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Different Behavioural Patterns Induced by the Dopamine Agonist Apomorphine Analysed by Multivariate Statistics

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STÅHLE, L AND U UNGERSTEDT *Different behavioural patterns induced by the dopamine agonist apomorphine analysed by multivariate statistics* PHARMACOL BIOCHEM BEHAV **24**(2) 291–298, 1986 —Exploratory behaviour was recorded in an automatic holeboard apparatus measuring activity, locomotion, rearing, hole exploration and corner restricted behaviour Multivariate statistical methods were used to analyse the results Low doses of the dopamine agonist apomorphine (0 01–0 1 mg/kg SC) dose-dependently reduced most variables measured, although the pattern of behaviour resembled that of normal animals High doses of apomorphine (0 2–1 0 mg/kg SC) induced a qualitative change in the pattern of behaviour with a stereotyped locomotion and a reduced mean time of exploration of holes The described methods for detection and statistical analysis of behaviour differentiates between the behavioural patterns induced by high and low doses of apomorphine and may be useful in finding and analysing patterns of drug induced behaviour related to various mechanisms of action

Apomorphine Autoreceptors Behaviour Dopamine Exploration Holeboard Locomotion Multivariate statistics Postsynaptic receptors

THE existence of dopamine receptors on the dopamine neurons and their functions in regulating release [11, 26, 29, 34, 38], synthesis [8, 16, 30, 33] and firing rate [1,2] has become widely accepted The receptors have been termed autoreceptors [5] and are believed to mediate the decrease in neurotransmission caused by dopamine agonists such as apomorphine (APO) and certain ergot derivates

Low doses of APO are known to inhibit spontaneous activity of rats [15, 18, 21, 24, 25] and this effect has been explained as a selective action on the dopamine autoreceptor [5, 8, 18, 30] In higher doses the inhibition changes into an activation of the animal Strombom [30] found that mice returned to normal levels of activity while Puesch *et al* [25] reported a biphasic inhibition curve It is well known that higher doses of APO induce stereotyped behaviour [10] and the discrepancy between the two reports is most probably due to the properties of the particular recording apparatus [17], its ability to quantitate different motor patterns and differences in the experimental design The problem can be partly solved by using a "stereotypy rating scale" [7] which classifies the "intensity" of the stereotyped behaviour, however, an ordinal scale of that kind makes the statistical treatment of the observation difficult Ljungberg and Ungerstedt [20] used another approach, a "holeboard" [4] where eight different components of motor activity were recorded automatically with a combination of photocells together with a vibration sensor This system made possible the detection of qualitative shifts in behaviour by relating the different components to each other while still maintaining the quantitative recording of each component

Multivariate statistical methods make possible a detailed analysis of the pattern of behaviour [23, 28, 37] We have used this approach to study the qualitative and quantitative shift in exploratory behaviour after various doses of APO In this way it is possible to objectively separate behavioural patterns related to different dopamine receptor populations This may be of particular relevance in view of the recent attempts to relate behavioural patterns to specific subpopulations of dopamine receptors (see Cools [6] and Herrera-Marschitz and Ungerstedt [14]) Such a model should be particularly interesting in view of recent discussions on the use of autoreceptor agonists as a new type of neuroleptics [22] The aim of the present study has been to evaluate the strength of these behavioural and statistical methods It was found that they clearly separate the different patterns of behaviour produced by low and high doses of APO

METHOD

Subjects

Male Sprague-Dawley rats (Anticimex, Sweden), weighing 140–240 g, were used throughout They were delivered to the animal department at least two days before the experiment and were housed five per cage (Macrolon 56×40×16 cm) The animals had access to ordinary lab chow and tap water (ad lib) except during experimental procedures They were kept on a constant 7 a m /7 p m light dark schedule Each animal was used only once

Experimental Procedure and Drugs

All rats were weighed and put into individual cages, and

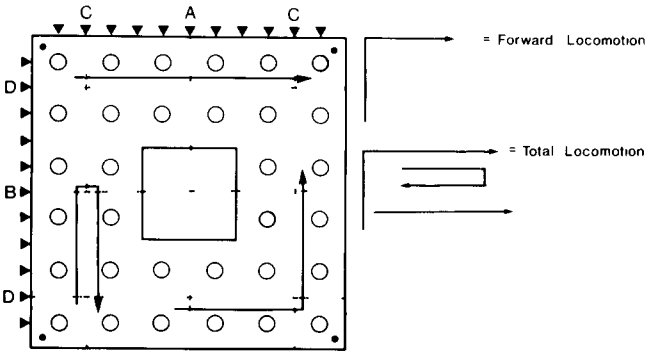

FIG 1 The holeboard from above schematically The black triangles indicate ACTIVITY phototransistors 4 cm above the floor emitting infra-red light detected on the opposite side The arrows indicate the path animals have to move in order to reach the criteria set to get a FORWARD LOCOMOTION or a TOTAL LOCOMOTION count (see also the text) 0 5 cm under the floor there is a photobeam under each of the six rows of holes, detecting the HOLE variables The CORNER variables are detected by vertical photobeams in the corners (black dots) and the REARING variables are detected by a field of photobeams 12 5 cm above the floor

