous release (product moment correlation test: \( r = 0.995, p < 0.05 \)).

b. Release in the presence of a retaining current

The efficacy of retaining currents was evaluated using two micropipettes (15 and 19). First an ejecting current (+100 nA) was applied and the output during a 10 min retention period was measured. The release curve for +100 nA was then repeated, and the retaining current of -25 nA was applied again, this time for 20 min. The output during the 10 min retention period was subtracted from the output during the 20 min retention period in order to obtain an estimate of the output during the final 10 min of the 20 min retention period. This procedure was repeated, using successively increasing retention times up to 60 min.

It was found that the output of NA decreased in time during the prolonged application of a retaining current. The results obtained from micropipette no. 19 are illustrated in Fig. 13. Similar results were obtained with micropipette no. 17.

During the first 30 min retention with -25 nA the amount of NA released from the 0.2 M solution was 72% (micropipette no. 17) and 44% (micropipette no. 19) of the amount released during an equivalent period when no current was applied. Comparison of Fig. 13 with Fig. 12 shows that this retaining current was
FIG. 13. The effect of a retaining current on the rate of spontaneous release. Micropipette no. 17 (see Table 2).

Lower graph: electrophoretic current applied to each of 3 barrels containing NA (0.2 M, pH 3.5) (ordinate: current intensity, nA, positive upwards; abscissa: running time, min). Hatched area: ejecting current; stippled area: retaining current. Upper graph: output of NA during sequential 10 min periods of sample collection (ordinate: output of NA, p mole/barrel/10 min); abscissa: running time, the same scale as in the lower graph). Broken line indicates the steady-state rate of release.

The rate of release gradually declined during the application of the retaining current.
NA RELEASED
(p mole/barrel/10 min)

CURRENT
(nA)

[Graph showing the release and current over time]
much less effective in counteracting spontaneous release from the 0.2 M solution than were similar retaining currents in the case of the 0.02 M solution. With the 0.2 M solution, the minimal effective retention time for -25 nA was more than 50 min (micropipette no. 19, see Fig. 13), whereas a much weaker retaining current (-5 nA) totally abolished spontaneous release from the 0.02 M solution within 20 min of its application (micropipette no. 12, see Fig. 12/c).

c. Release in the presence of an ejecting current

Electrophoretic release was measured using a range of ejecting currents (+12.5 to +200 nA). The rate of electrophoretic release was linearly related to the intensity of the ejecting current (see Fig. 14, filled circles).

The transport numbers calculated for each micropipette are shown in Table 2. The mean transport number of Na obtained from all five micropipettes was 0.29. This value is significantly higher than the mean transport number of Na obtained using the 0.02 M solution (0.17) (t test; p < 0.05).

I:2,3,5. Effect of the addition of other ions.

The effect of the addition of other ions upon the release of Na from an 0.2 M solution was investigated by comparing the output from a solution prepared by dissolving Na bitartrate in double glass-distilled water (the pH of this solution was 3.5) with the output from a similar solution to which NaOH had been added in order to raise the pH to 5.0. The concentration of Na⁺ in the latter solution was 0.1 M.
FIG. 14. Rate of release of NA at different pH values.

Micropipette no. 17 (see Table 2). Ordinate: rate of release of NA (p mole/barrel/min); abscissa: intensity of ejecting current applied to each of three NA-containing barrels. Closed circles: release from barrels containing NA at pH 3.5; open circles: release from barrels containing NA at pH 5.0. Each point is the mean of four measurements (standard error is indicated except when it was less than ± 0.25 p mole/barrel/min. Both lines were obtained by linear regression. The rate of spontaneous release is indicated by the broken line.

The total rate of release of NA is greater from the solution at pH 3.5 than from the solution at pH 5.0.
RATE OF RELEASE OF NA

![Graph showing the rate of release of NA vs. current (nA). The graph has a y-axis labeled "p mole/barrel/min" ranging from 0 to 30 and an x-axis labeled "CURRENT (nA)" ranging from 0 to 200. The graph includes three lines with data points, indicating a linear relationship between the rate of release and current.]
NaOH was used in these experiments since it had been suggested that in *in vivo* experiments, the electrophoretic application of NA is accompanied by artefactual excitatory neuronal responses due to the release of H⁺, and that in order to eliminate these responses it is necessary to raise the pH of the NA solution by the addition of NaOH (Frederickson et al., 1971; see II:3.1.2).

Some of the characteristics of the five micropipettes used are summarized in Table 2. The electrical resistance was lower in the case of barrels containing NA at pH 5.0 (Wilcoxon test: p < 0.005).

a. **Spontaneous release**

There was no significant difference between the rate of spontaneous release of NA from the solution at pH 3.5 and from the solution at pH 5.0.

b. **Release in the presence of a retaining current**

The efficacy of retaining currents was studied in detail using only two micropipettes. The output of NA from the pH 5.0 solution was 2.6 times (micropipette no. 17) and 2.5 times (micropipette no. 19) greater than the output from the pH 3.5 solution during the application of -25 nA for a standard period (40 or 60 min). Thus the retaining current was less effective with the pH 5.0 solution.

c. **Release in the presence of an ejecting current**

Electrophoretic release was measured using a range of ejecting currents (+12.5 to +200 nA). With all the five micropipettes tested, any given ejecting current was less
effective in releasing NA from the solution at pH 5.0 than from the solution at pH 3.5. The relationship between current intensity and rate of electrophoretic release for one micropipette is shown in Fig. 14. The transport number of NA was calculated individually for each sample collected from each micropipette. The mean values for the transport number obtained from each micropipette are shown in Table 2. In every case the transport number of NA was lower when release was measured from the pH 5.0 solution. The overall mean values from the five micropipettes were 0.29 (pH 3.5 solution) and 0.11 (pH 5.0 solution); the difference between these two values is statistically significant (t test: p < 0.01).

II:2.4. DISCUSSION

The release of a drug from a micropipette consists of spontaneous release (resulting from diffusion and hydrodynamic outflow of the solution), and electrophoretic release (resulting from iontophoresis and electro-osmosis (Curtis, 1964). In the following discussion we will adopt the assumption made previously in the derivation of the model (II:1.2.) that spontaneous release results virtually entirely from diffusion and that electrophoretic release results virtually entirely from iontophoresis.

Spontaneous release due to hydrodynamic outflow depends upon the size of the tip orifice and the concentration of the drug solution within the micropipette (Krnjević et al., 1963b). When a micropipette of small tip diameter is filled with a drug solution of low molarity, the contribution of hydrodynamic
outflow is likely to be very small (Curtis, 1964). The rate of release due to hydrodynamic outflow can be estimated (Krnjević et al., 1963b): if we assume that one of the Na-containing barrels of our micropipettes has an internal tip diameter of 1 μ, that the hydrostatic driving force results from the weight of a water column 5 cm in height, and that the viscosity of our Na solution is similar to that of a 3 M acetylcholine solution, the rate of the outflow of the solution from one barrel will be about $2 \times 10^{-10}$ ml/min. (This estimation, however, involves two major sources of error. Firstly, the viscosity of our dilute Na solution is probably lower than that of a 3 M acetylcholine solution; this factor would tend to increase the outflow due to hydrostatic pressure in our experiments. Secondly, this calculation does not take into account the opposing force of capillarity, which would tend to reduce the outflow.) This estimated outflow of the solution would release Na from the 0.02 M and 0.2 M solutions at the rate of 0.004 and 0.04 p mole/min respectively. This release would account for less than 2% of the total rate of spontaneous release. Thus, the contribution of hydrodynamic outflow to spontaneous release will be ignored in the following discussion.

The release resulting from the application of the ejecting current is mostly iontophoretic release and, to a much smaller extent, electro-osmotic release. This latter has been estimated to be about 11% of the total drug release from a 3 M acetylcholine solution, (Krnjević et al., 1963b). In our experiments much weaker solutions were used, but it was not possible to differentiate between iontophoretic and electro-osmotic release. For this reason electrophoretic release is
regarded as resulting entirely from iontophoresis.

In the first part of the discussion (I:2.4.1.) the present results are interpreted in terms of the theoretical model of ion movements (see I:1.2.) and in terms of the theoretical 'release curve' (see I:1.3.). In the second part of the discussion (I:2.4.2.), some practical implications of the results are considered.

I:2.4.1. Interpretation of the results on the basis of the model.

It is apparent that the model offers a number of predictions about the shape of the release curve and how it is determined by the parameters of the ejecting and retaining current. The main obstacle in the path of quantification of these predictions is the discrepancy between the cylindrical shape of the hypothetical micropipette and the conical and highly variable shape of real micropipettes. For given values of the ejecting and retaining currents and concentration of the drug solution, the effect of this factor will be to increase enormously the time scale on which the processes of ejection and retention take place. In the present discussion no attempt has been made to relate theoretical and empirical release curves in a quantitative fashion; rather, the results are discussed in relation to the general concepts of the model.

A. Release in the absence of an electrophoretic current

The model predicts that the rate of diffusional release from a micropipette is linearly proportional to the concentration
of the drug solution, the diffusion coefficient of the drug ions, and the surface area of the tip orifice, and is inversely related to the thickness of the interphase layer (cf. equation (3)).

In our experiments, the tip of the micropipette was broken (i.e. the surface area of the tip orifice was increased) immediately before spontaneous release was measured. The increase in the size of the tip orifice would upset the steady-state relationship which presumably existed before the tip was broken. Thus, it would take some time, before an interphase layer of unchanging thickness could be re-established at a different level (Fig. 1/I). This could explain the finding, with most of the micropipettes, that after the tip of the pipette had been broken the rate of release gradually decreased until a steady-state rate of release was established (Fig. 5).

Diffusional leakage from micropipettes can be significant. Using a 0.02 M NA solution, the average rate of spontaneous release was 0.36 p mole/barrel/min, which is approximately 10% of the total rate of release during the passage of +25 nA. However, when a 0.2 M solution was used (as is often the case in in vivo experiments) spontaneous release (8.8 p mole/barrel/min) accounted for as much as 65% of the total release in the presence of +25 nA.

The model predicts that diffusional release will be altered by the prior application of an electrophoretic current: (a) When a retaining current is applied, at first the thickness of the interphase layer is increased, and later the interphase
layer is removed from the tip boundary (Fig. 1, II). Thus, it is predicted that after the termination of the retention period, it will take some time before the steady-state rate of diffusional release is re-established. This prediction is confirmed by our observations (see Fig. 7)

(b) Castillo & Katz (1957) have suggested that there was an enhanced diffusional release ('after-diffusion') at the end of an ejection period. After-diffusion is also predicted by the present model: following a period of steady-state electrophoretic release it is assumed that the boundary of the bulk of the solution is situated at the tip boundary (Fig. 1, III/3). Thus it is expected that when the ejecting current is turned off the rate of diffusional efflux will gradually decline until the interphase layer is re-established (Fig. 1, I/2). The occurrence of after-diffusion is confirmed in the present experiments (Fig. 8).

B. Release in the presence of a retaining current

The model proposes that drug retention is a time dependent process, and that any retaining current will completely abolish diffusional efflux if it is applied for a long enough period of time. It is therefore predicted that the effect of using a retaining current of high intensity is to reduce the time required to abolish diffusional release. A retaining current of sufficiently high intensity ('critical retaining current') should counteract diffusional release instantaneously i.e. \( t_{\text{min}} = 0 \).

The results obtained in our experiments are in
agreement with these predictions. Fig. 12/C shows that a weak retaining current gradually became completely effective during the period of retention (see also Fig. 13). The results shown in Fig. 9 also support the predictions: as higher retaining currents were tested, the amount of drug released during the first 16 min retention was progressively reduced. Since there was no release at all in the presence of -25 nA it is possible to conclude that this current abolished release as soon as it was applied.

According to equation (10), $t_{\text{min}}$ is directly proportional to the square of the concentration of the drug solution contained in the micropipette. Thus, any given retaining current should be less effective in counteracting release from more concentrated solutions. Fig. 13 offers qualitative confirmation of this expectation: in contrast to the pipette shown in Fig. 9 (0.02 M), Fig. 13 shows that even after 50 min a retaining current of -25 nA had not succeeded in completely abolishing spontaneous release from a pipette containing a 0.2 M solution (the two pipettes had similar tip diameters).

Equation (10) also indicates that retaining currents should take longer to become effective if the transport number of the drug ion is reduced. This prediction is also confirmed by the present experiments (see I:2.3.5.).

The fact that any given retaining current was less effective if it followed an ejection period than if it followed a period of spontaneous release (Fig. 9) lends further support to the concept of 'after-diffusion' (Castillo & Katz, 1957), and is readily explicable in terms of the model. During
steady-state electrophoretic drug release the bulk of the solution, assumed to be at the tip boundary (Fig. 1, III/3). When an ejecting pulse is followed immediately by the application of a retaining current, the boundary of the bulk of the solution has to migrate through a relatively greater distance ($\delta c$ compared to $\delta c - \delta c$), before that particular retaining current becomes effective. Thus it is suggested that the reduced efficacy of a retaining current after an ejecting pulse is a reflection of the prolongation of $t_{\text{min}}$.

C. Release in the presence of an ejecting current

a. The transport number of NA. As a preceding retaining pulse can interfere with the release during an ejection period (see below), it was necessary to examine electrophoretic release when no retaining current had been applied previously. In these experiments, there was a linear relationship between the intensity of the ejecting current and the rate of drug release (Figs. 10 and 14). This shows that drug release, under these circumstances, can be described by Faraday's Law.

The data obtained from these experiments were used to calculate the transport number of NA. When transport numbers are calculated, it is necessary to know the contribution of spontaneous release to total release. Our practical procedure was to subtract the steady-state rate of spontaneous release from the total rate of release during an ejection period. This was, however, a necessary simplification since the application of the ejecting current may result in local changes in the concentration of drug ions inside and outside the tip, which in turn would
alter the rate of diffusional release.

The mean transport number of NA (0.02M NA bitartrate, pH 3.0), for the 13 micropipettes tested, was 0.17. When a more concentrated solution was used, however (0.2 M NA bitartrate, pH 3.5) the transport number was considerably greater. A possible reason for this difference is that there were proportionately fewer 'foreign' ionic species in the stronger NA solution. Other workers have determined the transport number of NA on the basis of electrophoretic release experiments, using a NA hydrochloride solution. Values of 0.34 and 0.37 were obtained from two micropipettes (1.7 M pH 3.0 - 4.0) using a fluorimetric method for the assay of NA (Krnjevic et al., 1963), and values of 0.09 (0.03 M, pH 5.5), 0.19 (0.3 M, pH 5.5) (Bradley & Candy, 1970) and 0.05 - 0.30 (0.5 M) (Hoffer et al., 1971a) were obtained using a liquid scintillation method for the assay of H\textsuperscript{3}-NA. Our results are within the range of values obtained by these workers. More precise comparisons, however, are not possible because of the use of different experimental parameters in these studies. (Different NA salt, H\textsuperscript{3}-labelled NA, the application of retaining currents between ejecting pulses).

b. The effect of retaining currents. It has been suggested (Curtis, 1964) that 'the use of an excessively strong retaining current for prolonged periods ... (may) ... prevent the substance under test from being ejected from the micropipette during the application of short current pulses'. Our results confirm this suggestion (Fig. 11).
In order to avoid this unfortunate effect of strong retaining currents, Curtis, (1964) has recommended the use of a 'just-adequate' retaining current, i.e. a retaining current which exactly counteracts spontaneous leakage without interfering with subsequent electrophoretic release. However, the concept of such a 'just-adequate' retaining current is incompatible with the proposed model, according to which every retaining current, however weak, will eventually become completely effective in counteracting diffusional efflux. Furthermore, the model proposes that if a retaining current is applied beyond the point in time at which it successfully counteracts diffusional efflux (minimal effective retention time, \( t_{\text{min}} \)) it will necessarily begin to deplete the pipette tip of drug ions, thus inevitably interfering with electrophoretic release during the next ejection period.

The model is apparently vindicated by the present results. Fig. 12 shows that when no retaining current had been applied previously, the release curve was rectangular in shape. The effect of a pre-ejection retaining current was to prolong the rising phase of the release curve. Both the intensity (Fig. 12B) and the duration of application (Fig. 12A) were important in determining the magnitude of this distortion, thus supporting the proposal that drug retention is a time dependent process. Fig. 12C shows that a weak retaining current (-5 nA) was not initially effective in counteracting spontaneous release, but became so before the end of the fourth 5 min collection period. (Thus, \( t_{\text{min}} \) for this
removing current was between 15 and 20 min). The continued application of this current for a further 60 min resulted in a significant distortion of the release curve during the next ejection period.

I:2.4.2. Some practical implications

(a) Faraday's Law, of course, applies only to drug ions released from the micropipette by iontophoresis. Even if we are prepared to neglect the contribution of electrophoresis, there still remains the awkward problem of diffusional release. This is necessarily an unknown factor during the application of an ejecting current. As mentioned earlier, our procedure has been to subtract the steady-state rate of spontaneous release from the total rate of release in order to estimate the 'true' rate of electrophoretic release. Yet, according to the model, the rate of diffusional release should be higher during an ejection period since the bulk of the solution would be situated at the tip boundary (Fig. 1, III/3). In reality, however, the situation is even more complicated since the high rate of release during the passage of an ejecting current will raise the concentration of drug ions just outside the tip, and this will tend to reduce the rate of diffusional efflux.

