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THE EFFECTS OF MESCALINE, AMPHETAMINE AND FOUR-RING SUBSTITUTED AMPHETAMINE DERIVATIVES ON SPONTANEOUS BRAIN ELECTRICAL ACTIVITY IN THE CAT

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Summary—Alterations in frequency distribution of spontaneous brain electrical activity were measured following administration of mescaline, amphetamine, trimethoxy-amphetamine (TMA), methoxy-methylenedioxy-amphetamine (MMDA), methylenedioxy-amphetamine (MDA) and dimethoxy-amphetamine (DMA) to unanesthetized and unrestrained cats.

Two orthogonal patterns of frequency change were found which were correlated with dosage of the test compounds. It is proposed that one of these patterns represents central alerting in the cat, while the other is a correlate of the hallucinogenic effect produced in man by some of the drugs used. An analysis of the effects produced by individual compounds for their content of each pattern revealed that only amphetamine produced significant alerting; in terms of hallucinogenic effect, activities of the drugs decreased in the order TMA > MDA > MMDA > mescaline > DMA.

INTRODUCTION

THIS report is concerned with alterations in spontaneous electrical activity produced in the brain of the unanesthetized and unrestrained cat by the central stimulant amphetamine, the hallucinogenic agent mescaline and four ring-substituted amphetamine derivatives, at least three of which are known to be hallucinogenic in man. Administration of mescaline and the amphetamine derivatives resulted in the appearance of hypersynchronous slow-wave activity in the CNS which was associated with certain abnormal and stereotyped changes in the behavior of the animal. Amphetamine, at comparable dose levels, produced a desynchronized electrical record concomitant with behavioral alerting and hyperactivity. The present study is concerned with the quantitative measurement of dose related changes in frequency pattern and the estimation of relative potency of the test compounds for producing distortions of brain electrical activity which are characteristic of the hallucinogenic drugs on one hand and amphetamine on the other.

METHODS AND MATERIALS

Cats of both sexes weighing between 2·8 and 3·2 kg were prepared with electrodes chronically implanted in various cortical and subcortical brain areas. Depth electrodes were bipolar and consisted of two insulated 40 gauge stainless steel wires fixed to a section

*Deceased.

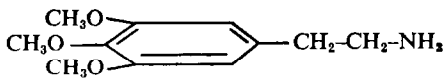
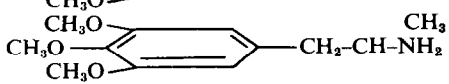
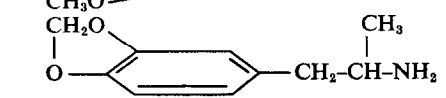
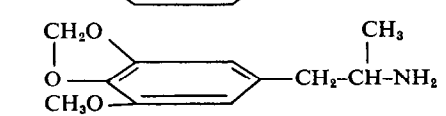
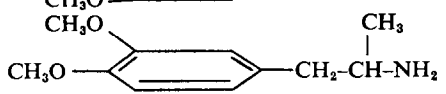
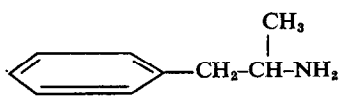
of insulated 26 gauge stainless steel tubing. The wires were cut 2 mm below the central shaft and these sectioned ends represented the only electrically exposed surface. The distance between the tips was approximately 1 mm. Cortical electrodes consisted of nickel plated screws placed into but not through the bony table of the skull. Wires leading from the electrodes were soldered into miniature female plugs which were permanently fixed to the skull with acrylic plastic.

Table 1 shows the structural formulae, chemical nomenclature, abbreviations and equivalent weights of the compounds employed in this study. With the exception of MMDA, previously described procedures were employed for the synthesis of all of these compounds. MMDA was prepared by lithium aluminum hydride reduction of 5-methoxy-3,4-methylenedioxy phenylnitropropylene obtained by the condensation of nitroethane with myristic aldehyde. This synthetic sequence was analogous to that reported by HEY (1947) for the synthesis of TMA. The synthesis of MMDA has recently been described elsewhere (SHULGIN, 1964).

In order to provide a consistent behavioral and electrical baseline for measurement of drug effect the cats were maintained alert prior to injection of the test compounds. Two separate procedures were employed for insuring arousal. Three cats comprising one group were presented with a variety of sensory stimuli such as a loud buzzer, voice commands, hand claps and sudden changes in cage illumination. Three animals in a second group were housed in a sound attenuated chamber and alerted by presentation of a conditioned stimulus (CS) which had been paired with an adversive unconditioned stimulus (US). The CS was a 3000 c/s tone and the US a blast of air delivered from two lengths of soft rubber tubing fixed to both sides of the dorsal straps of an ordinary small animal harness and connected through a length of plastic tubing to a source of compressed air. The tubes hung limply down each side of the neck until air was delivered to the system, whereupon they flayed violently about with the generation of considerable noise. Adaptation to this adversive stimulus was slight and its use avoided the electronic recording difficulties associated with the more commonly employed electrical shock. The animals were conditioned on a fixed-ratio, variable-interval schedule in which CS–US pairings were randomly presented during one third of the trials. The CS–US interval was 5 sec and the intertrial interval was 1 min. All sequences were controlled by automatic programming equipment. The conditioned response (CR) was defined as EEG alerting or desynchronization occurring within 5 sec after presentation of the CS. Thirty trials per day were given and 90 per cent correct response was taken as the criterion for conditioning. The CR developed quickly and most animals were adequately conditioned by the second or third test session.