brought to the experimental room in order to habituate for 60–80 min Apomorphine or 0 9% saline was injected 15 min before a trial in a volume of 1 ml/kg (SC in the flank) Apomorphine was dissolved by rapid heating in 0 9% saline

Recording of Behaviour

An automatic holdboard, essentially similar to the one described by Ljungberg and Ungerstedt [20] was used The apparatus consisted of an open field (70×70 cm) with a transparent plastic cube situated in the centre in order to restrict the movements of the rat to the four arms where 32 holes were evenly distributed in the floor (see Fig 1) Three rows of photobeams were placed at two different levels above and one under the floor allowing seven different behavioural variables to be defined and quantitated With four vertical photobeams, one in each corner, two other variables could be detected A vibration sensor, not used in the present study, allowed the recording of gnawing behaviour directed towards the edges of the holes Impulses from the photobeams were fed into a digital logic in order to define the nine variables (see below) and these were collected by a PCS microcomputer programmed to store 20 periods of 30 seconds When an experiment was finished the data were transferred to a Wang 2200 computer and stored on diskette Statistical analysis was performed on a Gemini microcomputer

Definition of the Recorded Behavioural Variables

ACTIVITY Interruption of any of the 22 (11+11) horizontally oriented photobeams (black triangles in Fig 1) placed 4 cm above the floor gives a count Thus horizontal movements are registered

FORWARD LOCOMOTION Interruption of an ACTIVITY photobeam A (or B) gives a count if it is preceeded by interruption of ACTIVITY photobeam B (or A) on either of the adjacent sides This component corresponds to the rat walking from the middle of one of the arms to the middle of the next arm

TOTAL LOCOMOTION Interruption of ACTIVITY photobeam A (or B) gives a count if it is preceeded by an interruption of an ACTIVITY photobeam C (or D) A FORWARD LOCOMOTION will also give a TOTAL LOCOMOTION This corresponds to the rat walking the length of one arm either by walking from one end of the arm to the other or by turning back from the middle of the arm

HOLE COUNT Interruption of any of the photobeams (there is one under each of the six rows of holes) situated 0 5 cm under the hole gives a count This is nearly always caused by a head dip into the hole, an action suggested to reflect exploration [12]

HOLE TIME The accumulated time that any of the HOLE photobeams is interrupted

CORNER COUNT Interruption of any of the four vertically oriented photobeams in the corners gives a count This is a measure of corner restricted behaviour

CORNER TIME The accumulated time that any of the CORNER photobeams is interrupted

REARING COUNT Interruption of the horizontally oriented field of photobeams 12 5 cm above the floor gives a count, e g , when a rat raises on its hindlegs In order to receive only one count per rearing a time delay function was introduced Due to this delay the photobeam field must be *un*interrupted for 1 5 seconds *before next* REARING COUNT can be recorded

REARING TIME The accumulated time that a REARING photobeam is interrupted

Three ratios have been calculated from the variables described

FORWARD LOCOMOTION/TOTAL LOCOMOTION A measure of the variability of locomotor behaviour, the higher the ratio the less varied the locomotion

HOLE TIME/HOLE COUNT A measure of how fast a hole will be visited on average

CORNER TIME/CORNER COUNT A measure of the mean time of corner restricted behaviours

HABITUATION Defined as the ratio between the second and the first five-minute period of ACTIVITY, which is a measure of the within session habituation Low values correspond to a low ACTIVITY in the second five-minute period compared to the first and consequently a faster rate of habituation The reason that HABITUATION was defined in this way is that very low or zero ACTIVITY was sometimes recorded in the second five-minute period If the ratio had been inverse it would in such cases have been extremely large or undefined

Experiments

The pattern of behaviour in the holeboard during a 90-min trial, divided into eighteen five-minute periods was examined and analysed in untreated rats (n=11) With the exception of this experiment, all trials in the holeboard lasted for ten minutes A dose-response curve for APO using a constant pretreatment time of 15 min was constructed on the basis of the following doses 0 005 (n=5), 0 01 (n=7), 0 05 (n=8), 0 1 (n=7), 0 2 (n=7), 0 5 (n=7) and 1 0 (n=7) mg/kg A group of 13 rats served as saline treated controls At least two treatments were tested on the same occasion