In the in vivo situation one has not even the satisfaction of knowing the steady-state rate of spontaneous release, and thus the contribution of diffusion is especially problematical. One solution has been proposed by Curtis et al., (1971): they suggest that the rate of spontaneous release may be assessed
in terms of the magnitude of the retaining current needed to curb it. Thus, these workers have added the intensity of the 'just-adequate' retaining current to the intensity of the ejecting current in order to calculate the 'true' dose of drug applied. This procedure is obviously rendered invalid by the present observation that drug retention is a time-dependent process, and by the consequent invalidation of the concept of 'just-adequate' retaining currents. There would seem to be no easy answer to this problem. However, it may be possible to circumvent it by using very dilute drug solutions, so that the contribution of diffusion to total release is relatively small. The rate of release would then be almost entirely iontophoretic, and thus governed by Faraday's Law.

(b) Faraday's Law is applicable only to the plateau rate of release. However, the present results show that the prior application of a retaining current distorts the rising phase of the release curve. The concentration achieved at receptor sites presumably increases during this period of increasing rate of release, but unfortunately the Carslaw-Jaeger equations offer no description of this changing concentration. Certainly the relationship between time and concentration is likely to be highly complex. Even if the rate of release were a step function of time, the change in concentration would not be linear (see equation (13)) and in fact Fig. 12 would seem to indicate a non-linear relationship between rate of release and time. I shall have more to say about this relationship in Section 1:3.2.2. but suffice
it to say at this point that there would seem to be little justification for the a priori assumption that log concentration is linearly related to time during an ejecting pulse (Hill & Simmonds, 1973).

(c) The present results indicate that if it is intended to deliver standard pulses of a drug it is essential that not only the intensity and duration of the ejecting pulses, but also the intensity and duration of the retaining pulses are kept scrupulously constant. Furthermore, the results shown in Fig. 11 suggest that after a protracted retention period it may be necessary to apply standard ejecting pulses several times before they can be expected to result in standard pulses of drug release.

(d) Frederickson et al., (1971) have suggested that the excitatory responses of single cortical neurones to microelectrophoretically applied noradrenaline (NA) may be artefacts produced by the ejection of H\(^+\) from acidic NA solution. In contrast to Frederickson et al., (1971), we have found that the direction of responses to NA was not influenced by the pH of the solution (the results of these experiments are described below, II:3.1.2.). However, NA ejected from solutions at pH 3.1 often appeared to be more potent than NA ejected from solutions at pH 5.0. A possible basis for this apparent difference in potency could be a lowering of the transport number of NA in the pH 5.0 solution produced by the addition of NaOH. The results of the present experiments (I:2.3.5.) support this suggestion. The lower transport number of NA in the pH 5.0 solution observed in
these experiments is most likely to be due to the introduction of Na\(^+\) into the solution when the pH was adjusted. By titrating a 0.2 M NA bitatrate solution with 1 N NaOH, it was found that NA bitartrate solution behaves as a buffer within the pH range 2 - 5. This presumably reflects the two pK\(_a\) values for tartaric acid (2.98 and 4.34; see Weast, 1972). Thus, approximately 110 times more NaOH had to be added to the pH 3.5 solution in order to raise the pH to 5.0 than would have been predicted on the basis of one-to-one acid-base titration. Indeed, the concentration of Na\(^+\) in the final solution at pH 5.0 was 0.1 M.

The reduction in the transport number brought about by the addition of other ions is reflected in a diminished efficacy of both ejecting and retaining currents. The addition of extraneous ions should therefore be avoided wherever possible. In the case of NA there would seem to be no advantage to the practice of raising the pH by the addition of NaOH (see II:3.1.2.). However, the addition of NaCl may be necessary in order to assist the passage of electrophoretic currents when poorly ionised compounds are applied (e.g., see Curtis et al., 1971).
THE MEASUREMENT OF NEURONAL RESPONSES

I:3. Introduction

In the previous section we have seen how ejecting and retaining currents interact in determining the shape of the release curve. We have seen that the plateau rate of release is determined by the intensity of the ejecting current, whereas the rising and declining phases of the release curve are also determined by the retaining currents applied before and after the ejecting pulse. In this section we shall examine the relevance of these factors for pharmacological studies of single neurones. We will see that the kinetics of neuronal responses to microelectrophoretically applied drugs (the 'response curve') are influenced by the same factors as influence the release curve. (I:3.3.1.) The significance of these findings for the construction of dose-response curves will be discussed in I:3.3.2. Here it will be shown that there are several possible ways of constructing dose-response curves, and that these are by no means equivalent. Finally (I:3.3.3,) we will examine how the action of antagonists can be studied with the microelectrophoretic technique, and what constraints are placed upon drug interaction studies by the practical difficulties associated with the technique.

I:3.2. METHODS

I:3.2.1. Preparation of the animals.

Cats of either sex weighing between 2.0 and 3.5 kg were used in these experiments. Two different techniques were
used to prepare the animals.

(a) In earlier experiments the cats were prepared according to the methods of Roberts & Straughan (1967). Anaesthesia was induced using halothane (approximately 2.5%) delivered via a face mask, and was maintained throughout the experiment with halothane (about 1%) delivered via a tracheostomy cannula with an Ayer's T-piece-like arrangement. Catheters were inserted into the cephalic vein and femoral artery for the injection of drugs and recording of blood pressure. ECG was continuously monitored. Two silver ball electrodes were inserted through burr holes in the skull contralateral to the site of single unit recording for monitoring epidural EEG. Rectal temperature was maintained between 37 and 38°C with a heating pad controlled by a thermosensitive rectal probe.

The cat's head was held rigidly in a stereotaxic frame, and the muscles of the back of the neck were cauterised transversely to expose the atlanto-occipital membrane. The membrane was then incised, allowing CSF to run out. A hole about 1 cm in diameter was cut in the skull overlying the anterior sigmoid gyrus, the dura was removed, and 1% agar (at 38°C) was poured into the hole in order to reduce pulsation of the brain during recording.

(b) During the course of the project, several modifications were made to above technique.

Under local anaesthesia, a Portex cannula was inserted into the cephalic vein for the injection of thiopentone, which was used to induce general anaesthesia. It was found that
this method of induction was less stressful for the animals and obviated the need for high concentrations of halothane during the surgery. High concentrations of halothane were associated with hypotension, cardiac dysrhythmia and raised intra-cranial pressure. After the surgical preparation of the animal had been completed, the administration of thiopentone was discontinued (total amount injected 40-140 mg), and anaesthesia was maintained with halothane, administered via a tracheostomy cannula, for the rest of the experiment. Halothane was delivered from a temperature and flow-rate compensated vapouriser (Fluotek Mk III, Cyprane Ltd.), enabling us to administer a constant concentration of halothane (0.6 - 1.2%) throughout the experiment. No cell recordings were made until at least 3 hours after the discontinuation of thiopentone.

As well as the continuous monitoring of ECG, EEG, blood pressure and rectal temperature, CO₂ (%) levels at the bifurcation of the trachea were also continuously monitored using a Beckman gas analyser (LB-1). The animals respired spontaneously, inspiring the oxygen-halothane mixture from a reservoir bag via a low resistance non-return valve, and expiring into the atmosphere via another non-return valve. Constant inflation of the inspiratory reservoir bag was maintained by adjusting the flow rate of the oxygen supply. The flow rate thus yielded a continuous monitor of the respiratory minute volume. Artificial ventilation was used if the spontaneous respiration became irregular, if the respiratory minute volume was markedly reduced, or if the end-tidal CO₂ exceeded 5%.

During the course of the experiment a continuous infusion of
a dextrose-saline solution (5% dextrose, 0.9% NaCl) was administered at a rate of 4 ml hour, and the bladder was continuously drained using an indwelling catheter.

Modifications were also made to the method of exposing the area of cerebral cortex for recording. A small hole was made in the skull overlying the anterior sigmoid gyrus using a dental burr. An incision was made in the dura (0.5 - 1 mm in length) under microscopic control, and the arachnoid ruptured using a glass probe, allowing CSF to leak out. The micropipette could then be introduced under microscopic control. This technique has the advantage that it is not necessary to remove CSF and thus the skull behaves as a practically closed box, in this way problems associated with pulsation are minimised.

In a number of experiments, an osmotic diuretic (Urevert, 1 gm/kg) was administered prior to opening the skull in order to reduce intracranial pressure. In order to determine whether this procedure influences the pharmacological responses of cortical neurones, comparisons were made between the proportions of neurones excited and depressed by acetylcholine (ACh), 5-hydroxytryptamine (5-HT), noradrenaline (NA) and mescaline in animals treated with Urevert and animals which did not receive this treatment. The results of these comparisons are shown in Table 3. ACh excited all the cells studied in both groups of animals; the proportions of cells excited and depressed by 5-HT, NA and mescaline did not differ significantly between the two groups. It was therefore concluded that Urevert did not affect the responses of neurones
TABLE 3. Comparison of neuronal responses to ACh, 5-HT, NA and mescaline in Urevert-treated and non-Urevert-treated animals.

Animals treated with Urevert: 28
Animals not treated with Urevert: 49

+ : number of cells excited. - : number of cells depressed. (biphasic responses are not included).

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<th></th>
<th>ACh</th>
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$\chi^2 = 0.0029$ : N.S.

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$\chi^2 = 0.0270$ : N.S.

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$\chi^2 = 0.0290$ : N.S.
studied in these experiments.

Further statistical tests were carried out to determine whether the use of thiopentone altered the direction of responses to microelectrophoretically applied drugs in our experiments. Thiopentone is a short-acting barbiturate, and in the present experiments was only used as a surgical anaesthetic and was always discontinued several hours before any pharmacological studies were conducted. However, barbiturates are known to alter pharmacological responses of neurones in the CNS. Johnson et al., (1969b) observed that the proportions of cortical neurones excited by the monoamines was reduced in thiopentone anaesthesia, and that excitatory responses to monoamines and ACh could be abolished by the systemic injection of thiopentone.

Barbiturates administered intravenously or microelectrophoretically were found to block selectively excitatory responses of brain stem neurones to ACh (Bradley & Dray, 1973). In the cortex, microelectrophoretically applied barbiturates were found to abolish responses to homocysteate as well as those to ACh (Crawford, 1970). In view of these findings, the proportions of cells excited and depressed by ACh, 5-HT, NA and mescaline were compared in cats which had received thiopentone during surgery and cats which had not been so treated. The results of these comparisons are shown in Table 4. It is apparent that, except in the case of responses to mescaline, the response distribution did not differ significantly between the two groups of animals. A greater proportion of cells were excited by mescaline in the thiopentone-treated
TABLE 4. Comparison of neuronal responses to ACh, 5-HT, NA and mescaline in those animals which received thiopentone during surgery and those animals which did not.

Animals treated with thiopentone : 40
Animals not treated with thiopentone : 37

+ : number of cells excited. - : number of cells depressed. (biphasic responses are not included.)

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<tr>
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<tr>
<td>B: 5HT</td>
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<td>D: Mescaline</td>
<td>+</td>
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</tr>
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<td>10</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>24</td>
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\[ \chi^2 = 0.0083 : \text{N.S.} \]

\[ \chi^2 = 2.360 : \text{N.S.} \]

\[ \chi^2 = 4.660 : p < 0.05 \]
preparations. Previous reports on the effects of barbiturates would lead one to expect the opposite of this finding, and so it is possible that the present findings are fortuitous and unrelated to the administration of thiopentone. This is supported by the fact that responses to NA and 5-HT did not differ significantly between the two types of preparation, although mescaline appeared to act on receptors similar to those activated by NA and 5-HT (see part II). A possible explanation for the greater predominance of excitatory responses to mescaline in the thiopentone-treated animals may be that the general condition of these animals was better (due to improvement of technique during the course of the project). Excitatory responses were encountered infrequently in preparations of poor general condition. In all subsequent analysis of the results, the cells studied in both types of preparation are treated as though they belonged to a single population.

Halothane anaesthesia was used in all the in vivo experiments described in this thesis. Thus it is not possible to say whether the responses described here reflect the pattern of responses that would have been seen in unanaesthetised brains. However, halothane would seem to be a reasonable choice of anaesthetic. Johnson et al., (1969b) found that, in contrast to barbiturates and chloralase, halothane did not prevent the occurrence of excitatory responses to monoamines, which were frequently observed in unanaesthetised encepalé isolé preparations. Halothane also was found to have less depressant effect on responses to 'excitant' amino acids and
ACh (Crawford, 1970). Furthermore, in a recent study of brain stem neurones Bradley & Dray (1972) found that halothane, as opposed to barbiturates, did not distort the pattern of responses to ACh and the monoamines.

I:3.2.2. Preparation of the micropipettes

The five-barrelled micropipettes used in these experiments were similar to those used in the in vitro experiments (see I:2.2.). One of the five barrels was always filled with 3 M or 4 M NaCl for recording action potentials. In most of the experiments a second barrel was also filled with NaCl for use in 'current balancing' (Roberts & Straughan, 1967). In some experiments, however, four drug-containing barrels were needed; in these experiments the procedure of current balancing was dispensed with. The drug solutions used in the micropipettes were as follows: noradrenaline bitartrate (0.2 M pH 3.0 - 3.5), 5-hydroxytryptamine bimaleate (0.2 M pH 3.0 - 3.5), mescaline hydrochloride (0.2 M pH 3.5 - 4.5), acetylcholine chloride (0.2 M pH 4.0 - 4.5 or 0.05 M pH 4.0 - 4.5), carbachol chloride (0.2 M pH 4.0 - 4.5), and sodium glutamate (0.2 M pH 8.5). With the exception of the sodium glutamate solution, all the solutions were prepared by dissolving the drug salt in double-glass-distilled water. The pH of the sodium glutamate solution was adjusted to 8.5 by the addition of NaOH.

I:3.2.3. Recording of action potentials

The recording techniques used in these experiments have been described by Roberts & Straughan (1967).
Extracellular action potentials were recorded via a 3 or 4 M NaCl solution contained in one barrel of the micropipette (resistance: 3-10 megohm). The action potentials were amplified using a Bioelectric Electrometer and a Tektronix pre-amplifier, and were fed into an oscilloscope (Tektronix 565) for visual observation. Spikes of equal amplitude were isolated from the noise using a pulse height selector. The pulse height selector generated two sets of standard pulses. One set of pulses was fed back into the oscilloscope to provide a visual monitor of the isolation of spikes from the noise. The other set of pulses was fed into a ratemeter (Ekco, N522C) with a time-constant of 1 sec, and a cumulative recorder (Grass Unit Integrater UI-1). The outputs of the ratemeter were displayed on a pen-writing oscillograph with curvilinear write-out (Grass, model 7).

I:3.2.4. Response measures

In these experiments, action potentials generated by a single unit were defined as spikes of equal amplitude which were clearly separated from the noise level and from spikes of lower amplitude. Pharmacological responses were evaluated on the basis of the ratemeter and cumulative spike counter records. The following parameters were used (see Fig. 15):

a) The spontaneous (baseline) firing rate (F1).

b) The latency of onset of excitation, measured from the onset of the ejecting pulse (response latency) \( T_0 - T_1 \).
c) The maximum (equilibrium) firing rate obtained by the cell during the ejection period (F2). The maximum firing rate was measured under equilibrium conditions, i.e. when prolongation of the ejection period led to no further change in the firing rate. The baseline firing rate was subtracted from the maximum (equilibrium) firing rate in order to obtain the maximum change in firing rate (F2-F1).

d) The time taken to achieve the maximum firing rate (T₀-T₂).

e) The time taken for the recovery of the spontaneous firing rate after the termination of the ejecting pulse (recovery time) (T₃-T₄).

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 time taken to achieve the maximum firing rate, 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.
FIG. 15. Parameters of an excitatory response to ACh.

A. Electrophoretic current passed through the solution of ACh chloride (0.2 M) contained in one barrel of the micropipette (ordinate: current intensity, nA, positive upwards; abscissa: running time, min).

B. Ratemeter recording of the firing rate of a single cortical neurone (ordinate: firing rate, spikes/sec; abscissa: running time, min).

C. Cumulative record of the total number of action potentials generated by the neurone (ordinate: total spikes; abscissa: running time, min).

Response parameters are indicated by letters above and to the right of the traces. See text for explanation.
RESULTS AND DISCUSSION

All the neurones studied in these experiments were spontaneously active, and in no case was the firing rate controlled by the 'background' application of excitant amino acids. Only excitatory responses were used. In these experiments, we followed the usual practice of passing a retaining current through the drug-containing barrel continuously when ejecting currents were not being passed.

Kinetics of neuronal responses

A. The role of ejecting currents

In these experiments, the parameters of the retaining pulse (intensity, and retention time) were kept constant, and the intensity of the ejecting pulse was increased with successive applications.