Animals from both groups were maintained alert for periods of 15 min during which frequency analysis (see below) was performed on the electrical activity emanating from the dorsal hippocampus; standard EEG recordings were also taken from a number of cortical and subcortical sites. Following the period of control recording the cats were injected intraperitoneally with the test compounds. Mescaline and the four ring-substituted amphetamines were administered in at least two, and usually three, graded doses ranging between 0·005 and 0·04 m-mole/kg. Doses of the individual compounds varied at least two-fold at levels determined by their potency for producing hypersynchronous bursting; amphetamine was administered in two doses corresponding to the extremes of this range (0·005 and 0·04 m-mole/kg). Control experiments were conducted with injections of normal saline solution. A comparable 15-min of frequency analysis

TABLE 1. THE TEST COMPOUNDS

Chemical name	Structural formulae of base	Abbreviation or common name	Equivalent weight of salt
d,l-3,4,5-trimethoxyphenethylamine sulfate hydrate		mescaline	278
d,l-3,4,5-trimethoxyphenisopropylamine hydrochloride		TMA	262
d,l-3,4-methylenedioxyphenisopropylamine hydrochloride		MDA	216
d,l-3,4-methylenedioxy-5-methoxyphenisopropylamine hydrochloride		MMDA	246
d,l-3,4-dimethoxyphenisopropylamine-hydrochloride		DMA	232
d,l-phenisopropylamine sulfate		amphetamine	184

was performed during the period of peak drug effect, usually between 30 and 60 min following injection. At least 24 hr elapsed between test sessions and the compounds were administered to the cats in random sequence. Between twelve and seventeen tests were conducted on each animal comprising a total of eighty-seven experiments in all.

A frequency analyzer was designed and constructed for the purpose of obtaining quantitative information regarding the frequency distribution of brain electrical activity. The preamplifier output of one channel of a Grass six channel electroencephalograph was connected in parallel to eight frequency selective amplifiers through a cathode follower stage. Each amplifier employed two stages of amplification, each with negative feedback through a parallel T-filter. The nulls of the parallel T-filters were staggered so that the net frequency response of the amplifier was relatively flat within a frequency range of half an octave but was attenuated rapidly outside this interval. The output of each amplifier was rectified and fed into an integrator of essentially infinite time constant. At intervals of 1 min, the integrators were connected sequentially by a system of cam driven microswitches to an Esterline-Angus Milliammeter recorder and immediately reset. The resulting record represented the mean amplitude of the frequency components within the range of each of the eight amplifiers. The total frequency range covered was 2·5–40 c/s, in eight half octave steps.

RESULTS

General observations. Injections of mescaline and the amphetamine analogs produced initial restlessness accompanied by occasional urination, defecation and vomiting. Within 30 to 60 min after injection these effects had abated and the animal was usually in a prone position with its head erect and forelimbs rigidly extended. Although appearing highly alert the animal seldom changed its position, and at higher doses of the test compounds it was not uncommon for this relative immobility to persist for several hours. During this period mydriasis, hyperpnea, piloerection and other indications of sympathomimetic activity were frequently observed. In addition, certain bizarre reactions occurred such as hissing, extending a forepaw as if to touch an imaginary object and staring intently at some particular portion of the environment.

Coexistent with the abnormal and somewhat stereotyped behavioral changes observed, high-amplitude, low-frequency activity appeared in the EEG. Emanating from a background of low voltage fast activity these drug-induced changes were characterized by unusually regular or hypersynchronous wave forms occurring at frequencies varying between approximately 3 to 10 c/s. Particularly following higher doses of the test compounds such patterns would often occur in trains which remained uninterrupted for many seconds. They were recorded from widely divergent locations in the brain including the hippocampus, midbrain reticular formation, amygdaloid area, supraoptic region of the hypothalamus, basal forebrain area, caudate nucleus, the region of the medial–dorsal thalamus, and to a somewhat smaller degree in various cortical areas. A representative record of these effects following TMA is compared in Fig. 1 with brain electrical activity seen in slow-wave sleep and in the alert state.

Amphetamine, in contrast to its ring-substituted analogs given at comparable doses, produced only the relatively flat, desynchronized EEG record characteristic of an alert state with a marked increase, rather than decrease, in general activity.

In the three animals subjected to adversive conditioning the CS normally elicited EEG desynchronization for the entire 5 sec of its presentation and usually for a number of

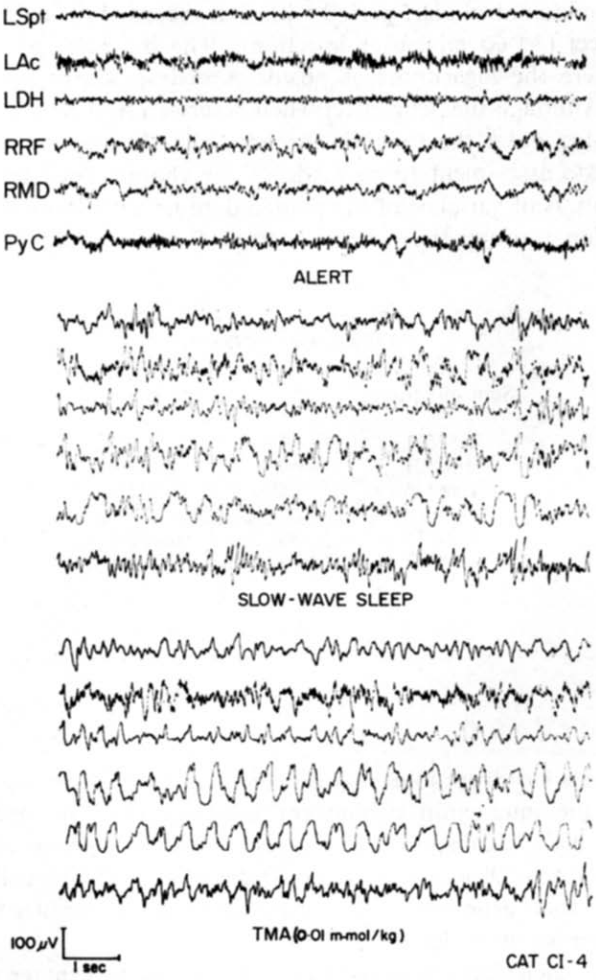


FIG. 1. Hypersynchronous, slow-waves produced by trimethoxy-amphetamine (TMA) in brain electrical activity of the unanesthetized and unrestrained cat as compared to patterns seen in spontaneous slow-wave sleep and the alert state.
LAc, left amygdaloid complex; LDH, left dorsal hippocampus; LSpt, left septal area; RMD, right medial-dorsal thalamus; RRF, right reticular formation; PyC, transparietal cortex.

seconds thereafter. Anticipatory behavior such as crouching and flattening of the ears was also observed during this period. In contrast, following administration of mescaline and the ring-substituted amphetamines presentation of the CS usually resulted in only momentary EEG desynchronization and few behavioral reactions were observed. Amphetamine resulted in continuous low-voltage, fast activity in the EEG and thus the CS produced little additional effect.

Quantitative frequency analysis of the EEG

In each experiment the drug effect on the EEG was quantitatively estimated with the electronic analyzer described under "Methods," by comparing the amplitude spectrum

obtained for a 15 min control alert period with that recorded for a 15 min period during the peak drug effect (30–60 min after injection). The measures of drug effect used in further analysis were the logarithms of postdrug/predrug means for each of the eight frequency bands. Although the drug effect when described in this way showed an obvious trend with increasing dose, the variation among individual experiments was too great to allow an accurate assessment to be made of the characteristic changes produced by the drugs. An analysis of variance of the pooled data nevertheless demonstrated a highly significant regression through the origin of drug effects on drug dose (Table 2). The

TABLE 2. ANALYSIS OF VARIANCE FOR REGRESSION ON DOSE OF DRUG EFFECTS ON THE HIPPOCAMPAL EEG FREQUENCY SPECTRUM

Source	Sum of squares	D.F.	Mean square	F	P
Regression	0·57571	1	0·57571	26·506	$<0·001$
Between channels	1·38921	7	0·19846	9·137	$<0·001$
Between drugs	0·66115	5	0·13223	6·088	$<0·001$
Interaction channels × drugs	2·24115	35	0·06403	2·948	$<0·001$
TOTAL	4·86722	48			
Deviations from regression	2·25893	104	0·021720		
TOTAL	7·12615	152			

regression coefficients on drug dose also differed significantly among drugs and among channels; finally, the interaction component indicates that the pattern of regression coefficients for the eight channels varied significantly from drug to drug. Table 3 summarizes the regression coefficient matrix for all drugs and all channels. Standard errors were calculated for each drug using as an estimate of error variance the component for deviations from regression in Table 2.

These regression coefficients were used to calculate the percentage amplitude changes which would be expected in each frequency band following approximately equiactive doses of the test drugs, and the results are shown in graphic form in Fig. 2. In general, mescaline and the ring-substituted phenylisopropylamines produced an increase in middle and low frequencies, and a decrease or no change in the higher frequency range, while amphetamine reduced the mid-frequency amplitude.

It was of interest to determine whether these six patterns of change could be satisfactorily represented as combinations of fewer basic patterns, which might reflect specific pharmacodynamic actions. A canonical reduction of the regression structure was therefore carried out by means of a canonical correlation analysis using an IBM 7094 computer.

ANDERSON (1958, p. 228–297) has discussed this use of canonical analysis to achieve a canonical resolution of a multivariate regression structure. The procedure identifies those linear combinations of the response variables and the dose variables which have the highest correlation with each other. The coefficients of the former will define a specific frequency pattern, and a score representing the content of this pattern in a given set of values for the response variables; the coefficients of the new dose variable will be proportional to the regression coefficients of this score on the dose of each drug (see Appendix).