The following changes in the excitatory responses were observed:

a. The response latency became progressively shorter.
b. The latency to plateau became progressively shorter.
c. The maximum firing rate attained by the cell increased.
d. The recovery time was prolonged (in the presence of post-ejection retaining current of standard intensity).
e. The total spike number increased (when a standard ejection time was used).

Ratemeter recordings of the responses of a single
cortical neurone to 5-HT, released from a micropipette with a range of different current intensities, are displayed in Fig. 16. Response latencies and latencies to plateau of responses to ACh, carbachol (CCh), 5-HT, NA and mescaline are shown in Fig. 17. Responses to glutamate behaved similarly, but it was not possible to measure the recovery time of these responses, due to their fast time-course.

B. The role of retaining currents

a. Pre-ejection retaining currents. When the intensity of the retaining current applied between ejecting pulses was increased, the response to the standard ejecting pulse was markedly reduced, and, in some cases, completely abolished. This observation is illustrated in Fig. 18. In this experiment, a single cortical neurone was excited by ACh, released from one barrel of the micropipette with an electrophoretic current of +50 nA applied for 20 sec. A retaining current of -25 nA was passed through the ACh-containing barrel during the 80 sec period which separated successive applications of the ejecting current. After three applications of ACh, which evoked approximately equivalent excitatory responses, the intensity of the retaining current was increased to -100 nA (A). This resulted in a complete abolition of the neuronal response to ACh. After the retaining current had been reduced to -25 nA again (B), the original response to ACh recovered.

These observations were extended further, using excitatory responses to glutamate. Because of their fast time-course, these responses are particularly suitable for such studies. It was found that a reduction in the size
FIG. 16. The excitatory responses of a single cortical neurone to 5-HT applied microelectrophoretically with different ejecting currents. Lower traces: electrophoretic currents passed through the solution of 5-HT bimaleate (0.2 M) contained in one barrel of the micropipette (ordinates: current intensity, nA; positive upwards; abscissae: running time, min). Stippled area: retaining current; hatched area: ejecting current. Upper traces: excerpts from the recording of the firing rate of the neurone (ordinates: firing rate, spikes/sec; abscissae: running time, the same scale as in the lower traces). Arrows above the trace indicate the onset of the response and the recovery of the baseline firing rate. When the intensity of the ejecting current was increased with successive applications, the response latency became shorter, the maximum firing rate attained during the response was increased, the recovery time was prolonged, and the total spike number was increased. Note that the application of +50 nA was terminated prematurely because the cell became over-excited and the spike amplitude was reduced.
FIG. 17. The relationship between the intensity of the ejecting current and the latencies of neuronal responses. Each graph shows the latencies to onset and latencies to plateau of responses of one individual neurone. Ordinates: latency (sec); abscissae: intensity of ejecting current (nA). Closed circles: latency to onset of the response; closed triangles: latency to plateau.
In the case of all four agonists the latencies were reduced when higher ejecting currents were passed.
FIG. 16. The effect of an increase in the intensity of the retaining current upon neuronal responses
to ACh. Lower trace: Electrophoretic current passed through the solution of ACh chloride (0.2 M) con¬
tained in one barrel of the micropipette (ordinate: current intensity, nA, positive upwards; abscissa: running time, min). Stippled area: retaining current; hatched area: ejecting current. Upper trace: continuous recording of the firing rate of a single cortical neurone (ordinate: firing rate, spikes/sec; abscissa: running time, the same scale as in the lower trace). Figures above the trace represent the total number of spikes generated in response to each ejecting pulse. The parameters (intensity and duration) of the ejecting pulse were kept constant.
of the response to a standard ejecting pulse could be achieved by increasing the retention time, as well as by increasing the intensity of the retaining current. This observation is illustrated in Fig. 19. When the cell studied in this experiment was first isolated, a retaining current of +25 nA had been applied to one of the barrels of the micropipette, which contained sodium glutamate for more than 3 min. An ejecting pulse (-200 nA, 20 sec) evoked a small excitatory response. This standard ejecting pulse was repeated several times, successive ejecting pulses being separated by 30 sec application of the retaining current of +25 nA. Progressively larger responses were evoked by the repeated applications of the ejecting pulse, until an equilibrium response was achieved (A). The retention time was then increased to 80 sec with the intensity of the retaining current maintained at +25 nA; this resulted in a decrease in the number of spikes elicited by the standard ejecting pulse (B). A further increase in the retention time to 130 sec further reduced the magnitude of the responses (C). After the 15th ejecting pulse (D) the intensity of the retaining current was increased to +50 nA and the retention time was restored to 30 sec. Under these circumstances the size of the responses increased up to a constant level. Here the responses were smaller than when successive ejecting pulses were separated by the smaller retaining current (+25 nA) applied for the same period of time (cf. A). When the retention time was increased to 80 sec with the retaining current maintained at +50 nA, the response completely disappeared (E). The intensity
FIG. 19. The effect of changes in the parameters (intensity and duration) of the retaining pulse upon neuronal responses to glutamate. Lower traces: electrophoretic current passed through the solution of sodium glutamate (0.2 M) contained in one barrel of the micropipette (ordinates: current intensity, nA, negative upwards; abscissae: running time, min). Stippled area: retaining current; hatched area: ejecting current. Upper traces: continuous recording of firing rate of a single cortical neurone (ordinates: firing rate, spikes/sec; abscissae: running time, the same scale as in the lower traces). Figures above the traces represent the total number of spikes generated in response to each ejecting pulse. 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. Before the beginning of the study a retaining current of +25 nA had been applied for more than 3 min.

A: retaining pulse: +25 nA, 30 sec; responses progressively increase, until a standard response is established.

B: retaining pulse: +25 nA, 80 sec; responses are reduced.

C: retaining pulse: +25 nA, 130 sec; responses are further reduced.

D: retaining pulse: +50 nA, 30 sec; responses increase, but are still smaller than in A.

E: retaining pulse: +50 nA, 80 sec; responses are abolished.

F: retaining pulse: +75 nA, 30 sec; no responses.

G: retaining pulse: +25 nA, 30 sec; original responses (compare A) recover.
of the retaining current was then increased to +75 nA; now the standard ejecting pulse failed to evoke any response, even when the retaining time was again reduced to 30 sec (F). Finally, when the original retaining current of +25 nA was restored, and the retention time was maintained at 30 sec (G), complete recovery of the responses was observed.

When the intensity, or duration of application of the retaining current was increased, the reduction in the total spike number was accompanied by an increase in the response latency.

In the studies shown in Fig. 18 and 19, 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. In order 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. 20.

When either parameter of the retaining current was increased the response latency and the latency to plateau increased (Figs. 20 & 21), 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. 20). In the
The effect of changes in the duration of application of the pre-ejection retaining current upon neuronal responses to ACh. Lower traces: electrophoretic current passed through the solution of ACh chloride (0.2 M) contained in one barrel of the micropipette and the action of the retaining current, current intensity, n£, positive upwards; abscissae: running time, min. Stippled area: retaining current; hatched area: ejecting current.

Upper traces: excerpts from the recording of the firing rate of a single cortical neurone (ordinates: firing rate, spikes/sec; abscissae: running time, min). Arrows above the traces indicate the onset of the response, the attainment of the maximum 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.

A: Pre-ejection retention time: 5 min, the response latency and the time taken to attain the maximum firing rate were further increased, with no change in the maximum firing rate and the recovery time. B: Pre-ejection retention time: 10 min, the response latency and the time taken to attain the maximum firing rate were increased. D: Pre-ejection retention time: 5 min. A: Pre-ejection retention time: 5 min, the response latency and the time taken to attain the maximum firing rate were further increased, with no change in the maximum firing rate and the recovery time.
FIG. 21. The effect of retention time on response latency and latency to plateau of the responses of a single cortical to ACh. Closed circles: response latency; closed triangles: latency to plateau. The intensity of the retaining current was -100 nA, and the intensity of the ejecting current was +25 nA. An increase in the retention time resulted in an increase in the response latency and the latency to plateau.
study shown in these figures the retention time was increased; similar observations were made when the intensity of the retaining current was increased.

b. Post-ejection retaining currents. After any given ejecting current, the recovery time of the response was constant in the presence of a standard post-ejection retaining current (see previous section). However, 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. An example of this observation is displayed in Fig. 22, which shows the recovery times of responses of a cell to Ach.

I:3.3.1.2. Discussion

The results presented in the preceding pages support the suggestion that the response curve closely follows the concentration curve. The changes in the response curve observed when increasingly higher ejecting currents were used are consistent with the predictions presented in I:1.4. for the concentration curve: the latency is reduced, the plateau is raised, and the recovery time is prolonged.

Since it is proposed that the concentration curve reflects in part the shape of the release curve, it is to be expected that all the distortions imposed on the release curve by the passage of retaining currents would be manifested in the response curve. Such is indeed the case:

a. Pre-ejection retaining currents. An increase in either the intensity or the duration of the pre-ejection retaining
FIG. 22. The effect of the intensity of the post-ejection retaining current upon the recovery time of responses of a single cortical neurone 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.
current results in an increase in the response latency and latency to plateau, but does not alter the plateau firing rate itself. These findings are complementary to the observations of the release curve measured in vitro (e.g. compare Figs. 12 and 20). When standard ejecting pulses are used the distortions in the response curve are reflected in a decrease in the total spike number; this presumably results from a decrease in the total drug output during the ejection period (compare Figs. 11 and 19).

It is apparent from Fig. 19 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.

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 already been shown 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 (I:1.2, I:2.3.3).

b. Post-ejection retaining currents. The post-ejection retaining current can modify the recovery time of the response
(Fig. 22). 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 became completely effective (cf. Fig. 12C). The progressive shortening of the recovery time in the presence of retaining currents of higher intensities is presumably a reflection of progressive shortening of the minimal effective retention time \( t_{\text{min}} \). The fact that -25 nA was practically as effective as -100 nA suggests that -25 nA almost instantaneously abolished spontaneous release (i.e. \( t_{\text{min}} = 0 \)), 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 \textit{in vivo} observations reported in this section confirm the practical relevance of the \textit{in vitro} studies for the use of the microelectrophoretic technique under 'normal' experimental conditions. It is important to note that despite the fact that more dilute drug solutions were used in most of the \textit{in vitro} experiments, the effects of retaining currents on the release curve are paralleled in every way by their effects on the response curve. Retaining currents are universally used in microelectrophoresis experiments. There is little, if anything, to be gained from the use of weak retaining currents, for if a retaining current is to be effective in counteracting spontaneous release it must necessarily distort the rising phase of the subsequent release curve (and hence also the response curve). The use of a
relatively high retaining current has the advantage that it may abolish release promptly and thus allow time for the neurone to recover before the next ejecting pulse. Sustained diffusional release may give rise to subthreshold concentrations of drugs whose presence becomes apparent only in the presence of a protagonist (Curtis et al., 1970); it may also be source of desensitisation (Roberts & Straughan, 1967).

It was mentioned in I:1.5. that many workers have attributed some biological significance to the different time-courses observed with different drugs. The alternative position considered here is that differences between time-courses of responses to different drugs are to a great extent due to differences between the mobilities of the drug molecules. It was suggested earlier, on theoretical grounds, that the concentration curve of a more mobile drug will have a 'faster' time-course than that of a less mobile drug released under identical conditions (I:1.4.). These hypothetical differences between the concentration curves correlate well with actual differences between the response curves; more mobile drugs tend to evoke 'faster' responses than less mobile drugs (see Table 5). It is also relevant that the same drug applied microelectrophoretically in different tissues tends to evoke responses of similar time-courses (Stone, 1971, 1972a). Of course, on the basis of the presently available evidence, one cannot conclude with certainty that physical factors alone are responsible for the shape of the response curves. However, any a priori assumption about the involvement of biological factors would seem to be unwarranted at this stage.
<table>
<thead>
<tr>
<th>TIME COURSE</th>
<th>DRUG</th>
<th>RESPONSE</th>
<th>TRANSPORT NUMBER</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>'FAST'</td>
<td>GLYCINE</td>
<td>-</td>
<td>0.5</td>
<td>Werman et al., (1966)</td>
</tr>
<tr>
<td></td>
<td>GLUTAMINE</td>
<td>+</td>
<td>0.4</td>
<td>estimated from Ziegengansberger et al., (1969).</td>
</tr>
<tr>
<td>'INTERMEDIATE'</td>
<td>ACh</td>
<td>+</td>
<td>0.24, 0.48</td>
<td>Bradley &amp; Candy (1970)</td>
</tr>
<tr>
<td>'SLOW'</td>
<td>NA</td>
<td>+,-</td>
<td>0.05-0.30</td>
<td>Hoffer et al., (1971)</td>
</tr>
<tr>
<td></td>
<td>5-HT</td>
<td>+,-</td>
<td>0.4</td>
<td>Knjevic et al., (1963)</td>
</tr>
</tbody>
</table>


There is a significant positive correlation between the 'speed' of response and the transport number of the drug ion (Spearman Rank Correlation test: $r_s = 0.73$, $p < 0.01$).
I:3.3.2. **Dose-response curves**

I:3.3.2.1. **Plateau responses**

The Carslaw-Jaeger equation for diffusion from a continuous point source (equation (13a)) provides a theoretical basis for the construction of dose-response curves, in which the intensity of the ejecting current is used as the measure of dose, and the plateau of the response curve (expressed as a percentage of the maximum change in firing rate) is used as the measure of response. The relationship which exists under equilibrium conditions between current intensity and concentration is expressed in equation (14). Usually the logarithm of the ejecting current intensity is plotted on the abscissa, in accordance with conventional dose-response curves in pharmacology in which percent response is plotted against log dose.

In the case of depressant responses, total cessation of firing is normally taken as 100% response. Although this does not represent the greatest possible suppression of cellular excitability, it does provide a convenient operational definition of a maximum response with which intermediate responses may be compared. The magnitude of each response is thus expressed as:

\[
\frac{\text{change in firing rate}}{\text{Max. change in firing rate}} \times 100
\]

On this basis, a number of workers have been successful in constructing current-response curves for depressant responses of central neurones to certain amino-acids (GABA, glycine)
In the case of excitatory responses, the situation is more problematical, since it is rarely possible to establish a maximal excitatory response which cannot be superseded when higher ejecting currents are passed (Boakes et al., 1970). Usually when the firing rate is increased beyond a certain value the spike amplitude is reduced, and eventually it may become impossible to detect any action potentials at all. However, just as the maximal depressant response may be operationally defined as the total cessation of firing, the maximal excitatory response may be operationally defined as either (i) that firing rate which cannot be increased by the application of higher ejecting currents, or (ii) that firing rate at which the spike amplitude is reduced to some arbitrary level (say, when the spikes can no longer be distinguished from the background noise).

In the present experiments, current-response curves have been constructed for excitatory responses of individual cells to 5-HT, NA, mescaline, ACh and CCh and two curves obtained with ACh can be seen in Fig. 23. In the case of ACh and CCh maximum response occurred without a significant change in the spike amplitude, but in the case of all the other drugs 100% responses were taken as the point at which the spike amplitude was reduced.

The greatest difficulty encountered in the construction of these curves was the very long time for which weak ejecting currents needed to be applied before plateau responses were
FIG. 23. Antagonism by atropine of excitatory responses of two cortical neurones to ACh. Ordinates: response to ACh, plateau firing rate minus baseline firing rate (expressed as a percentage of the maximum change in firing rate). Abscissee: intensity of ejecting current used to apply ACh (nA, log. scale). Closed circles: control current-response curve. Closed triangles: current-response curve obtained in the presence of atropine (0.01 M 0 nA). Atropine was applied continuously and the response to a standard application of ACh was measured at regular intervals. The current-response curve was constructed when there appeared to be no further change in the response to this standard application. open circles: recovery response.

A: study conducted on one neurone, to which ACh was applied from a 0.2 M solution.

B: study conducted on another neurone, to which ACh was applied from a 0.05 M solution. In both cases atropine shifted the current-response curve to the right. The antagonism was surmountable, and the dose-ratio measured at 50% response was about 12.
A

RESPONSE TO ACh

PERCENT MAX. INCREASE IN FIRING RATE

CURRENT (nA)

B

RESPONSE TO ACh

PERCENT MAX. INCREASE IN FIRING RATE

CURRENT (nA)
achieved (see Fig. 17). This was presumably an effect of the pre-ejection retaining current, since the actual shape of the release curve is likely to be determined not only by the retaining current but also by its interaction with the ejecting current (i.e. it should take a relatively long time for a weak ejecting current to move the boundary of the bulk of the solution down to the tip boundary (I:1,2)). One way of overcoming this difficulty might be to apply the agonist continuously, increasing the intensity of the ejecting current whenever a plateau is achieved. Current-response curves constructed in this way might be expected to differ from those shown in Fig. 23, since it is known from observations of peripheral systems that cumulative and non-cumulative dose-response curves are often not identical (Guimaraes, 1972). In the present series of experiments a few attempts were made to construct cumulative curves, but these met with little success since prolonged exposure of the neurone to the agonist usually resulted in desensitisation.