TABLE 3. REGRESSION COEFFICIENTS OF DRUG EFFECTS ON DOSE FOR ALL FREQUENCY BANDS OF THE ANALYZER

Channel	Frequency range (c/s)	Regression coefficient of drug effect on dose (m-mole/kg)						
		Mean difference after saline	TMA	MDA	MMDA	DMA	Mescaline	Amphetamine
1	2·5–3·5	−0·00273	13·353‡	11·206†	6·295‡	1·604	1·720*	−0·003
2	3·5–5·0	0·00225	6·543	4·073	5·007‡	1·311	2·368‡	−2·691
3	5·0–7·0	0·00220	3·639	4·231	5·433‡	1·631	2·491‡	−2·743
4	7·0–10·0	0·00183	0·350	2·510	4·310†	1·237	1·896†	−3·405*
5	10·0–14·0	−0·00063	− 2·726	−0·823	1·862	0·745	0·940	−3·317*
6	14·0–20·0	−0·00233	− 6·983*	−2·800	−1·227	0·145	0·018	−0·890
7	20·0–28·0	−0·00768*	−11·626‡	−4·642	−1·930	0·133	−0·047	−0·065
8	28·0–40·0	−0·00620	−11·587‡	−6·052	−4·080†	−0·397	−0·429	0·974
SE (N=104)	—	0·00368	3·43	3·43	1·30	0·834	0·673	1·48

Drug effect was estimated as the logarithm of the mean amplitude at the peak of drug effect divided by the amplitude during a predrug period.
*P<0·05, †P<0·01, ‡P<0·001.

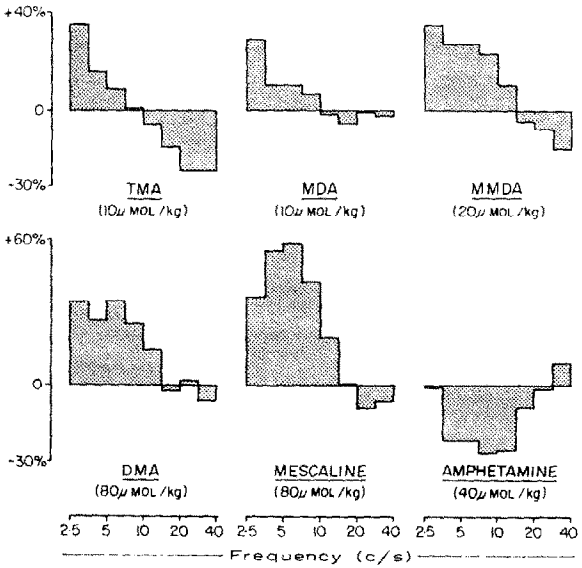


FIG. 2. Frequency pattern changes produced by the test drugs as percentages of the levels seen in the predrug alert state. The changes were calculated from the regression coefficients in Table 3 for doses which produced roughly equivalent behavioral effects.

Repetition of this process will yield a second variable, orthogonal to the first, and its regression coefficients on the drug doses. The process may be repeated until a series of new variables has been defined equal in number to the number of drugs (or measured variables, whichever is smaller). The significance of the corresponding correlation coefficients may now be assessed (ANDERSON, 1958, p. 329), and only those scores showing a significant correlation are utilized in further analysis.

This type of analysis was applied to the entire set of data comprising the results of eighty-seven experiments using six drugs on six cats at two or more dose levels each, and including saline controls in three of the cats.

Of the seven orthogonal scores yielded by the analysis, only two were significantly correlated with dose ($P<0{\cdot}001$ for each). The remaining five correlations were not significant ($P>0{\cdot}2$) and were not considered further. It can therefore be concluded that all the drug effects found may be adequately represented in terms of two independent patterns of change in the EEG frequency spectrum. The potencies of each drug in producing these two patterns may be conveniently represented as a point on a two-dimensional plot, the coordinates being given by the canonical coefficients of the dose variables corresponding to the first two correlations. Confidence limits were estimated (see Appendix) and may be described by ellipses with these points as their centers.

Figure 3 shows the results presented in this way. The saline controls did not differ from the origin significantly ($P>0{\cdot}2$) and are not shown. Of the six drugs studied, all but amphetamine showed significant activity in relation to the first score while only amphetamine and mescaline (in opposite directions) differed significantly from zero in regard to the second. In view of the fact that mescaline, TMA, MDA and MMDA are known to produce hallucinations, it seems reasonable to suppose that the first score may be associated

with hallucinogenic activity; the order of potency corresponding to it is TMA > MDA > MMDA > mescaline > DMA. The pattern corresponding to the second score and its production by amphetamine suggest that it is associated with alerting.

The frequency patterns measured by these scores were determined by finding the values of the original response variables corresponding specifically to vectors in the direction of the coordinates of Fig. 3 (see Appendix). They correspond approximately to the changes produced by TMA and amphetamine, respectively, and are shown as insets on the two coordinates of Fig. 3.