Clarke et al., (1973) have criticised the use of plateau responses on the grounds that diffusional release distorts the relationship between current intensity and rate of release. This is a serious problem which was indeed a confounding factor in the present experiments. Boakes et al., (1970) and Dreifuss & Matthews (1972) have found that as much as 20% of the maximum responses to glutamate and GABA could be evoked by removal of the retaining current, without the passage of any ejecting current. However, in both these studies rather concentrated drug solutions were used (0.6 M and 1.0 M respectively). In the present experiments in which more
dilute drug solutions were used (0.2 M or in some cases 0.05 M), spontaneous release may have been less important although it will be remembered that spontaneous release accounted for a considerable proportion of total release from a 0.2 M NA solution (see Fig. 14).

I:3.3.2.2. The initial phase of the response curve

Because of the considerable practical difficulties associated with the use of plateau responses, Hill & Simmonds (1973) have devised an ingenious method for constructing cumulative dose-response curves on the basis of a single drug application. The method is based on assumptions similar to those proposed in I:1.5., namely that the concentration of drug molecules present at neuronal receptor sites rises gradually during the passage of an ejecting current, and that the neurone responds virtually instantaneously to the presence of a given concentration of drug molecules (Cuthbert & Dunant, 1970). It follows from these assumptions that the initial phase of the response curve (response vs. time) is in fact a dose-response curve (response vs. concentration). Accordingly, Hill & Simmonds (1973) have plotted percent response against time (measured from the onset of the ejecting pulse) in order to produce curves analogous to conventional log. concentration-effect curves.

The shape of the initial phase of the response curve is determined by the interaction between the ejecting and retaining currents (I:3.3.1.). Indeed, Hill & Simmonds observed that a change in the intensity of either the ejecting
or the pre-ejection retaining currents could seriously distort
the shape of the time-response curve. It must be remembered
that the retention time is also a determinant of the shape
of the response curve (1:3.3.1., Figs. 19, 20, & 21), and thus
must be kept scrupulously constant in any attempt to apply
Hill & Simmonds' method.

It seemed to be of interest to compare the dose-response
curves obtained by Hill & Simmonds' method with those obtained
by plotting log. current against percent plateau response.
Accordingly, an attempt was made to compare the time-response
relationship with the current-(plateau) response relationship.

Let us, for the time being, accept the applicability
of the Carslaw-Jaeger equation(13a) to describe the steady-
state concentration of drug molecules at receptor sites.
On this basis we may assume that the intensity of the ejecting
current is directly proportional to the concentration under
equilibrium conditions (equation 14). Thus we may regard
current values on the log.current-plateau response curve as
being equivalent to 'concentration' values (expressed in
arbitrary units), and we may therefore assign one such 'con-
centration' value to each value of the plateau firing rate.
Now, if we are also willing to make the assumption that the
neurone responds instantaneously to the presence of a given
concentration of drug molecules (Cuthbert & Dunant, 1970;
Hill & Simmonds, 1973; see also I:1.5. and I:3.3.1.) we are
now in a position to take a time-response curve for a maximal
response and substitute 'concentration' values for response
values. This yields a plot of 'concentration' against time.
By plotting log. 'concentration' along the abscissa we can see whether, during the initial phase of the response curve of a maximal response, log. concentration does really increase linearly with time. Experiments were conducted on six neurones in an attempt to answer this question. On three neurones excitatory responses to ACh were studied; on another, excitatory responses to NA. (The data obtained from the other two neurones, responding to CCh and 5-HT respectively, had to be rejected because with these neurones spontaneous drug release evoked a considerable response). To date, Hill & Simmonds have only applied their time-response curves to depressant responses to GABA and glycine, but the method should be equally applicable to excitatory responses.

Fig. 24 shows the results obtained from one neurone with ACh. The current-plateau response curve is shown in A, the arbitrary 'concentration' values being indicated below the current axis. Time response curves of maximal responses to +60 nA and +100 nA are shown in B. In C, log. 'concentration' is plotted against time. It can be seen from this graph that the assumption of a linear relationship between log. concentration and time leads us to an impossible conclusion, namely that the concentration of drug molecules at receptor sites starts to rise some time before the ejecting current is turned on. In D, the same data are shown, this time with 'concentration' plotted on a linear scale. While a straight line still does not fit the points very well, this function does at least indicate that there is some latency before the concentration starts to rise, and that the rise in concentration
FIG. 24. Comparison of current-plateau response curves and time-response curves for the responses of a single cortical neurone to ACh.

A: current-plateau response curve (see Fig. 23). Current is expressed as 'concentration' (in arbitrary units) on the abscissa.

B: Time-response curves for two maximal responses.

C: Log. 'concentration' (from A) plotted against time (from B), on the basis of equivalent response values from the current-response and time-response curves.

D: 'concentration' plotted against time.

The straight lines in C and D for the two maximal responses were obtained by linear regression: C: closed circle, 
\[ y = 0.167 x; \]  closed triangle, 
\[ y = 0.016 + 0.033 x. \]  D: closed circle, 
\[ y = -14.765 + 0.516x; \]  closed triangle, 
\[ y = -18.390 + 0.973x. \]

See text for explanation.
ACh (0.05 M)

A CURRENT-PLATEAU RESPONSE

B TIME-RESPONSE

RETAINING CURRENT
-25 nA, 300 SEC

C TIME-LOG. "CONCENTRATION"

D TIME-"CONCENTRATION"
is more rapid when a higher ejecting current is used.

It should be noted that this kind of *reductio ad absurdum* argument does not indicate which of the premises is false. For instance, it might well be that the fundamental error lies in the assumptions underlying equations (13a) and (14) (i.e. the assumption that concentration is linearly related to the intensity of the ejecting current under equilibrium conditions). However, whether or not this is the case, the manipulations presented above do show one thing quite clearly - that log. current-plateau response curves and time-response curves are not equivalent. Since the former would seem to have a somewhat firmer theoretical foundation than the latter, there would seem to be some justification for treating time-response curves with reservation until further empirical evidence becomes available.

I:3.3.3. **Evaluation of antagonists**

I:3.3.3.1. **Continuous application**

The most widely used procedure in agonist-antagonist studies with the microelectrophoretic technique is for the agonist(s) to be applied repeatedly while the antagonist is being applied continuously from another barrel of the micropipette. Responses to the agonists before, during and after the application of the antagonist may then be compared. Several workers have endeavoured to construct current-plateau response curves for the agonists so that parallel or non-parallel displacement may be observed in the presence of the
antagonist (Davidoff et al., 1969; Johnson et al., 1970; Curtis et al., 1971; Dreifuss & Matthews, 1972; Haas & Hosli, 1973). (The fact that such studies have been entirely restricted to depressant responses to amino-acids is probably due to the long time-courses of responses to ACh and the monoamines which tends to made such studies impracticable). In studies of this kind it is essential that the antagonist be applied for a prolonged period so that the effect of the antagonist can be studied under equilibrium conditions. A further difficulty with these studies is that it is seldom possible to obtain high dose-ratios in the presence of the antagonist, because of the limited current carrying capacity of the drug solutions. Fig. 23 shows the antagonism of excitatory responses to ACh by atropine; the dose-ratios in these studies were about 12. Somewhat lower dose-ratios were obtained for the antagonism of depressant responses to glycine by strychnine (Johnson et al., 1970) and the antagonism of depressant responses to GABA by bicuculline (Dreifuss & Matthews, 1972; Kelly & Renaud, 1973). However, the studies by Johnson et al., (1970) were considerably strengthened by the observation of a non-parallel shift in the GABA dose-response curve by strychnine.

The time-response curves of Hill & Simmonds (1973) essentially preclude the possibility of observing non-parallel displacement since they are constructed in such a way as to ensure parallel displacement. However, the procedure has the very considerable practical advantage that the time required to conduct an antagonist study is much
reduced. Hill & Simmonds have used their method to compare the potencies of various antagonists of GABA, and were able to observe that picrotoxin was more potent than bicuculline in blocking the responses of cuneate neurones to GABA.

In the present project it was not considered necessary to compare the potencies of various antagonists on responses to a single agonist. Antagonists were used as tools to discriminate between different agonists. In these experiments, ejecting currents and ejection times for the various agonists were selected so that all the agonists evoked approximately equivalent submaximal responses. Then, the agonists were applied in a regular cycle, and the antagonist was applied continuously with a weak ejecting current. The time-course of the gradually developing antagonism of the responses to the agonists could then be compared. When the antagonism had reached an equilibrium level, the ejecting current used to apply the antagonist could, if necessary, be increased until complete blockade was achieved. After terminating the antagonist application, the time-course of recovery from antagonism for each agonist could be compared. The use of this procedure can be seen in Figs. 36–45 (Fig. 38B shows a complete ratemeter recording of an antagonist study). A further example is shown in Fig. 25. A noteworthy feature of the study shown in Fig. 25, is that when the response to the agonist is partially antagonised, the response latency is increased and the recovery time reduced. This is in keeping with the predictions made in I:1.5. for the effect of antagonists on the response curve.
FIG. 25. Antagonism of excitatory response to 5-HT by methysergide.

A, B, C, D: excerpts from the ratemeter recording of the firing rate of a single cortical neurone. Ordinates: firing rate (spikes/sec); abscissa: running time (min).

Time from the onset of the study is indicated below the abscissae. Horizontal bars indicate drug applications (numbers refer to the intensities of the ejecting currents (nA)).

A: control response to 5-HT. B: partial antagonism of response to 5-HT by methysergide (5 nA). C: almost complete abolition of response to 5-HT by methysergide (10 nA). D: recovery of response to 5-HT after the application of methysergide had been terminated.

Arrows indicate response latency and recovery times of the responses.

Lower graph shows the time course of the entire study.

Ordinate: size of the response to 5-HT (total spike number, expressed as a percentage of the mean control response); abscissa: running time (min). Solid bar above graph indicates application of methysergide (numbers refer to intensities of ejecting current). Each point refers to a single response to 5-HT. Letters A, B, C, D indicate the responses illustrated in the ratemeter tracings above.
I:3.3.2. **Pulse application**

An alternative way of applying an antagonist is to use a brief ejecting current pulse. Under these circumstances the micropipette may be regarded as an 'instantaneous point source', and the changing concentration of antagonist molecules at any fixed point in the external medium may be described by equation (12). If the agonist is applied at regular intervals after the antagonist pulse, it is possible to follow the response to the agonist during the course of the entire 'concentration curve' of the antagonist. There are, however, serious drawbacks to this procedure. Firstly, the antagonism is never evaluated under equilibrium conditions. Secondly, since the concentration of the antagonist is continuously, and fairly rapidly changing, it is not possible to examine the effect of a single pulse of antagonist on responses to more than one agonist. Nevertheless, in studies of the tricyclic antidepressants, which when applied for prolonged periods lead to a progressive decrease in cellular excitability and loss of spike height, the 'brief pulse' procedure has proved useful (Bradshaw et al., 1971, 1973; Bevan et al., 1973). Therefore this procedure was adopted in the present project when the effects of tricyclic antidepressants upon responses to mescaline were examined.
PART II

THE EFFECT OF MESCALINE ON CORTICAL NEURONES
II: 1. INTRODUCTION

II: 1.1. MICROTOELECTROPHORETIC STUDIES OF NA AND 5-HT

The technique of fluorescence histochemistry has demonstrated the existence of neurones in the mammalian CNS which contain NA and 5-HT. The somata of these cells are located in discrete nuclei in the brain-stem: NA-containing cell bodies in the locus coeruleus, and 5-HT-containing cell bodies in the raphe nuclei. Fibres project from these nuclei both posteriorly to the spinal cord and anteriorly to the forebrain. Terminals containing NA and 5-HT are disseminated throughout most structures in the CNS (Dahlstrom & Fuxe, 1965).

These histochemical findings boosted the already growing speculation that the monoamines might function as neurotransmitters in many areas of the mammalian nervous system (see McLennan, 1963; 1972). This supposed 'monoamine transmission' forms the basis of most current theories of the mode of action of psychoactive drugs including the antidepressants (see Davis, 1970), the neuroleptics (see Stein, 1971) and the hallucinogens (see Brawley & Duffield, 1972).

The postulated transmitter role of the monoamines and their implication in the action of psychoactive drugs forms the background to a plethora of investigations into the sensitivity of central neurones to NA and 5-HT. Neuronal responses to the monoamines have been observed in almost every structure studied, but disparate results have often been obtained by different workers studying the same structure. In many instances it is possible to attribute such
discrepancies to methodological factors; in particular, the widespread use of barbiturate anaesthesia has been responsible for many altercations amongst users of the micro-electrophoretic technique (see Curtis & Crawford, 1969).

a) Spinal cord

Curtis et al., (1961) found that NA had no effect on spinal neurones of barbiturate-anaesthetised cats. More recent work, however, demonstrated a depressant action of NA on spontaneous and chemically- or synaptically-driven firing of interneurones and motoneurones (Engberg & Ryall, 1966; Bruggecate & Engberg, 1969). This depressant action of NA was not modified by electrophoretically applied α- and β-adrenoceptor antagonists, strychnine, or bicuculline, (Engberg et al., 1971).

Engberg & Ryall (1966) and Weight & Salmoiraghi (1966) observed both excitatory and depressant responses to 5-HT. Recently, Barasi & Roberts (1972, 1973a, b) have reported that 5-HT can excite spinal motoneurones (as demonstrated by an increase in the amplitude of the orthodromic or antidromic field potentials). Stimulation of the raphe nuclei also increased the excitability of motoneurones. These excitatory effects of 5-HT and raphe stimulation could be antagonised by methysergide and cinanserin, and could be potentiated by imipramine.

b) Brain stem

Straschill & Perwein (1971) observed only depressant effects of NA and 5-HT on brain stem neurones. However,
most investigators have reported the occurrence of both excitatory and depressant responses to the monoamines (Boakes et al., 1970; Boakes et al., 1971; Hosli et al., 1971; Anderson et al., 1973). Biphasic responses have also been observed. Hosli et al. argued that the occurrence of biphasic responses might indicate that some brainstem neurones possess two populations of monoamine receptors: excitatory and depressant. In addition, the observation that many cells respond differently to NA and 5-HT may indicate that these monoamines act at different receptors, although as Hosli et al. pointed out, the possibility that some neurones possess receptors common to both monoamines cannot be excluded.

Excitatory and depressant responses to NA were not susceptible to antagonism by α- and β-adrenoceptor antagonists (Boakes et al., 1971). However, chlorpromazine (Bradley et al., 1966) and α-methyl-NA (Boakes et al., 1971) were able to block the excitatory effects of NA, while depressant responses could be antagonised by mescaline (Gonzalez-Vegas, 1971), 3,4-dimethoxyphenylethylamine (Gonzalez-Vegas & Wolstencroft, 1971a) and bulbocapnine (Gonzalez-Vegas & Wolstencroft, 1971b). Anderson et al. (1973) observed that the depressant but not the excitatory effects of NA were mimicked by cyclic AMP, but they pointed out that it was unlikely that NA-induced depression was mediated by cyclic AMP since theophylline, aminophylline and nicotine were without effect on these responses (see below: e. Cerebellum).

Excitatory responses to 5-HT could be blocked by LSD, methysergide and 2-bromolysergic acid diethylamide (BOL) (Boakes et al., 1970), LSD being the most potent antagonist tested. Excitatory responses to glutamate were also
antagonised by LSD, but depressant responses to 5-HT, excitatory responses to ACh, and excitatory and depressant responses to NA were unaffected by LSD and methysergide. In contrast to these observations, Hosli et al., (1970) found that LSD antagonised excitatory responses to NA as well as those to 5-HT.

The studies discussed so far were concerned with unidentified neurone in the brain-stem. However, there are two reports in the literature about the effects of monoamines on neurones of the midline raphe nuclei. Couch (1970) observed both excitatory and depressant responses to NA and 5-HT. Both kinds of response to NA were evenly distributed amongst all the cells studied, but excitatory responses to 5-HT occurred predominantly on cells which were also excited by stimulation of the nucleus paragigantocellularis lateralis, while depressant responses to 5-HT were confined to cells which were inhibited by stimulation of this nucleus. Excitatory responses to 5-HT were blocked by LSD.

In contrast to Couch's observations, Aghajanian et al., (1972) found only depressant responses to 5-HT on raphe neurones, although both excitatory and depressant responses to NA were seen. Since LSD also was invariably depressant, Aghajanian et al., suggested that LSD might act by stimulating 5-HT receptors. The implications of these observations are discussed at greater length in II:1.2.

c) Diencephalon

Phillis & Tebecis (1967a, b) investigated the
responsiveness of thalamic neurones to NA and 5-HT. Both excitatory and depressant responses were observed, but the distribution of these effects was dependent upon the site of recording. Both amines were predominantly depressant in the more dorsally located nuclei; excitatory responses were more common in the ventral nuclei.

Tebecis (1970) reported that medial geniculate cells were mostly depressed by monoamines, although excitatory and biphasic responses were seen on a small proportion of the cells. There was no consistent correlation between the effects of the two monoamines when tested on the same neurone. Repeated applications of the monoamines led to desensitisation of excitatory, but not of depressant responses. Strychnine blocked the depressant effects of NA and 5-HT, but only when applied with much higher electrophoretic currents than were required to antagonise depressant responses to glycine.