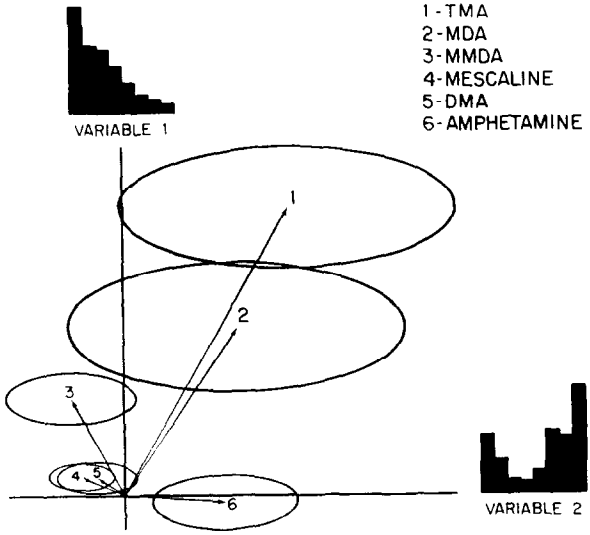


FIG. 3. Summary of canonical analysis of drug effects on spontaneous electrical activity in the dorsal hippocampus using pooled data from all experiments on all cats.
The coordinates are the values of the canonical coefficients of dose in the first two canonical variables, both of which were statistically significant ($P < 0{\cdot}001$). They are proportional to the regression coefficients of two corresponding response patterns, indicated as inserts, on dose, and may be considered as estimates of drug activity in producing each of these patterns. The ellipses depict 95% confidence regions.

The six cats used in this study fell into two groups, depending upon the procedures used for maintaining an alert control state (see above); it is therefore pertinent to inquire whether these groups differed in regard to the apparent potency of the drugs assessed by the procedures described above. Regression coefficients for each of the two scores on drug dose were calculated separately for each cat and each drug, and a series of "t"-tests was made to determine whether differences between groups were significantly greater than those to be expected from variation between cats within groups. The twelve such tests (six drugs, two scores for each drug) yielded P values ranging from 0·133 to 0·967. A second series of tests was made comparing the relative potency of all possible pairs of drugs for the two groups in a similar manner. In thirty tests of this kind (fifteen pairs of drugs, two scores for each pair) P values ranging from 0·053 to 0·941 were obtained. It may be concluded that the results provide no evidence of differences between the two groups of cats insofar as the relative potencies of the drugs are concerned.

DISCUSSION

This work has demonstrated differences in the potencies of mescaline, amphetamine and the ring-substituted amphetamine derivatives for producing patterns of distortion in the frequency distribution of spontaneous brain electrical activity in the cat which are consistent with available information concerning the hallucinogenic and central stimulant properties of these compounds in man.

A good deal of discussion has been devoted to the numerous pitfalls associated with the use of subhuman species to investigate drugs which produce largely subjective effects in man; nevertheless, the obvious limitations associated with clinical investigations continue to spur work in this area. An alteration in spontaneous brain electrical activity has frequently been observed in experimental animals in response to hallucinogenic agents. Usually recorded in deep and particularly temporal lobe brain structures in unanesthetized animals and variously described as epileptiform spiking or hypersynchronous bursting, it has been associated with a number of hallucinogenic substances. Abnormal brain electrical activity has been recorded in the cat with psychotomimetic cyclohexamines (ADEY and DUNLOP, 1960), LSD-25, psilocybin and psilocin (ADEY *et al.*, 1964) and following intraventricular injection of mescaline (SCHWARZ *et al.*, 1956). RINALDI and HIMWICH (1955) reported a similar phenomenon in conscious, but curarized, rabbits with LSD-25 and mescaline. According to a report by SPECK (1958) the conscious rat also exhibits paroxysmal spiking activity in the EEG following relatively high doses of mescaline. An interesting human corollary is provided by HEATH (1954) who recorded slow-wave and spiking electrical activity from certain subcortical structures in schizophrenic patients, Following mescaline or LSD this abnormal electrical activity increased and took the form of slow wave, paroxysmal bursts which were said to occur in conjunction with overt psychotic behavior (MONROE *et al.*, 1957).

During the course of the present investigation, mescaline and the ring-substituted amphetamine derivatives were observed to produce a slow-wave hypersynchronous bursting pattern in the brains of unanesthetized and unrestrained cats. Amphetamine, on the other hand, produced a desynchronized brain electrical pattern, an effect of this drug mentioned in a number of reports (i.e. BRADLEY and ELKES, 1957; BRADLEY and KEY, 1958; WHITE and DAIGNEULT, 1958). Although hypersynchronous bursting activity was recorded from a number of subcortical sites, the dorsal hippocampus was selected for frequency analysis since other workers (ADEY and DUNLOP, 1960; ADEY *et al.*, 1964) had described abnormal electrical activity occurring in this region of the brain following the administration of a variety of hallucinogenic substances.

With the six test compounds employed in the present study it was found that two orthogonal variables could represent all measured, drug-induced changes in hippocampal electrical activity which had a significant correlation with dose. The calculation of the potency of each compound with respect to these two variables revealed that amphetamine differed significantly from zero only in terms of one, while the ring-substituted amphetamine derivatives had a similar difference only in terms of the other. Mescaline produced significant variation in both variables but its effect was opposite in sign to that of amphetamine while being similar to that of the amphetamine derivatives. The projections of the vectors for mescaline and amphetamine analogs on the ordinate in Fig. 3 are measures of the relative potency of these compounds for producing a particular type of dose-related distortion in the frequency pattern of the cat brain which is positively correlated with their hallucinogenic activity in man.

Medical science has been aware of the properties of the mescale button since the early reports of LEWIN (1887, 1888, 1894) and there has been extensive documentation of the hallucinogenic or psychotomimetic potency of the pure mescaline alkaloid over the intervening years. Amphetamine, introduced by ALLES in 1932, is recognized as a powerful central stumilant and, although some reports (i.e. MONROE and DRELL, 1947; CONNELL, 1958) have implicated acute and particularly chronic intake of high doses in the development of psychotomimetic reactions, it is usually not regarded as a hallucinogenic agent. On the other hand, a combination of the isopropyl side-chain of amphetamine with the trimethoxy-substituted benzene ring of mescaline results in TMA, a compound which is 3 to 5 times more potent as a hallucinogen than mescaline itself (PERETZ *et al.*, 1955; SHULGIN *et al.*, 1961). Replacement of two of the three methoxy groups on TMA with a methylenedioxy bridge produces MMDA which has recently been reported (SHULGIN, 1964) to be approximately 3 times more potent than mescaline. Elimination of the single methoxy radical from MMDA apparently does not markedly decrease activity, since ALLES (1959) experienced hallucinogenic phenomena with MDA at a dose 4 to 5 times less than would be necessary for mescaline. Little information is available concerning the clinical properties of DMA although on the basis of threshold effects ALLES (1962) judged this compound to be 2 or 3 times less active than MDA.

Therefore, while documentation of the hallucinogenic effects of the amphetamine analogs in man is far from extensive, TMA, MDA and MMDA have been reported to be several times more potent than mescaline while DMA appears less active than the other three derivatives. A similar ranking resulted from the ability of the test compounds to induce a specific pattern of frequency distribution in the spontaneous brain electrical activity of the cat.

Spectral analysis of the EEG has frequently been employed in attempts to extract useful information from it. Most of these are subject to KNOTT's criticism (1953) that "an instrument designed to complement visual analysis should not lead to further visual analysis"; the present work represents an attempt to analyze the frequency spectrum of the EEG in such a way that the results are amenable to direct statistical evaluation. The use of broad band filters has been criticized in the past because it loses some ability to detect small shifts in dominant frequency; however, this disadvantage has probably been overemphasized (KAISER *et al.*, 1964). An averaging interval of one minute might also be questioned because of the information it rejects in the form of more rapid changes; this nevertheless appears both justified and desirable when the objective is to identify and measure changes in EEG occurring over long periods of time. A few attempts have been published to condense the information from broad band frequency analysis into specific parameters (GLESSER, 1954; KAISER *et al.*, 1964); multivariate analysis is ideally suited for this purpose since it substitutes an objective maximization procedure for a subjective and arbitrary decision. Its application to large sets of data with many variables has been limited in the past by the tedious and extensive computations involved, but is now quite feasible when a large electronic computing facility is available.

The current investigation has demonstrated that qualitative differences in the central nervous system activity of a series of compounds having similar chemical structures can be accurately described by the application of multivariate statistical techniques to the results of broad-band frequency analysis of spontaneous brain electrical activity. The degree of positive correlation between the central activity of these compounds in the

cat and their relative hallucinogenic potency in man lends support to the concept that certain frequency patterns, particularly when recorded from subcortical brain structures, may represent an electrophysiologic correlate of the hallucinogenic state.

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APPENDIX

The relationship between dose and response was developed by the method of canonical reduction of the multivariate regression of response on dose (ANDERSON, 1958). The idea of the analysis can be outlined as follows. Let y_{ij} denote the response of the j^{th} response variable (e.g. log ratio post-drug/pre-drug mean amplitude for j^{th} frequency band) for the i^{th} test compound administration, and let χ_{ik} denote the dose of the k^{th} compound used in the i^{th} administration; in the series of administrations reported here, one and only one compound was given at a positive dose for each administration. The regression relationship can be expressed

$$y_{ij} = \sum_{k=1}^{q} \chi_{ik} b_{kj} + \epsilon_{ij} \qquad \begin{matrix} j = 1, \ldots, p \\ i = 1, \ldots, n \end{matrix} \tag{A-1}$$

in which the b_{kj} are the regression coefficients of response on dose and the ϵ_{ij} are the residuals. Suppose a set of numerical weighting coefficients, $w_1, \ldots, w_p$, were used to form a single response

$$z_i = \sum_{j=1}^{p} y_{ij} w_j = \sum_{k=1}^{q} \chi_{ik} \sum_{j=1}^{p} b_{kj} w_j + \sum_{j=1}^{p} \epsilon_{ij} w_j \qquad i = 1, \ldots, n. \tag{A-2}$$

The regression coefficients of the response, z, on dose for the test compounds are obtained as c_k, $k = 1, \ldots, q$, where

$$c_k = \sum_{j=1}^{p} b_{kj} w_j \tag{A-3}$$

The numerical values of the c_k would be the same as those obtained as the regression coefficients of z on the dose variables χ_{ik}. An index of the effectiveness of the set of weights, $w_1, \ldots, w_p$, is the ratio of the mean square attributable to regression to the mean square of deviation from regression,

$$F = \frac{SS_R/q}{SS_D/n-q}, \tag{A-4}$$

where the sum of squares due to regression is

$$\begin{aligned} SS_R &= \sum_k c_k \sum_i z_i \chi_{ik} \\ &= \sum w_j w_{j'} \Big(\sum_{i,k} \sum y_{ij} \chi_{ik} b_{kj'} \Big) \end{aligned} \tag{A-5}$$

and the sum of squares of deviations from regression is

$$\begin{aligned} SS_D &= \sum_i z_i^2 - SS_R \\ &= \sum w_j w_{j'} \left[\Big(\sum_i y_{ij} y_{ij} \Big) - \Big(\sum_{i,k} \sum y_{ij} \chi_{ik} b_{kj'} \Big) \right]. \end{aligned} \tag{A-6}$$