Curtis & Davis (1962) and Philis et al., (1967) found that NA and 5-HT depressed the orthodromic field potential and spontaneous firing of lateral geniculate neurones. LSD did not antagonise this action; in fact it evoked a similar depressant response itself. These findings have been extended in a recent study by Tebecis & Di Maria (1972). Two different depressant responses to 5-HT were observed. Applied with low ejecting currents, 5-HT and LSD depressed firing induced by light and optic nerve stimulation; applied with higher currents 5-HT also depressed ACh- and glutamate-induced firing and antidromic field potentials. Tebecis & Di Maria concluded that 5-HT and LSD at low concentrations inhibited
transmitter release and at higher concentrations blocked post-synaptic receptors.

d) **Corpus Striatum**

Microelectrophoretic studies of striatal neurones have mostly been concerned with the effects of dopamine. However, two papers report the effects of NA. Bloom et al. (1965) observed both excitatory and depressant responses; depressant responses being more common. York (1970) observed excitatory and depressant responses to both NA and 5-HT. Phentolamine blocked excitatory responses, and sotalol blocked depressant responses to both amines. INPEA blocked depressant responses to NA, but did not affect depressant responses to 5-HT. Excitatory responses could sometimes be potentiated by monoamine oxidase inhibitors.

e) **Cerebellum**

Yamamoto (1967) found that neurones in the cerebellar flocculus were usually excited by NA, while neurones in the cerebellar cortex were invariably depressed by NA. More recent studies of cerebellar Purkinje cells have confirmed that depression is the most frequent response to NA (Hoffer et al., 1971b; Siggins et al., 1971; Godfraind & Pumain, 1972). Hoffer et al. (1971b) reported that depressant responses to NA could be blocked by sotalol and potentiated by desipramine and that these interactions were not affected by pretreatment of the animals with 6-hydroxydopamine, a fact which was taken to indicate that NA acted postsynaptically. The depressant effects of NA were mimicked by cyclic AMP, and
the effects of both NA and cyclic AMP were potentiated by theophylline and aminophylline, drugs which inhibit the metabolism of cyclic AMP by phosphodiesterase. Furthermore, prostaglandins E₁ and E₂ and nicotinate, which reduce cyclic AMP levels in the periphery, blocked NA and cyclic AMP depressions (Siggins et al., 1971). On the basis of these findings, Siggins et al., argued that the effects of NA on Purkinje cells were mediated by cyclic AMP.

Contrary findings were obtained by Godfraind & Pumain (1972). These workers were unable to replicate the observation that cyclic AMP depressed Purkinje cells. Moreover, they reported that theophylline was without effect on NA-induced depressions. The controversy remains unresolved.

f) Olfactory bulb

Bloom et al., (1964) observed depressant responses of olfactory bulb neurones to NA and 5-HT. Dibenamine blocked the responses to NA but did not affect responses to 5-HT or ACh. Dichlorisoprenaline was not an effective antagonist of NA. LSD and BOL blocked responses to both NA and 5-HT, but were more effective on responses to NA. The same authors (Salmoiraghi et al., 1964) found that stimulation of the lateral olfactory tract (LOT) inhibited olfactory bulb neurones. Since this inhibitory response was also blocked by dibenamine, LSD and BOL, it was suggested that the LOT inhibitory response was mediated by NA.

McLennan (1971) confirmed that mitral cells could
be depressed by NA and by LOT stimulation. However, these depressant responses were often accompanied by the excitation of other simultaneously recorded cells. Since the depression of mitral cells by NA could be blocked by bicuculline, McLennan proposed that NA acted by activating 'granule cells' which in turn inhibited the mitral cells via a GABA-mediated synapse.

g) Cerebral cortex

Krnjević & Phillis investigated the response of cortical neurones to a wide range of drugs applied by microelectrophoresis. These cells were excited by glutamate and homocysteate (Krnjević & Phillis, 1973a) and also by ACh (Krnjević & Phillis, 1963b). NA, 5-HT and a number of other amines had a predominantly depressant action (Krnjević & Phillis, 1963c). LSD was also depressant. Excitatory responses to NA and 5-HT were occasionally observed when high ejecting currents were used; however, Krnjević & Phillis suggested that these were probably of a non-specific nature. LSD failed to antagonise depressant responses to 5-HT.

Johnson et al., (1969b) have criticised Krnjević & Phillis' exclusive use of barbiturate-anaesthetised and cerveau isolé preparations. Johnson et al., confirmed the finding that monoamines were predominantly depressant in barbiturate-anaesthetised cortices, but reported that in halothane-anaesthetised and encéphale isolé preparations cortical neurones responded to NA and 5-HT more frequently with excitation than depression.
Roberts & Straughan (1967) found that while depressant responses to 5-HT were resistant to antagonists, excitatory responses could be reversibly antagonised by LSD, BOL, methysergide and cinanserin. Antagonism of responses to 5-HT occurred without there being any change in responses to ACh.

Johnson et al., (1969a) reported that α-adrenoceptor antagonists (phentolamine, dibenamine and thymoxamine) and β-adrenoceptor antagonists (propranolol, sotalol and INPEA) reversibly antagonised excitatory responses to NA. Depressant responses were infrequently affected, although antagonism of depressant responses by thymoxamine and propranolol was observed on a few cells. In the case of all the antagonists tested, NA-induced excitations were blocked without any change occurring in responses to ACh. Two antagonist (Phentolamine and sotalol) were shown to block responses to NA without affecting responses to 5-HT.

A recent series of experiments (Bradshaw et al., 1971, 1973) has shown that the tricyclic antidepressants imipramine and desipramine can modify both excitatory and depressant responses to NA and 5-HT. A brief pulse was used to apply the antidepressant, and the time-course of its effect on responses to the monoamine was observed. A small 'dose' of the antidepressant usually potentiated the response to the monoamine; when a larger 'dose' was applied, this potentiation was preceded by a period during which the responses were antagonised. Desipramine proved to be more potent than imipramine in modifying responses to NA, while imipramine was more potent in modifying responses to 5-HT.
Responses to ACh and CCh were affected in a similar manner by the antidepressants (Bevan et al., 1973).

Johnson et al., (1969a) conducted careful controls for the possible effects of $H^+$ released during the electrophoretic ejection of NA. Since NA released from solutions at pH 3 had identical effects of NA released from solutions at pH 5, these workers concluded that $H^+$ was unlikely to play a role in responses to NA. Their findings have been confirmed by Stone (1972c).

Frederickson et al., (1971) obtained results which were in direct conflict with those of Johnson et al., (1969a). They reported that NA released from solutions at pH 4 or pH 5 was predominantly depressant. However, if solutions at pH 3 or less were used, excitatory responses were frequently encountered. Similar results were obtained with 5-HT (Jordan et al., 1972a). In a subsequent paper, Jordan et al., (1972b) reported that $H^+$ released electrophoretically from HCl solutions could also excite cortical neurones. These authors concluded that their results 'clearly show that hydrogen ions applied iontophoretically from drug solutions or from solutions of HCl can cause excitation of neurones, and that such hydrogen ion effects are responsible for a major portion of the excitation observed in the cerebral cortex when NA is applied from acidic solutions' (Jordan et al., 1972b). They further argued that 'the true action of 5-HT (and NA) on cortical neurones .... is inhibitory'.

Frederickson et al., (1972) found that both excitatory and depressant responses to NA, together with depressant
responses to 5-HT, could be antagonised by phentolamine, phenoxybenzamine, dibenamine and INPEA. They suggested that in cases where excitatory responses were antagonised, the excitation was brought about by the depression of a nearby inhibitory interneurone, and that the antagonist acted by preventing this depressant action of the amine. There is at present no evidence either for or against this ad hoc speculation.

In view of the controversy about the nature of excitatory responses to the monoamine, it was decided during the present project to conduct a further investigation of the involvement of $H^+$ in these responses. The results of these experiments are described below (II:3.1.2.).

What general conclusions can be drawn about the action of NA and 5-HT on central neurones? It would seem that when the effects of barbiturate anaesthesia have been eliminated, excitatory as well as depressant responses to the monoamines can be observed in most areas of the CNS. A notable exception is the cerebellar cortex, where only depressant responses have been seen. It is unlikely that all the excitatory responses seen in various regions of the CNS are artefacts due to the electrophoretic release of $H^+$, since many observations of excitatory responses have been made using 5-HT and NA solutions at pH 5 - pH 6 (Boakes et al., 1970; Boakes et al., 1971). According to Jordan et al., (1972b), less than 2% of cortical neurones are excited when NA solutions of pH 5 are used.
In general, excitatory responses seem to be more susceptible to blockade by conventional agonists, although some reports indicate that depressant responses can also be affected. Johnson et al., (1969a) suggested that depressant responses to the monoamines might be non-specific in nature. However, the observation that substances such as bulbocapnine, 3,4-dimethoxyphenylethylamine and mescaline can block depressant responses to NA without affecting depressant responses to 5-HT or excitatory responses to either NA or 5-HT raises the possibility that the depressant actions of the monoamines are brought about by specific receptors of a type quite dissimilar from the receptor type underlying excitatory responses.

Another interesting question is whether excitatory and depressant receptors might co-exist on the same neurone. The technique of microelectrophoresis can never offer direct confirmation of such a postulate, since indirect effects cannot be excluded. For example, the initial depressant phase of a biphasic response might reflect the stimulation of inhibitory receptors on the cell under investigation, while the delayed excitatory phase might result from the depression of a more remote inhibitory interneurone (cf. Frederickson et al., 1972). On the other hand, the suggestion that both receptor types may be located on the same cell is not implausible, since such a situation is known to exist on certain molluscan neurones (Gerschenfeld, 1970; Ascher, 1972).

The fact that monoamine-sensitive neurones are
found throughout the CNS does not necessarily indicate that these cells receive input from aminergic terminals. In fact it has only been possible in a few instances to relate the effects of microelectrophoretically-applied monoamines to synaptic transmission in a specific pathway. However it is not unlikely that more aminergic pathways will be identified since monoamine-containing terminals are found in most structure in the CNS. In the present project it was not intended to relate the action of mescaline specifically to monoamine transmission, but rather to determine how mescaline interacts with NA and 5-HT receptors (sub-synaptic or otherwise) on single cortical neurones.

II:1.2. THE PHARMACOLOGY OF MESCALINE

Mescaline (3,4,5-trimethoxyphenylethylamine) belongs to a structurally heterogenous group of drugs known as the hallucinogens. The term 'hallucinogen' refers to the striking psychological effects which these drugs have in man, including hallucinations in one or more sensory modality, thought blocking, depersonalisation, and affective lability (Hoffer & Osmond, 1967; Freedman, 1969; Brawley & Duffield, 1972). Usually included among the hallucinogens are the indolic or '5-HT-like' hallucinogens (lysergic acid diethylamide (LSD), psilocybin and N,N-dimethyltryptamine (DMT)), and the phenylethylamine or 'NA-like' hallucinogens (mescaline, and a number of substituted amphetamines) (Freedman, 1969; Aghajanian et al., 1970). Excluded from this classification are the 'anticholinergic hallucinogens', tetrahydrocannabinol (THC) and amphetamine. The exclusion of these drugs by many
writers is based upon certain subtle differences between
the psychological effects of these drugs and those of the
'true hallucinogens' (Smythies, 1968; Freedman, 1969;
Brawley & Duffield, 1972), and upon the observation that
behavioural cross-tolerance which exists between mescaline,
LSD and psilocybin does not extend to THC and amphetamine
(Balastrieri, 1957; Wolbach et al., 1962; Rosenberg et al.,

It has frequently been assumed (Snyder & Richelson,
1968; Chothia & Pauling, 1969; Smythies, 1970) that the
hallucinogens share a common mode of action at the receptor
level. However, there is little evidence on which to
evaluate this claim. Most pharmacological investigations
of the hallucinogens have been concerned with one hallucinogen
only, LSD: there is a paucity of information about the
pharmacology of other hallucinogens, such as mescaline.
Moreover, as Brawley & Duffield (1972) have pointed out,
the similar phenomenological effects of the hallucinogens,
and the existence of behavioural cross-tolerance between
the various drugs of this class, does not preclude the
possibility that dissimilar drug-receptor interactions are
involved.

The work of Gaddum in the 1950's established LSD
as a potent antagonist of 5-HT on smooth muscle preparation
(Gaddum, 1955; Gaddum et al., 1957). The antagonistic
action of LSD has since been confirmed in the CNS using
the microelectrophoretic technique (Roberts & Straughan,
1967; Boakes et al., 1970). Since LSD is usually
regarded as the prototype hallucinogen, it has been proposed that antagonism of 5-HT at central receptor sites is a necessary and sufficient condition for hallucinogenic activity (Smythies, 1970). However, the evidence necessary for this hypothesis, that mescaline can antagonise the actions of 5-HT, is unclear. Costa (1956) observed that mescaline facilitated the action of 5-HT on uterine muscle, and that this action of mescaline could in fact be antagonised by LSD. On the other hand, Kawai & Yamamoto, (1966) found that mescaline and psilocybin shared with LSD the ability to antagonise the depressant effects of 5-HT on sections of guinea pig superior colliculus.

Yet antagonism of the effects of 5-HT is by no means the only pharmacological action of LSD. The excitatory effects of 5-HT on the clam heart are mimicked by LSD (Welsh, 1957; Wright et al., 1962). Similarly the contractile effect of 5-HT on human and sheep umbilical vascular smooth muscle is also reproduced by LSD (Dyer & Gant, 1973). These effects of LSD are shared by other hallucinogenic drugs, including mescaline, psilocybin and bufotenine, and the actions of both 5-HT and the hallucinogens are susceptible to antagonism by 2-bromolysergic acid diethylamide (BOL), a non-hallucinogenic congener of LSD (Greenberg, 1960; Dyer & Gant, 1973).

Aghajanian et al., (1970) observed that a wide range of intravenously administered hallucinogens, including LSD and mescaline, could inhibit the firing of single units in the raphe nucleus. These authors attributed this effect of the
hallucinogens to an action on an inhibitory neuronal feedback loop brought about via the direct stimulation of 5-HT receptors on raphe neurones. There was, and is, little evidence for such a feedback circuit in the raphe nucleus (see Aghajanian, 1972). However, the postulate that LSD stimulates 5-HT receptors on raphe neurones was in some measure supported by the observation that both drugs depressed the firing of raphe cells when applied microelectrophoretically (Aghajanian et al., 1972). On the other hand, it should be noted that LSD has been shown to depress the firing rate of neurones in many other areas of the CNS (Bloom et al., 1964; Roberts & Straughan, 1967; Boakes et al., 1970), but this action appears to be unrelated to the agonistic effects of 5-HT since it occurs on neurones that are excited by 5-HT. Furthermore, a considerable proportion of neurones in the raphe nucleus can be excited by 5-HT, and these excitatory responses can in fact be antagonised by LSD (Couch, 1970). The effect of microelectrophoretically applied mescaline on raphe neurones has not been examined.

The controversy as to whether LSD mimics or antagonises the actions of 5-HT (Boakes et al., 1970; Aghajanian, 1972) would not seem to be very fruitful. LSD apparently does both. Indeed, the two actions of LSD may not be mutually exclusive even on one preparation. For example, Cottrell (1970a, b) found that high concentrations of LSD blocked, but low concentrations mimicked the post-synaptic effects of stimulating the giant 5-HT containing neurones in the cerebral ganglion of Helix.
So far as theories of hallucinogenesis are concerned, there would seem to be no sound basis for the preferential acceptance of either the agonistic or the antagonistic action of LSD as being the relevant action, since other hallucinogens such as mescaline and psilocybin share both these effects of LSD depending on the particular preparation studied.

Most pharmacological studies of LSD have centred around its effects on 5-HT systems. However in the case of mescaline several authors have drawn attention to the structural resemblance between this drug and NA (Hoffer & Osmond, 1967; Freedman, 1969; Aghajanian et al., 1970; Brawley & Duffield, 1972), and it has been suggested that mescaline might exert its central effects via an action on adrenergic receptors (Speck, 1957; Hoffer & Osmond, 1967; Dill, 1972). Unfortunately, most of the studies which purport to show that mescaline can act at adrenergic sites have been conducted on highly complex test systems, so that any interpretation in terms of receptor mechanisms is at best dubious. Speck (1957) reported that mescaline produced bradycardia and hypoglycaemia in rats; these effects were blocked by adrenaline, suggesting that mescaline acted as an adrenergic antagonist. Morton & Malone (1969), however found that mescaline-induced bradycardia was not due to simple \( \beta \)-adrenoceptor blockade, since isoprenaline-induced tachycardia was not affected by mescaline. Parker & Hildebrand (1962) found that pretreatment with \( \alpha \)-benzamine blocked the hypertensive effect of mescaline in anaesthetised
cats and suggested that mescaline was an agonist at adrenergic receptors. Yet many years previously Raymond-Hamet (1932) and Grace (1934) had shown that the hypertensive effect of mescaline was of complex origin; Grace had tentatively suggested that cholinergic receptors at sympathetic ganglia might be involved.