Because the weighting coefficients, w_j, are arbitrary, they may be chosen to maximize the F-ratio. The relevant sums of squares are non-negative quadratic functions of the w_j and are expressed in matrix form as

$$SS_R = w' S_R w$$
$$SS_D = w' S_D w. \tag{A-7}$$

The w_j that yield the largest value for the F-ratio are the components of the characteristic vector corresponding to the largest root of the determinantal equation

$$|S_R - \lambda S_D| = 0 \tag{A-8}$$

and can be obtained numerically. One can form a second score by taking as weighting coefficients components of the characteristic vector corresponding to the second largest root of the equation. The process can be continued to obtain a canonical representation of the problem. A gain in simplicity arises if at some stage, all further roots are of a magnitude that can be attributed to sampling variation. In the present paper, for example, only the two largest roots were retained, indicating that an appropriately chosen two-dimensional response is an adequate summarization of the response as assessed by the eight frequency channels.

The numerical calculations were made using the Canonical Correlation Analysis program BMDX75, prepared by Paul Sampson of the UCLA Health Sciences Computing Facility. The program provided the option of using deviations from the origin instead of deviations about the mean. This option was desirable for the present problem, since a regression through the origin reflects zero response at zero dose.

The canonical correlation analysis is equivalent to a canonical reduction of the multivariate regression. The canonical variables formed from the response variables in the correlation analysis remain canonical response variables for the regression analysis; the program BMDX75 computes the weighting coefficients w_j such that the average square of the score for the several administrations has the value unity for each canonical response variable. The canonical variables formed from the dose variables χ_{ik}, apart from a normalizing factor, are the regression predictors of the corresponding response variables. The regression of a canonical response variable upon the corresponding canonical predictor variable has as a regression coefficient the corresponding canonical correlation, r. Consequently, the values of the regression coefficients of a canonical response variable on the dose variables χ_{ik} are obtained as $c_k = rc_k^*$, where c_k^* denote the coefficients that define the canonical dose variable in the correlation analysis, and are available in the computer output. The roots of the determinantal equation are $\lambda = r^2/(1-r^2)$, where r denotes the value of a canonical correlation. An approximate test of significance of the λ is outlined by ANDERSON (1958, pages 326–327).

After the canonical response variables have been obtained, it is of interest to obtain the configurations, or profiles, that correspond to each of the canonical response variables. What set of values of the observed response variables will yield the value unity for a given canonical variable, and zero for all other canonical response variables? The result is simply obtained by computing the average products

$$y_{\alpha j}^* = 1/n \sum_i y_{ij} z_{i\alpha}, \qquad j = 1, \ldots, p \tag{A-9}$$

where $y_{\alpha j}^*$ is the profile value of the j^{th} response variable corresponding to the α^{th} canonical

response variable, y_{ij} is (as before) the value of the j^{th} response variable at the i^{th} administration, and z_{i_α} is the value of the α^{th} canonical response variable for the i^{th} administration.

The correctness of the result follows from the property of the canonical variables

$$1/n\sum_{i=1}^{n} z_{i_\alpha}z_{i_\beta} = \begin{cases} 1 & \alpha = \beta \\ 0 & \alpha \neq \beta \end{cases} \tag{A-10}$$

Replace z_{i_β} in the sum of products (A–10) by the defining relation

$$z_{i_\beta} = \sum_j w_{j\beta}y_{ij} \tag{A-11}$$

where $w_{j\beta}$, $j = 1, \ldots, p$, are the weighting coefficients for the β^{th} canonical variable, to obtain

$$\begin{aligned} 1/n\sum_{i=1}^{n} z_{i_\alpha}z_{i_\beta} &= 1/n \sum_i z_{i_\alpha}\sum_j w_{j\beta}y_{ij} \\ &= \sum_j w_{j\beta}(1/n \sum_i z_{i_\alpha}y_{ij}) \\ &= \sum_j w_{j\beta}y_{\alpha j}^*. \end{aligned} \tag{A-12}$$

It follows from (A–10) that the latter term is zero or one accordingly as α differs from or is equal to β, so that the computed $y_{\alpha j}^*$ possess the desired property

$$\sum_{j=1}^{p} w_{j\beta}y_{\alpha j}^* = \begin{cases} 1 \text{ if } \alpha = \beta \\ \\ 0 \text{ if } \alpha \neq \beta \end{cases}, \quad \alpha, \beta = 1, \ldots, p. \tag{A-13}$$

Confidence regions. The regression coefficients of the canonical response variables on dose provide a representation of the relative potencies of the test compounds, and this being the case it is of interest to consider the stability of the estimated regression coefficients. This stability can be represented in terms of confidence regions.

Confidence regions can be developed on the basis of the Hotelling T^2 statistic. Since only one test compound is tested at a time, the regression coefficients b_{kj} of equation (A–1) are estimated as

$$b_{kj} = \frac{\sum_{i=j}^{n} y_{ij}\chi_{ik}}{\sum_i \chi_{ik}^2}, \; j = 1, \ldots, p, \tag{A-14}$$

and the covariance matrix of the b_{kj}, for given k, is a multiple of the covariance matrix of the observed response

$$\sum b_k = \frac{1}{\sum_i \chi_{ik}^2} \sum y. \tag{A-15}$$

The covariance matrix Σ_y is estimated, equation (A-7), as the $p \times p$ matrix $S_D/(n-q)$, so that

$$T^2 = (\sum_i \chi_{ik}^2)(b_k-\beta_k)'[S_D/(n-q)]^{-1}[(b_k-\beta_k)] \tag{A-16}$$

has the Hotelling T^2 distribution, when b_k and β_k denote the estimated and "true" (column vector) regression coefficients of response on dose for the k^{th} test compound. Since the distribution of T^2 is that of $[p(n-q)/(n+1-p-q)]F$, where F has the Snedecor–Fisher F-distribution,

$$(\beta_k-b_k)'[S_D/(n-q)]^{-1}(\beta_k-b_k) \leq \frac{p(n-q)}{(n+1-p-q)\sum_i \chi_{ik}^2} F(\beta;p,n+1-p-q) \tag{A-17}$$

defines a 100β per cent confidence region, where $F(\beta;n_1, n_2)$ denotes the 100β percentage point of the F-distribution with n_1 degrees of freedom for the numerator and n_2 degrees of freedom for the denominator. Let W_0 be a $p_0 \times p$, $p_0 \leq$ p matrix of rank p_0, and let W_1 be a $(p-p_0)\times p$ matrix such that the matrix T,

$$T = \begin{pmatrix} W_0 \\ W_1 \end{pmatrix} \tag{A-18}$$

is nonsingular and that

$$W_0 S_D W_1' = 0. \tag{A-19}$$

Then

$$(\beta_k-b_k)'[S_D/(n-q)](\beta_k-b_k) = [T(\beta_k-b_k)]'[TS_DT'/(n-q)]^{-1}T(\beta_k-b_k) \tag{A-20}$$

$$= \sum_{l=0}^{1} [W_l(\beta_k-b_k)]'[W_l S_D W_l'/(n-q)]^{-1} W_l(\beta_k-b_k)$$

$$\geq [W_0(\beta_k-b_k)]'[W_0 S_D W_0'/(n-q]^{-1}[W_0(\beta_k-b_k)],$$

so that a conservative region is obtained as

$$[W_0(\beta_k-b_k)]'[W_0 S_D W_0'/(n-q)]^{-1} W_0(\beta_k-b_k) \leq \frac{p(n-q)}{(n+1-p-q)\sum_i \chi^2_{ik}} F \tag{A-21}$$

regardless of the transformation W_0 chosen. In particular, equation (A-21) applies using the matrix of canonical coefficients for retained variables for W_0. For the case of two retained canonical response variables,

$$W_0 S_D W_0' = n \begin{pmatrix} 1-r_1^2 & 0 \\ 0 & 1-r_2^2 \end{pmatrix},$$

for which (A-21) becomes

$$\frac{(n-q)}{n}\left[\frac{(\beta_{1k}^*-b_{1k}^*)^2}{1-r_1^2} + \frac{(\beta_{2k}^*-b_{2k}^*)^2}{1-r_2^2}\right] \leq \frac{p(n-q)}{(n+1-p-q)\sum_i \chi_{ik}^2} F(\beta;\, p,\, n+1-p-q)$$

or

$$\frac{(\beta_{1k}^*-b_{1k}^*)^2}{1-r_1^2}+\frac{(\beta_{2k}^*-b_{2k}^*)^2}{1-r_2^2}\leq\frac{pn}{(n+1-p-q)\sum_i\chi_{ik}^2}F \tag{A-23}$$

or

$$\frac{r_1^2}{1-r_1^2}(\gamma_{1k}^*-c_{1k}^*)^2+\frac{r_2^2}{1-r_2^2}(\gamma_{2k}^*-c_{2k}^*)^2\leq\frac{pn}{(n+1-p-q)\sum_i\chi_{ik}^2}F(\beta,\,p,\,n+1-p-q),$$

where γ^* and c^* denote the true and estimated canonical coefficients. Equation (A-23) is overly conservative since it makes no allowance for W_0 being canonical coefficients for canonical response variables corresponding to statistically significant canonical correlation coefficients. This conservativeness is slightly overcompensated for by replacing p, the number of response variables, by the number of retained canonical response variables, in the present case, two. The confidence regions presented in Fig. 3 were obtained in this way.

$$\frac{r_1^2}{1-r_1^2}(\gamma_{1k}^*-c_{1k}^*)^2+\frac{r_2^2}{1-r_2^2}(\gamma_{2k}^*-c_{2k}^*)^2\leq\frac{2n}{(n-1-q)\sum\chi_{ik}^2}F(0{\cdot}95,\,2,\,n-1-q). \tag{A-24}$$

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