Equally complex test systems have been used in studies of the central pharmacology of mescaline. Ratcliffe (1971) studied the influence of mescaline upon the gross effects of intracerebral injections of NA: the hypothermic response, and the potentiation of amylobarbitone-induced anaesthesia. Since systemically injected mescaline blocked both these actions of NA, Ratcliffe concluded that mescaline probably acts by antagonising catecholamines at central adrenergic receptor sites. Lill (1972) reached the same conclusion on the basis of his experiments in which he observed that dyskinesia induced by intrastriatally administered mescaline was reversed by concomitant intrastriatal injections of isoprenaline or dopamine.

In none of these studies purporting to show an action of mescaline at adrenergic sites was it possible to exclude an action at 5-HT receptors. Whenever it has been possible to differentiate reliably between the effects of NA and 5-HT, for example on the Venus heart preparation (Greenberg, 1960), the human umbilical vein or sheep umbilical artery (Dyer & Gant, 1973), or the rat stomach strip (Vane, 1960), the action of mescaline has been found to be related to that of 5-HT rather than to that of NA. In the case of the Venus
heart and the umbilical vasculature, the action of mescaline was shared by other hallucinogens including LSD and psilocybin (Wright et al., 1962; Dyer & Gant, 1973). In this context it is relevant to note that Snyder & Richelson (1968) have suggested that the preferred steric configuration of the mescaline molecule closely resembles the indole nucleus of the 5-HT molecule. However, the plausibility of Snyder & Richelson's conformations has been contested by Chothia & Pauling (1969).

To the best of my knowledge there have been only four reports of the effect of mescaline applied to neurones in the CNS by microelectrophoresis. Curtis & Davis (1962) used mescaline as one of a wide range of amines tested on orthodromically excited lateral geniculate neurones in the cat. Mescaline, like most of the compounds tested, including NA, adrenaline, isoprenaline, 5-HT and LSD had a depressant action on these cells. Krnjević & Phillis (1963c) found that the action of mescaline on cortical neurones was 'feebly depressant'. In both these studies barbiturate anaesthesia was used. Since excitatory responses to amines are particularly sensitive to barbiturates (Johnson et al., 1969), these studies are perhaps of less interest than two more recent investigations using unanaesthetised preparations.

Roberts & Straughan, (1968) observed that mescaline, like NA, could evoke both excitatory and depressant responses on cortical neurones, although on any one cell mescaline and NA did not always exert similar effects. Correlations
between the effects of mescaline and 5-HT were not studied. Somewhat dissimilar results were obtained by Gonzalez-Vegas (1971). This author found that the depressant, but not the excitatory responses of brain-stem neurones to NA and dopamine could be antagonised by mescaline; excitatory and depressant responses to 5-HT were not affected, neither were excitatory responses to ACh and depressant responses to glycine and GABA. This observation by Gonzalez-Vegas provides the only direct evidence in the literature that mescaline can act at adrenergic sites. This finding of an antagonistic effect of mescaline is in contrast to the observations of Roberts & Straughan, who reported that mescaline did not specifically antagonise responses to NA. A further discrepancy between the two studies is the infrequent occurrence of agonistic effects of mescaline in Gonzalez-Vegas' experiments (12% of the cells excited and 9% depressed by mescaline).

On the basis of the foregoing discussion one may draw the following general conclusions about the pharmacology of mescaline and its relationship to the more extensively studied hallucinogen, LSD.

1. Mescaline, like LSD can mimic or antagonise the actions of 5-HT on certain isolated preparations.
2. The possibility that mescaline interacts specifically with peripheral and/or central adrenergic receptors remains an open question.
3. The central pharmacology of mescaline has received little attention. When injected intravenously, both mescaline and LSD inhibit the firing of raphe neurones. When applied by microelectrophoresis, mescaline can excite and depress cortical neurones and can antagonise the depressant effects of NA on brain-stem neurones. LSD, on the other hand, is notable for its antagonistic effect on responses to 5-HT.

The experiments described below are a further investigation of the action of mescaline on cortical neurones. Specifically, it was intended to determine which receptors mescaline might act upon on these cells.
II:2. METHODS

All the experiments reported in Part II were conducted on single spontaneously-active neurones of the cerebral cortices of cats anaesthetised with halothane. The methods used for the preparation of the animals and the 5-barrelled micropipettes, for the recording of action potentials, and for the measurement of neuronal responses are described in I:3.2.

The following drug solutions were used in the micropipettes: mescaline hydrochloride (0.2 M, pH 3.5-4.5), noradrenaline bitartrate (0.2 M, pH 3.0-3.5), 5-hydroxytryptamine (0.2 M, pH 3.0-3.5), acetylcholine chloride (0.2 M pH 4.0-4.5), sotalol hydrochloride (0.1 M, pH 4.0-5.0), methysergide maleate (0.01 M, pH 3.0-4.0).

In one series of experiments in which the effects of pH were examined, a different method was used for the manufacture of the micropipettes. 5-barrelled pipettes were pulled, and their tips broken to the desired diameter (3-6 μ). Freshly prepared drug solutions were then introduced into the barrels, and air was expelled from the tips by centrifugation at 3000 RPM for 30 min (see Curtis, 1964). The solutions used in these experiments were: noradrenaline bitartrate (0.2 M, pH 3.1), noradrenaline bitartrate (0.2 M, pH 5.0), and hydrochloric acid (0.01 M, pH 2.0). The solution of noradrenaline bitartrate at pH 3.1 (± 0.1) was obtained by dissolving L-noradrenaline bitartrate in glass distilled water, whereas the solution at pH 5.0 (± 0.1) was obtained by adjustment with 1 N NaOH solution. The pH values of
these solutions were measured using a Pye Universal pH meter (model EJ 660). Hydrochloric acid solutions of 0.01 M were used because this was the lowest concentration which adequately carried electrophoretic currents.

When a well isolated cell was identified, the agonists were applied in a regular sequence. Ejecting currents of +50 or +100 nA were normally used to apply the agonists: ejection times varied between 30 and 100 sec. Between ejecting pulses a retaining current of -25 nA was passed. If the neurone appeared to be insensitive to an agonist, or if the responses to the various agonists differed very greatly in size, the ejecting currents and/or ejection times were adjusted in order to evoke comparable responses to all the agonists.

Except when it was necessary to use four drug-containing barrels, 'current balancing' was always employed (see I:3.2.2.). Current effects were also tested routinely by the passage of a current, equal to the ejecting currents used to apply the agonists, through the NaCl-containing barrel. If the passage of this current evoked a noticeable response, the cell was discarded.

In evaluating the actions of sotalol and methysergide, the method of continuous application was used (see I:3.3.3.1.). After control responses to all the agonists had been obtained, the antagonist was applied continuously and the time course of the developing antagonism was followed. If necessary, the intensity of the ejecting current used to apply the antagonist was increased, until complete antagonism was observed. Then
the application of the antagonist was stopped, and the time-
course of recovery was observed. The figures illustrating
the action of the antagonists (Figs. 36 - 46) show excepts
of the ratemeter tracings taken before, during and after the
application of the antagonist. (A complete ratemeter record
of an antagonist study is shown in Fig. 38B). Also shown in
these figures are the 'total spike numbers' (I:3.2.4.) of all
the responses observed throughout the study, expressed as
percentages of their respective control responses. Responses
were considered to be antagonised if the total spike number
was reduced to less than 50% of the control. Similarly,
responses were considered to be potentiated if there was an
increase of more than 50% in the total spike number.

In evaluating the effects of DMI on responses to
mescaline, the method of pulse application (I:3.3.2.2.) was
used. Although this method was thought to be inferior to
the method of continuous application, it was considered
suitable in the present studies since previous observations
of the actions of DMI on responses to NA and 5-HT had used
this method.
RESULTS

II: 3.1. THE EFFECT OF NA, 5-HT AND ACh.

II: 3.1.1. The occurrence of excitatory and depressant responses

The failure of a cell to respond to any particular drug may result from a multitude of causes. In particular, one can never be sure that an appreciable quantity of drug actually leaves the pipette; Table 1 shows that some pipettes exhibit low values of the transport number of NA despite apparently adequate passage of current. Thus the failure of a neurone to respond to the passage of an ejecting current cannot be considered a sufficient proof of neuronal insensitivity to the drug. Accordingly, only cells which exhibited clear responses to the drug in question are included in the following analysis.

Examples of the types of responses observed with ACh, NA and 5-HT are shown in Fig. 26.

a. ACh

10 cells were studied which yielded consistent responses to ACh. In every case the response was excitatory.

b. NA

Of 161 cells responding to NA, 126 were excited, and 29 were depressed by the drug. The remaining 4 cells responded in a biphasic fashion; a depression was followed by an excitation. The predominance of excitatory responses was highly significant \( (\text{Binomial test}; \ p < 0.0001) \).

c. 5-HT

Of 85 cells responding to 5-HT, 63 were excited and
FIG. 26. Examples of the types of response to ACh, 5-HT, NA and mescaline observed in these experiments. Each excerpt is taken from ratemeter recordings of the firing rate of an individual cortical neurone. Ordinates: firing rate (spikes/sec); abscissae: time (min). Horizontal bars indicate drug applications (numbers refer to intensity of ejecting current (nA)). Only excitatory responses to ACh were observed, whereas excitatory, depressant and biphasic responses were observed with all three monoamines.
20 were depressed by the drug. The remaining 2 cells showed biphasic responses. The predominance of excitatory responses was highly significant (Binomial test; \( p < 0.0001 \)).

A greater proportion of cells were depressed by 5-HT (24.1%) than were depressed by NA (18.5%).

II: 3.1.2. The effect of pH

The predominance of excitations to NA and 5-HT in the present experiments is in agreement with other studies of cortical neurones (Roberts & Straughan, 1967; Johnson et al., 1969a; Johnson et al., 1969b). In order to ascertain whether the predominance of excitatory responses was due to the acidic solutions used in the present experiments (Frederickson et al., 1971; Jordan et al., 1972b), the responses of sixteen neurones to NA released from solutions at pH 3.1 and 5.0 and to \( H^+ \) released from a HCl solution were compared.

a. Comparison of NA (pH 3.1) and NA (pH 5.0).

Of the 16 cells studied, the 13 neurones which were excited by NA (pH 3.1) were also excited by NA (pH 5.0), and the 3 neurones depressed by NA (pH 3.1) were also depressed by NA (pH 5.0). No cell responded differently to the drug at different pH values. An example of this finding is shown in Fig. 27.

b. Effect of \( H^+ \)

Of the 16 NA-sensitive cells, 15 did not respond to \( H^+ \) even when currents of up to +200 nA were used (see Figs. 27 and 29). One neurone, which was depressed by NA, was also
FIG. 27. Responses of a single cortical neurone to NA ejected from solutions at different pH. Continuous recording of the firing rate of a single cortical neurone. Ordinate: firing rate (spikes/sec); abscissa: time (min). Horizontal bars indicate drug applications (numbers refer to intensities of ejecting currents (nA)). The neurone was excited by NA (pH 3.1) and NA (pH 5.0), but did not respond to H⁺.
depressed by $H^+$. Changes in spike amplitude did not occur.

The application of $H^+$ frequently reduced the size of the response to subsequent application of NA. Furthermore, when NA (pH 5.0) and $H^+$ were ejected simultaneously from adjacent barrels of the micropipette there was a reduction in the size of the excitatory response (Fig. 28).

c. Current-response studies

It was observed on 9 cells that NA (pH 3.1) evoked a larger response than NA (pH 5.0) (see Fig. 27). As this suggested a difference in potency between NA (pH 3.1) and NA (pH 5.0), we conducted detailed comparative studies on three cells. A range of current intensities was used to compare the responses of the same cell to NA (pH 3.1) and NA (pH 5.0). The results obtained from one cell are displayed in Fig. 29 and Fig. 30. At the lower current intensities NA (pH 3.1) and NA (pH 5.0) were approximately equipotent, whereas NA (pH 3.1) was considerably more potent when currents of higher intensities were used.

II:3.2. RESPONSES TO MESCALINE

II:3.2.1. The occurrence of excitatory and depressant responses

Both excitatory and depressant responses to mescaline were observed. A total of 199 cells which yielded consistent responses to mescaline were observed in these experiments. 159 cells were excited, 34 were depressed, and 6 responded in a biphasic fashion. The predominance of excitatory responses is highly significant, (binomial test: $p < 0.0001$). Examples of these responses are shown in Fig. 26.
FIG. 28. Effect of H4 on excitatory responses of a cortical neurone to NA (pH 5.0). The size of the excitatory response to NA (nM) was reduced when it and H4 were applied simultaneously. Continuous recording of the firing rate of a single cortical neurone. Ordinate: firing rate (spikes/sec). Abscissa: time (min). Horizontal bars indicate drug applications (numbers refer to intensities of ejected currents (nA)).
FIG. 29. Excitatory responses of a cortical neurone to NA applied from solutions at different pH. Continuous recording of the firing rate of a single cortical neurone. Ordinate: firing rate (spikes/sec); abscissa: time (min). Horizontal bars indicate drug applications (numbers refer to intensities of ejecting current (nA)). A-D: NA (pH 3.1) and NA (pH 5.0) applied with successively higher currents evoked progressively larger responses. A: higher current intensities NA (pH 3.1) evoked larger responses than NA (pH 5.0). E-H: H⁺ (25 - 200 nA) failed to evoke responses.
FIG. 30. Magnitude of excitatory responses of a cortical neurone to NA applied from solutions at different pH.

Ordinate: total spike number; abscissa: intensity of ejecting current (nA), on a log. scale. Open circles: NA (pH 3.1); closed circles: NA (pH 5.0); open triangles: H⁺. Data are derived from the study shown in Fig. 29.

NA (pH 3.1) was more potent than NA (pH 5.0) at higher ejecting current intensities.
II:3.2.2. Qualitative comparison of the effects of mescaline with the effects of NA and 5-HT

a. Mescaline and NA (Table 6A)

163 cells were observed which yielded consistent monophasic responses to both mescaline and NA. With one exception, all these cells responded in the same 'direction' to the two drugs (i.e. either excited by both drugs or depressed by both drugs).

b. Mescaline and 5-HT (Table 6B)

90 cells were observed which yielded consistent monophasic responses to both mescaline and 5-HT. 19% of these cells responded in opposite directions to the two drugs.

c. NA and 5-HT (Table 6C)

Responses to NA and 5-HT were compared on 70 cells. 21% of these cells responded in opposite directions to these two agonists.

The comparisons are summarised in Table 6D. It is apparent that, while mescaline and NA behaved in a qualitatively similar way on nearly all the cells tested, mescaline behaved differently from 5-HT on a considerable proportion of the cells.

The NA-mescaline profile is significantly different from the NA-5-HT profile ($\chi^2$ test; $p < 0.01$). On the other hand, the mescaline-5-HT profile did not differ significantly from the NA-5-HT profile. This may be taken as a further indication of the similarity of action of mescaline and NA.

d. Mescaline, NA and 5-HT (Fig. 31)

66 cells were studied which yielded consistent responses to all three drugs. 51 of these cells responded in the same
FIG. 51. Comparison of the effects of 5-HT, NA and mescaline on cortical neurones.

Continuous ratemeter recordings of the firing rates of four individual cortical neurones.

A: a neurone which was excited by all three monoamines.

B: a neurone which was depressed by all three monoamines.

C: a neurone which was depressed by mescaline and excited by 5-HT.

D: a neurone which was excited by 5-HT and depressed by mescaline.

The four traces illustrate the four patterns of responses observed in these experiments.

Bars indicate drug applications (numbers refer to intensities of injecting currents (nA)).

Ordinates: Firing rate (spikes/sec) / Abscissae: Time (min).

The legend for the neurones is as follows:

A: B: C: D:
TABLE 6. Comparison of the effects of mescaline with the effects of NA and 5-HT.

+ : number of cells excited, - : number of cells depressed, (biphasic responses are not included).

<table>
<thead>
<tr>
<th></th>
<th>MESCALINE</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>NA</td>
<td>+ 134</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>- 1</td>
<td>28</td>
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N = 163 cells

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</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5-HT</td>
<td>+ 63</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>- 12</td>
<td>10</td>
</tr>
</tbody>
</table>

N = 90 cells

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
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<td>-</td>
</tr>
<tr>
<td>5-HT</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>7</td>
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</tbody>
</table>

N = 70 cells

<table>
<thead>
<tr>
<th></th>
<th>Same</th>
<th>Opposite</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA, 5-HT</td>
<td>79%</td>
<td>21%</td>
</tr>
<tr>
<td>NA, Mescaline</td>
<td>&gt; 99%</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>5HT, Mescaline</td>
<td>81%</td>
<td>19%</td>
</tr>
</tbody>
</table>

* Significantly different from NA, 5-HT ($\chi^2$ test: p < 0.001)
† Not significantly different from NA, 5-HT.
direction to all the drugs (Fig. 31A & B), whereas with the remaining 15 cells both excitatory and depressant responses were seen. In every one of these 15 cases, the cell responded in the same direction to mescaline and NA and in the opposite direction to 5-HT. 3 of these cells were depressed by mescaline and NA and excited by 5-HT (Fig. 31C); 12 were excited by mescaline and NA and depressed by 5-HT (Fig. 31D).

II:3.2.3. Potency comparisons

It was frequently observed that mescaline evoked somewhat smaller responses than NA or 5-HT when applied with equivalent ejecting pulses (e.g. see Fig. 40), and it was often necessary to apply mescaline with a higher ejecting current or a longer ejection time in order to obtain comparable responses (e.g. see Figs. 37, 41). As this suggested a difference in potency between mescaline and the other amines, detailed comparisons of the relative potencies of mescaline and NA were conducted on 9 cells, using a range of ejecting current intensities. With the exception of one cell, mescaline was invariably less potent than NA. This lower potency of mescaline is statistically significant (binomial test: p = 0.02). In the case of one cell on which mescaline and 5-HT were compared, mescaline was found to be less potent than 5-HT. Examples of these observations are shown in Fig.32.

II:3.3. THE EFFECT OF MESCALINE ON RESPONSES TO NA

The effects of mescaline on responses to NA were investigated in two ways. With cells which responded to
FIG. 32. Comparison of potencies of mescaline and 5-HT and mescaline and NA on cortical neurones.

A: Comparison of mescaline and 5-HT. Excerpts from ratemeter recording of the firing rate of a single cortical neurone. Ordinates: firing rate (spikes/sec); abscissae: time (min). Horizontal bars indicate drug applications (number refer to intensities of ejecting currents). Mescaline was less potent than 5-HT.

B: Comparison of mescaline and NA (different cell from A). Total spike number is plotted against intensity of ejecting current (nA), on a log, scale. Closed circles: NA; closed triangles: mescaline. Mescaline was less potent than NA.
mescaline, attempts were made to determine whether cross-desensitisation occurred between the two drugs. With cells which did not respond to mescaline, or on which mescaline evoked only very weak responses, mescaline was applied simultaneously with NA to see whether it could antagonise the response to NA.

II:3.3.1. Cross-desensitisation

Desensitisation to mescaline was observed on 12 of the 15 cells tested. In these experiments mescaline was either applied continuously for a prolonged period, or was applied repeatedly at brief intervals. With both these procedures a reduction in the size of the response to mescaline could be observed (Fig. 33). Recovery from this desensitisation was slow and erratic, and in some of the cells could not be observed at all.

Studies were conducted on 9 cells in which NA was applied immediately after the cell had been desensitised to mescaline. With 6 of these cells a reduction in the size of the response to NA was observed. This 'cross-desensitisation' did not appear to be specific, since responses to 5-HT and ACh were also affected. (In the case of one cell only, the response to NA was reduced without a significant change occurring in the response to ACh.) Examples of these observations are shown in Fig. 34, 35.

II:3.3.2. Antagonism

The effectiveness of mescaline as an antagonist of NA was tested on five cells which did not respond or which
FIG. 53. Desensitisation of excitatory responses to mescaline. Excerpts from ratemeter recording of the firing rates of three individual cortical neurones. Ordinates: firing rate (spikes/sec); abscissae: time (min). Horizontal bars indicate drug applications (numbers refer to intensities of ejecting currents (nA)).

A: two examples of the desensitisation of excitatory responses during the prolonged application of mescaline.

B: an example of the desensitisation of excitatory responses following repeated applications of mescaline at short intervals.
FIG. 34. Cross-desensitisation between mescaline and NA. Ratemeter recording of the firing rate of a single cortical neurone. Ordinate: firing rate (spikes/sec); abscissa: time (min). Horizontal bars indicate drug applications (numbers refer to intensities of ejecting currents (nA)).

Upper trace: control responses to ACh and NA; middle trace: repeated application of mescaline resulted in desensitisation of excitatory response; lower trace: following this desensitisation of the response to mescaline the response to NA was also abolished. The cross-desensitisation was not specific, since the response to ACh was also affected.
FIG. 35. Cross-desensitisation between mescaline and NA. Continuous ratemeter recording of the firing rate of a single cortical neurone. Ordinate: firing rate (spikes/sec); abscissa: time (min). Horizontal bars indicate drug applications (numbers refer to intensities of ejecting currents [nA]). Upper trace: control response to NA and ACh; middle trace: repeated application of mescaline resulted in desensitisation of excitatory response; lower trace: following this desensitisation of the response to mescaline the response to NA was also abolished. In the case of this cell the cross-desensitisation was specific, in that the response to ACh was not affected.
yielded only weak responses to mescaline. In four of these cells responses to NA were reduced in the presence of mescaline. Responses to 5-HT were also affected. Recovery from this antagonism was slow, the original responses re-appearing about one hour after the mescaline application. The observation is illustrated in Fig. 36.

II:3.4. THE EFFECT OF ANTAGONISTS AND PROTAGONISTS ON RESPONSES TO MESCALINE

II:3.4.1. The effect of a NA-antagonist: sotalol.

a. Effect on excitatory responses

The effect of sotalol upon excitatory responses to mescaline was investigated on 20 cells. On 17 of these cells, the response to mescaline was reduced in the presence of sotalol. Ejecting currents of +5 to +25 nA applied for 5 to 15 min were usually adequate to antagonise responses to mescaline. Occasionally, however, currents as high as +50 nA were needed to abolish the responses completely (e.g. see Fig. 37). Recovery from the antagonism usually occurred 5 to 20 min after terminating the application of sotalol, although after a prolonged application of sotalol recovery was sometimes slower (e.g. see Fig. 38). On three cells the development of antagonism was preceded by a potentiation of the response (as judged by an increase in the total spike number). This potentiation was only observed when sotalol was applied with ejecting currents of +10 nA or less, and was always superceded by antagonism when the intensity of
FIG. 36. Effect of mescaline on excitatory responses to NA and 5-HT. A, B, C, D: excerpts from the ratemeter recording of the firing rate of a single cortical neurone. Ordinates: firing rate (spikes/sec); abscissae: time (min). Time from the onset of the study is indicated below the abscissae. Horizontal bars indicate drug applications (numbers refer to ejection current intensities (nA)). A: NA and 5-HT evoked excitatory responses; mescaline failed to evoke a response. B: simultaneous application of mescaline abolished the response to NA and markedly reduced the response to 5-HT. C: partial recovery of response to NA, response to 5-HT still absent. D: recovery of response to NA, and partial recovery of response to 5-HT.

Lower graph shows the time-course of the entire study. Ordinate: size of response to agonist (total spike number, percent; abscissa: time (min). The response to each agonist is expressed as a percent of its respective control. Solid bars above the graph indicate applications of mescaline. Each point represents a single response to an agonist. Letters below the time base indicate sections of the study illustrated in the ratemeter tracings above.
the ejecting current was increased.

The effects of sotalol on responses to mescaline and on responses to NA were compared on 9 cells. In every case, when the responses to mescaline was antagonised the response to NA was also antagonised. The time-courses of the development of antagonism and of recovery were similar for the two drugs. An example is shown in Fig. 37.

Sotalol also antagonised responses to 5-HT. In the case of 7 cells on which mescaline and 5-HT were compared, it was found that sotalol was equally effective in antagonising the responses to both agonists. The time-courses of antagonism and recovery were similar for the two drugs. An example is shown in Fig. 38.

Responses to ACh were resistant to sotalol. ACh was used as a control agonist on 5 cells. In these studies sotalol abolished the responses to mescaline without affecting responses to ACh. An example is shown in Fig. 39.

b. Effect on depressant responses

On two cells, sotalol failed to antagonise depressant responses to mescaline and 5-HT.

II:3.4.2. The effect of a 5-HT-antagonist: methysergide

a. Effect on excitatory responses

The effect of methysergide upon excitatory responses to mescaline was investigated on 20 cells. On 15 of these cells, responses to mescaline were reduced in the presence of methysergide. On 4 of the remaining 5 cells methysergide caused a reduction in the spontaneous firing rate, from which
FIG. 37.  Effect of sotalol on excitatory responses to NA and mescaline.  A, B, C: excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in previous figs.).  A: control responses to NA and mescaline.  B: simultaneous application of sotalol abolished the responses to both monoamines.  C: partial recovery of responses after application of sotalol had been terminated.

Lower graph: time course of entire study (as in previous figs.).
RESPONSE TO AGONIST

A

spikes/sec

NA 100

MESC 150

B

spikes/sec

SOTALOL 50

C

spikes/sec

RESPONSE TO AGONIST

total spike number (percent)

SOTALOL

15 25 50

O MESC

△ NA

A

0 10 20 30 40 50 60 min

B

C
FIG. 38A. Effect of sotalol on excitatory responses to 5-HT and mescaline. A, B, C, D: excerpts from the rater-meter recording of the firing rate of a single cortical neurone (as in previous figs.). A: control response to 5-HT and mescaline. B: response to 5-HT and (to a lesser extent) response to mescaline were increased in size during the application of sotalol (5 nA). C: responses to both agonists were abolished in the presence of sotalol (20 nA). D: partial recovery of response to 5-HT in the presence of sotalol (5 nA). The increase in the size of the response to mescaline did not exceed the 50% criterion, and therefore was not classified as potentiation (see text). The complete rater-meter recording of this study is shown in Fig. 38B.
Response to Agonist

A

B

C

D

SOTALOL

RESPONSE TO AGONIST

total spike number (percent)

SOTALOL

○ MESC
□ 5-HT

min

min

min

min
FIG. 383. Effect of sotalol on excitatory responses to 5-HT and mescaline. Continuous ratemeter recording of the firing rate of a single cortical neurone. Excerpts from this record, and a graphical representation of the time-course of the study are shown in Fig. 38A.
FIG. 40. Effect of methysergide on excitatory responses to 5-HT and mescaline. A, B, C, D: excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in previous figs.). A: control responses to mescaline and 5-HT. B: partial antagonism of responses to both monoamines in the presence of methysergide (5 nA). C: complete antagonism of responses to both monoamines in the presence of methysergide (10 nA). D: partial recovery of responses after the application of methysergide had been terminated.

Lower graph: time-course of the entire study (as in previous figs.).
RESPONSE TO AGONIST

### Spike Number and Percentage

<table>
<thead>
<tr>
<th>OPCD</th>
<th>Spikes/sec</th>
</tr>
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<tbody>
<tr>
<td>ACh 50</td>
<td>100 spikes/sec</td>
</tr>
<tr>
<td>MESC 50</td>
<td>100 spikes/sec</td>
</tr>
</tbody>
</table>

### Graphs

- **A**: Graph showing spike frequency with time for ACh 50 and MESC 50.
- **B**: Graph showing spike frequency with time for Sotalol 10.
- **C**: Graph showing response to agonist over time for MESC and ACh.

**Graph Details**

- **Y-axis**: Spike number (percent)
- **X-axis**: Time (minutes)
- **Legend**:
  - ○ MESC
  - □ ACh

**Legend Notation**
- ACh 50
- MESC 50
- Sotalol 10
the cell did not recover, so that the studies had to be abandoned.

Methysergide usually antagonised responses to mescaline when ejecting currents of +5 to +25 nA were used. The development of antagonism was somewhat slower than was the case with sotalol. Similarly, recovery was slower after the termination of the methysergide application; in some cases two or three hours elapsed before the responses to the agonists reappeared.

Potentiation was seen on 5 cells. As was the case with sotalol, this potentiation occurred with lower ejecting currents and was invariably superseded by antagonism when the intensity of the ejecting current was increased. The degree of potentiation appeared to be greater with methysergide than with sotalol.

The effects of methysergide upon excitatory responses to mescaline and 5-HT were compared on 11 cells. Methysergide antagonised both responses with equal effectiveness. The time-courses of the development of antagonism and of recovery were similar for the two drugs. An example is shown in Fig. 40.

Responses to NA were also sensitive to methysergide. The effects of methysergide on responses to mescaline, 5-HT and NA were compared on 5 cells. Methysergide appeared to be equally effective in antagonising responses to all three agonists. Fig. 41 shows the results obtained from one cell on which antagonism alone was observed. Fig. 42 shows the results obtained from another cell, on which both potentiation and antagonism were seen. It is apparent that on this cell
FIG. 40. Effect of methysergide on excitatory responses to 5-HT and mescaline. A, B, C, D: excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in previous figs.). A: control responses to mescaline and 5-HT. B: partial antagonism of responses to both monoamines in the presence of methysergide (5 nA). C: complete antagonism of responses to both monoamines in the presence of methysergide (10 nA). D: partial recovery of responses after the application of methysergide had been terminated.

Lower graph: time-course of the entire study (as in previous figs.).
FIG. 41. Effect of methysergide on excitatory responses to 5-HT, NA and mescaline. A, B, C: excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in previous figs.). A: control responses to 5-HT, NA and mescaline. B: almost complete abolition of responses to all three monoamines in the presence of methysergide (10 nA). C: partial recovery of responses after the application of methysergide had been terminated. Lower graph: time-course of the entire study (as in previous figs.).
FIG. 42. Effect of methysergide on excitatory responses to 5-HT, NA and mescaline. Ordinate: size of response to agonists (total spike number, expressed as percentage of mean control); abscissa: time (hours). Closed circles: 5-HT; closed triangles: NA; open circles: mescaline.

The responses to all three monoamines were potentiated in the presence of methysergide (5, 10, 20 nA). When the ejecting current for methysergide was increased to 40 nA the responses were abolished. Partial recovery of the responses occurred after the application of the antagonist had been terminated.
RESPONSE TO AGONIST
TOTAL SPIKE NUMBER (PERCENT)

RUNNING TIME (HOURS)

METHYSERGIDE (nA)

° MESC
• 5HT
△ NA
potentiation was most pronounced in the case of the response to 5-HT, whereas there was little potentiation in the case of the response to NA.

Responses to ACh were resistant to methysergide. ACh was used as a control agonist on 7 cells. In every case, the response to mescaline was antagonised without any gross change occurring in the response to ACh. Fig. 43 shows the results obtained from a cell on which only antagonism was seen. The study illustrated in Fig. 44 shows potentiation of the response to mescaline followed by antagonism as the ejecting current used to apply methysergide was increased.

b. Effect on depressant responses

The effect of methysergide on depressant responses to mescaline was observed on 6 cells. On 4 of these cells the response to mescaline was antagonised. The antagonism was preceded by potentiation in two cases. Responses to 5-HT and NA were also affected; one study in which mescaline and 5-HT were compared is shown in Fig. 45.

When the application of methysergide was continued after the responses had been antagonised, 'reversal' of the responses was sometimes observed: the depression was replaced by excitation. An example of this observation is shown in Fig. 46. In this study the depressant responses to mescaline, NA and 5-HT were all antagonised, and the responses to mescaline and NA were reversed. After the application of methysergide had been terminated, the depressant responses to mescaline and 5-HT reappeared and the excitatory response to NA was reduced in size.
FIG. 43. Effect of methysergide on excitatory responses to mescaline and ACh. A, B, C: excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in previous figs.). A: control responses to ACh and mescaline. B: in the presence of methysergide (10 nA), the response to mescaline was antagonised, but the response to ACh was relatively unaffected. C: recovery after the application of methysergide had been terminated. Lower graph: time-course of the entire study (see previous figs.).
FIG. 44. Effect of methysergide on excitatory responses to mescaline and ACh. A, B, C, D: excerpts from the rate-meter recording of the firing rate of a single cortical neurone (as in previous figs.). A: control responses to ACh and mescaline. B: in the presence of methysergide (5 nA) the response to mescaline was potentiated, but the response to ACh was not affected. C: when the ejecting current for methysergide was increased to 10 nA the response to mescaline was abolished, but the response to ACh was unchanged. D: recovery, after the application of methysergide had been terminated.

Lower graph: time-course of the entire study (as in previous figs.).
FIG. 45. Effect of methysergide on depressant responses to 5-HT and mescaline. Excerpts from the ratemeter recording of the firing rate of a single cortical neurone. A: effect on depressant responses to 5-HT. Left hand trace: control response to 5-HT. Centre trace: during the application of methysergide (25 nA, 15 min) the response to 5-HT was markedly reduced. Right hand trace: partial recovery of response 14 min after the application of methysergide had been terminated. B: a second study carried out on the same neurone showing effect on depressant responses to mescaline. Left hand trace: control response to mescaline. Centre trace: during the application of methysergide (25 nA, 5 min) the response to mescaline was abolished. Right hand trace: partial recovery of the response 7 min after the application of methysergide had been terminated.
FIG. 46. Effect of methysergide on depressant responses to mescaline, NA and 5-HT. Ratemeter recording of the firing rate of a single cortical neurone (as in previous figs.).

A: depressant responses to mescaline, NA and 5-HT.

B, C: during the application of methysergide (5 nA) the depressant responses to mescaline and NA were reversed and the depressant response to 5-HT was abolished.

D, E: after the application of methysergide had been terminated, the depressant responses to mescaline and 5-HT reappeared and the excitatory response to NA was reduced.
II:3,4,3. The effect of a tricyclic antidepressant: desipramine

The effects of desipramine (DMI) upon excitatory responses to mescaline were observed on 5 cells. DMI was always applied in the form of a pulse of +25 nA applied for 20 to 35 sec. In all 5 cases the responses to mescaline was at first reduced in size. Later a potentiation of the response could be seen. Recovery of the original response occurred 30 to 60 min after the application of DMI. An example is shown in Fig. 47.
FIG. 47. Effect of DMI on excitatory responses to mescaline. A, B, C, D: excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in previous figs.). A: control response to mescaline. B: immediately after the application of DMI the response to mescaline was somewhat reduced in size. C: the response to mescaline was later potentiated. D: recovery of the response to mescaline. Lower graph: time-course of the entire study (as in previous figs.).
II:4. **DISCUSSION**

II:4.1. **THE EFFECT OF NA, 5-HT AND ACh**

The finding that ACh had only an excitant action in these experiments is in keeping with the observations of Krnjević & Phillis (1963b) and Johnson et al., (1969a). Depressant responses of cortical neurones to ACh, such as have been described by Stone (1972b) and Jordan & Phillis (1972), were not seen.

The predominance of excitatory responses to NA and 5-HT in these experiments is in agreement with the findings of Roberts & Straughan (1967) and Johnson et al., (1969a). Johnson et al., (1969b) reported that excitatory responses to the monoamines occurred more frequently in halothane-anaesthetised cats than in unanaesthetised encephale isolé preparations. It is not possible to tell the extent to which halothane contributed to the response distribution in the present experiments. However, it may be worth mentioning that excitatory responses occurred somewhat more frequently in the present experiments than they did in Johnson et al.'s halothane-anaesthetised animals.

The present results confirm the observations of Johnson et al., (1969a) and Stone (1972) that NA ejected from solutions at high and low pH values has qualitatively the same effect. Thus it is unlikely that the occurrence of excitatory responses to NA is due to its use in solutions at low pH values. This conclusion is further supported by the frequent observation of excitatory responses of brain-stem neurones
to NA applied from solutions at pH 5.0 - 6.0 (Boakes et al., 1971).

Furthermore, we were unable to confirm the findings of Jordan et al., (1972b) that cortical neurones can be excited by H+ ejected from HCl solutions. Indeed, H+ reduced the size of excitatory responses to NA when H+ and NA were ejected simultaneously from adjacent barrels of the micropipette.

Although NA ejected from solutions at different pH values evoked qualitatively similar responses, the size of the depressant or excitatory responses of any neurone to NA ejected from a solution at pH 3.1 was usually greater than that of the response to NA ejected from a solution at pH 5.0. The current-response studies show that when ejected from solutions at pH 3.1 NA appeared to be more potent than when ejected from solutions at pH 5.0, especially when electrophoretic currents of higher intensities were applied. The difference between the actions of NA released from solutions at different pH values was interpreted by Frederickson et al., (1971) in terms of an excitatory action of H+ released from solutions at lower pH values. Such an explanation is no longer tenable in the light of the present results. However, the apparent difference in potency may result from a lower rate of NA release from the solution at pH 5.0. This would be due to the large number of Na+ introduced into the solution when the pH was adjusted to 5.0 (0.1 M in the final solution). This would tend to reduce the transport number of NA. The in vitro experiments described in I:2.3.5. show that this is indeed the case.
It is apparent, therefore, that there are no good reasons for avoiding the use of NA solutions at pH 3.1 in microelectrophoresis experiments. However, there are disadvantages associated with the adjustment of the pH to a higher level by the addition of NaOH: firstly, dose-response relations are distorted, and secondly, there is a greater risk of obtaining misleading results due to the application of a biologically active product of oxidation.

II:4.2. RESPONSES TO MESCALINE

The observation that mescaline can evoke both excitatory and depressant responses on cortical neurones is in agreement with the observations of Roberts & Straughan (1968) and Gonzalez-Vegas (1971).

Roberts & Straughan (1968) observed that some cells responded in opposite directions to NA and mescaline. The present results indicate that such cells are extremely rare, since only one cell out of a total of 163 cells tested with NA and mescaline showed opposite responses to the two drugs.

However, a substantial proportion of cells (19%) responded differently to mescaline and 5-HT. In fact the proportion of cells responding in opposite directions to mescaline and 5-HT was similar to the proportion of cells responding differently to NA and 5-HT (21%). Fifteen of the cells on which NA and 5-HT had opposite effects were also tested with mescaline. In every case the cells responded to mescaline in the same direction as they did to NA.

Taken together these findings suggest that whenever NA
and 5-HT act at different sites, mescaline acts at the NA site. However, on the majority of cells NA and 5-HT evoke similar responses, and in these cases it is not possible to determine from agonist studies alone whether mescaline acts at NA sites rather than 5-HT sites, or even whether NA and 5-HT themselves act at the same or at different sites.

It is for this reason that antagonist studies were conducted.

Roberts & Straughan (1968) reported that mescaline was less potent than NA. The present results confirm this statement and also indicate that mescaline can be less potent than 5-HT. However, the significance of this observation is unclear. An apparent difference in potency could result from a lower transport number of mescaline, since if this were the case any given ejecting current would give rise to a lower rate of release of mescaline than of NA or 5-HT. No experiments were conducted in vitro to determine the transport number of mescaline, but is likely that the three ring-methoxy groups would render the mescaline molecule less mobile than the NA

+ The word 'site', as I use it here, is intended to be theoretically neutral. I use the word 'site' rather than 'receptor' since the microelectrophoretic technique cannot differentiate between the case where two drugs act at different receptors on the same cell and the case where they act at pharmacologically identical receptors on different structures (e.g. the recorded cell and a more remote inhibitory interneurone: see above II:1.1.).
or 5-HT molecule. On the other hand, the possibility that there is a pharmacological basis for the lower potency of mescaline cannot be ruled out (see below, II:4.3.).

II:4.3. THE EFFECT OF MESCALINE ON RESPONSES TO NA AND 5-HT

Desensitisation to the effects of the monoamines has been observed in many tissues (Gaddum, 1953; Brownlee & Johnson 1963). Gaddum (1953) reported that exposure of the guinea pig ileum to high concentrations of 5-HT rendered the tissue insensitive to tryptamine as well as 5-HT, but that sensitivity to histamine was not affected. However, when very high concentrations of 5-HT were used the desensitisation was nonspecific, in that histamine and substance P also became ineffective.

Desensitisation to the effects of 5-HT and dopamine has also been observed on certain molluscan neurones (Gerschenfeld & Stefani, 1966; Ascher, 1972). Excitatory responses were found to be more susceptible to desensitisation than depressant responses. The desensitisation appeared to be specific, since it did not extend to excitatory and depressant responses to ACh. Desensitisation to the excitatory effects of NA and 5-HT has also been observed in microelectrophoretic studies of neurones in the mammalian CNS, although the specificity of this desensitisation has not been examined (Roberts & Straughan, 1967; Johnson et al., 1969a; Boakes et al., 1970; Boakes et al., 1971).

The present results show that repeated or prolonged exposure of cortical neurones to mescaline resulted in desensitisation to the excitatory effects of this drug. However,
this desensitisation did not seem to be specific, since in all but one of the studies cross-desensitisation to ACh as well as to NA was observed. The effects of ACh can readily be distinguished from the effects of NA, 5-HT and mescaline using a variety of antagonists (Roberts & Straughan, 1967; Johnson et al., 1969a; Boakes et al., 1970; see also below, II:4.4.). Therefore it would appear that cross-desensitisation is not a reliable method for discriminating between the effects of different agonists on cortical neurones.

It was observed on some cells that mescaline had a very much lower agonistic potency than NA and 5-HT, but was able to antagonise responses to these amines (see Fig. 36). This finding might argue in favour of a pharmacological explanation for the lower potency of mescaline: namely that mescaline has a lower intrinsic activity than either NA or 5-HT. If this were the case, the antagonistic action of mescaline could be explained in terms of simple competition for NA and 5-HT sites. In the present project it was not possible to obtain any direct confirmation of this hypothesis. Moreover, the very long time needed for the NA and 5-HT effects to recover after antagonism by mescaline casts some doubt upon this simplified explanation. It is also noteworthy that an application of mescaline on its own did not prevent the occurrence of an excitatory response to a subsequent application of 5-HT. One possible explanation for these findings could be that simultaneous exposure of the cell to both mescaline and NA (or 5-HT) resulted in the formation of a semi-irreversible drug-receptor complex.
The observation that mescaline can antagonise the effects of NA is in agreement with the findings of Gonzalez-Vegas (1971). However, this worker found that the antagonistic effects of mescaline were restricted to depressant responses to catecholamines, whereas in the present experiments excitatory responses were also blocked and responses to 5-HT, as well as those to NA, were affected.

In the experiments of Gonzalez-Vegas (1971) mescaline was only infrequently observed to have an agonistic effect of its own. In the present experiments mescaline usually behaved as an agonist, and this prevented investigations of its antagonistic effects on all but a few cells.

II:4.4. THE EFFECTS OF ANTAGONISTS AND PROTAGONISTS ON RESPONSES TO MESCALINE

The NA antagonist used in these experiments was sotalol. This drug is a specific $\beta$-adrenoceptor antagonist in the periphery (Lish et al., 1965). Johnson et al., (1969a) found sotalol to be a reliable antagonist of NA which did not affect excitatory responses to ACh and 5-HT. For this reason it was selected for use in the present project.

Although Johnson et al., (1969a) found that sotalol had no effect on depressant responses to NA in the cerebral cortex, Hoffer et al., (1971b) reported that sotalol was a potent antagonist of depressant responses of cerebellar Purkinje neurones to NA. York (1970) found sotalol to be an effective antagonist of depressant responses of caudate neurones to dopamine, NA and 5-HT as well as excitatory responses to dopamine.
In contrast, Boakes, et al., (1971), found that both excitatory and depressant responses of brain stem neurones to NA were resistant to sotalol.

In the present studies, sotalol antagonised excitatory but not depressant responses to mescaline. In confirmation of the observations of Johnson et al., (1969a), sotalol blocked the actions of NA, but did not affect responses to ACh. The similarity of the effects of sotalol on responses to mescaline and responses to NA seemed at first to confirm the suspicion (see above II:4.2.) that mescaline acted at NA sites. However, further experiments showed that responses to 5-HT could also be antagonised by sotalol. Thus an action of mescaline at 5-HT sites could not be excluded.

The observation that sotalol antagonised responses to 5-HT is in conflict with the findings reported by Johnson et al., (1969a). The reason for this discrepancy is not clear, although random variation between cells may well be an important factor since comparatively small samples of cells were used in both investigations.

The 5-HT antagonist selected for use in these experiments was methysergide. Roberts & Straughan (1967) found methysergide to be an effective 5-HT antagonist on cortical neurones. Its action was specific insofar as responses to ACh were concerned, but the effect of methysergide on responses to NA was not examined. Boakes et al., (1970) reported that methysergide was an effective 5-HT antagonist on brain stem neurones, and that responses to ACh and NA were resistant to it.

In agreement with earlier studies, methysergide discriminated between ACh and the monoamines in the present experiments.
However, methysergide antagonised responses to mescaline and NA as well as responses to 5-HT. Thus, as was the case with sotalol, it was not possible using methysergide to determine whether the action of mescaline was more closely related to that of NA or to that of 5-HT.

There is more than one possible explanation for the failure of sotalol and methysergide to discriminate between NA and 5-HT. On the one hand is the possibility that these antagonists in particular are not specific. If this were the case, then further experiments using a range of antagonists should succeed in separating the effects of the two monoamines. On the other hand there is the possibility that NA and 5-HT might act at the same sites. If this were the case, then a continued search for a specific antagonist would obviously be fruitless. A further possibility is that the truth lies somewhere between these two extremes. One could postulate the existence of two 'overlapping' populations of excitatory sites for the monoamines (depressant sites would constitute a separate population). Both monoamines would stimulate both sites, although NA would have a somewhat greater affinity for one and 5-HT a somewhat greater affinity for the other. The antagonists might or might not be specific for one site.

This hypothesis, though unparsimonious, has the advantage that it can accommodate the observation that antagonists can discriminate between the monoamines on some cells but not on others, so long as it is assumed that the distribution of the two excitatory sites can vary from cell to cell.

Roberts & Straughan (1967) and Boakes et al., (1970) found that depressant responses to 5-HT were relatively
resistant to antagonism by methysergide. In the present experiments methysergide antagonised the depressant effects of the monoamines on a considerable proportion of the cells tested. On some cells, excitatory responses appeared when the depressant responses were antagonised. An explanation for this could be that the monoamines stimulated both excitatory and depressant sites, and that the effects of the excitatory sites were 'masked' by the dominant depressant sites; in blocking the depressant sites methysergide may have 'unmasked' the effects of the excitatory sites.

The proposal that the monoamines might act simultaneously at both excitatory and depressant sites could provide an explanation for the unexpected finding that the antagonists, when applied with low electrophoretic currents, often potentiated the excitatory effects of the monoamines. This effect was particularly striking in the case of methysergide. A number of monoamine antagonists, including phentolamine, phenoxybenzamine and ergotamine, are known to potentiate the actions of NA and 5-HT on certain peripheral preparations (e.g., the nictitating membrane and perfused spleen of the cat). The established explanation for this phenomenon is that the antagonists are also inhibitors of the uptake of monoamines into stores (Iversen, 1967; Salzmann et al., 1968). This explanation is less plausible in the case of methysergide, since this drug has little or no uptake blocking activity (Born et al., 1972).
An alternative explanation is that the potentiation is brought about by the blockade of 'masked' depressant sites. When the intensity of the ejection current used to apply the antagonist is increased, the increased concentration of the antagonist may also affect the less sensitive, dominant excitatory sites. It should be noted that this hypothesis requires that depressant sites are more sensitive to methysergide than excitatory sites. This is in apparent contradiction of reports that methysergide is a more effective antagonist of excitatory than of depressant responses (Roberts & Straughan, 1967; Boakes et al., 1970), although in the present experiments methysergide did antagonise depressant responses.

Some interesting questions are raised by the observations that the tricyclic antidepressant, DMI, can both antagonise and potentiate responses to mescaline. The same effects had previously been observed on responses to NA and 5-HT (Bradshaw et al., 1971; 1973). Following a pulse application of the

+ I am grateful to Elemer Szabadi for the suggestion that masked depressant sites could explain the potentiating effects of the antagonists. The same explanation has been offered for the potentiating effects of the tricyclic antidepressants on excitatory responses to ACh (Bevan et al., 1973).
antidepressant, antagonism of the response to mescaline was always observed to precede the potentiation of the response. This suggests that antagonism occurred when the concentration of DMI was at its highest and that potentiation appeared as the concentration fell below the 'threshold' for antagonism (see I:3.3.3.2.). The antagonism may well be due to the blockade of excitatory monoamine sites since DMI is known to have a powerful α-adrenolytic action (Callingham, 1967). However, the origin of the potentiation is not clear. The potentiating effects of the antidepressants are usually ascribed to inhibition of uptake. Yet Iversen (1967) found that mescaline had virtually no affinity for the NA uptake mechanism in sympathetic nerves. A small fraction of systemically injected mescaline can be recovered from the brain (Denber & Teller, 1967) and much of this is located in synaptosomes (Denber & Teller, 1970). However, the amount of mescaline taken up into synaptosomes is extremely small and is linearly related to the injected dose, so that an active transport mechanism is probably not involved (Denber & Teller, 1967). An alternative possibility, of course, is that potentiation by DMI is brought about by blockade of masked depressant sites. This possibility has interesting implications for the pharmacology of the antidepressants.

II:4.5. CONCLUSIONS

The experiments described in the preceding pages show that mescaline can excite and depress cortical neurones, and that the action of mescaline resembles that of NA and 5-HT.
When NA and 5-HT have opposite effects, the effect of mescaline resembles that of NA and not that of 5-HT. Sotalol and methysergide antagonised responses to mescaline, NA and 5-HT without affecting responses to ACh. Thus the antagonist studies confirmed that mescaline behaves like NA and 5-HT on cortical neurones, but failed to discriminate between the actions of the two monoamines.

The antagonistic effects of mescaline are in contrast with the predominantly antagonistic effects of LSD on cortical neurones (Roberts & Straughan, 1967). However, if any common mode of action for the two hallucinogens is to be sought at the level of the cerebral cortex, it could be that both drugs act by reducing the effectiveness of monoamine transmission. LSD might achieve this by blocking post-synaptic receptors; mescaline might compete with the natural transmitter for these receptor sites, substituting its own weak agonistic effects for the more powerful effects of the transmitter. Obviously a great deal of further experimentation is needed before such speculations can deserve serious consideration.
REFERENCES
REFERENCES


Bloom, F. E., Costa, E. & Salmoiraghi, G. C. (1965). Anaesthesia and the responsiveness of individual neurones of the caudate nucleus of the cat to acetylcholine, norepinephrine and dopamine administered by microelectrophoresis. J. Pharm. Exp. Ther., 150, 244-252.