Statistical Analysis

Multivariate analysis of variance (MANOVA) and Hotellings T^2 test was used for hypothesis testing [23] HABITUATION but not the ratios were included in the MANOVA In addition, principal component analyses (PCA) were made The principal component scores were calculated for unro-

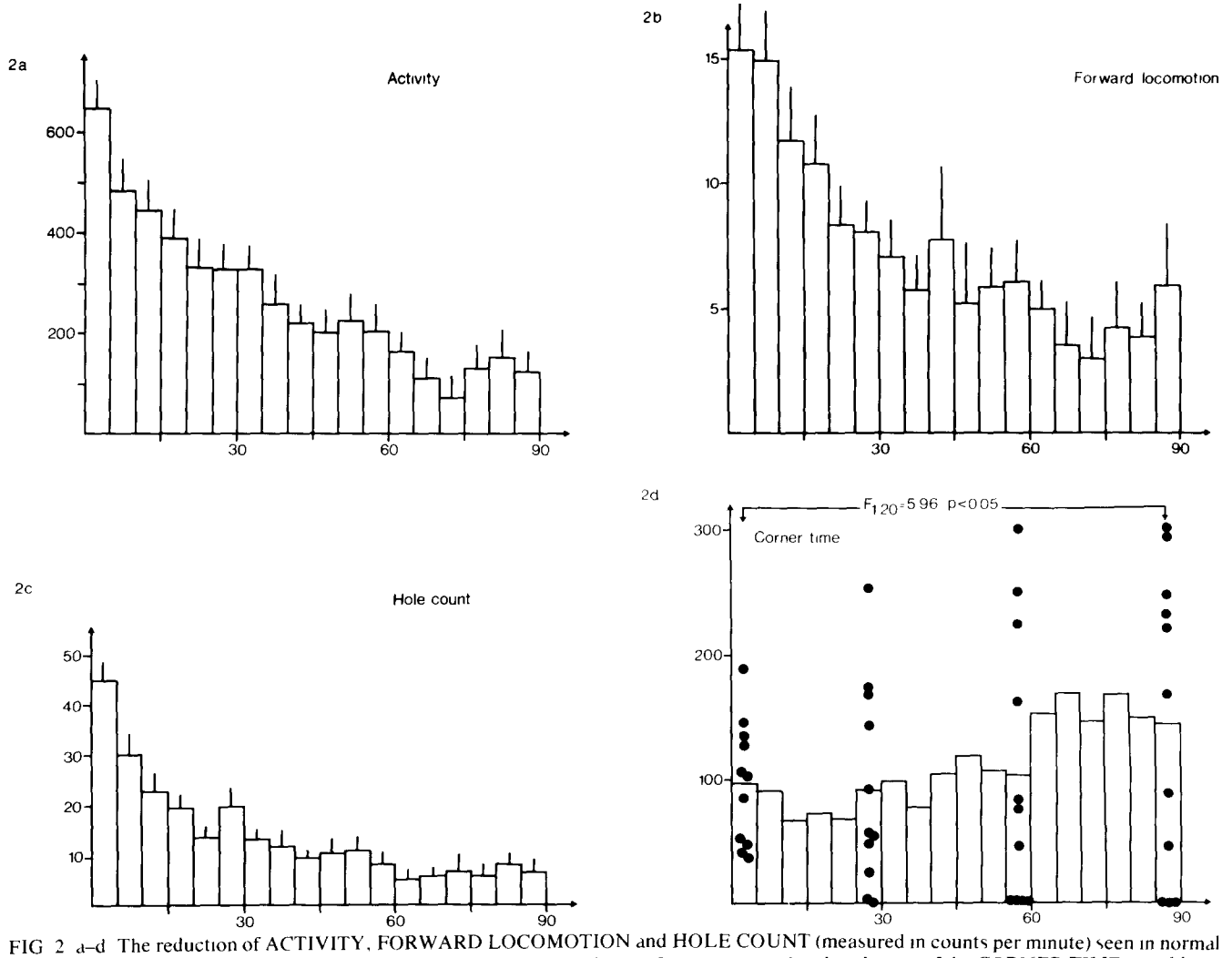

FIG 2 a–d The reduction of ACTIVITY, FORWARD LOCOMOTION and HOLE COUNT (measured in counts per minute) seen in normal rats exploring the holeboard for 90 min is seen in a, b and c The tendency of an extreme value distribution of the CORNER TIME variable as the rats become inactive is seen in d, where the value of CORNER TIME from each individual rat is marked by a dot in the figure

tated solutions and analysed for group differences by one-way analysis of variance (ANOVA) and Dunnett's test [9] In an attempt to interpret the behavioural effects in functional terms, a varimax rotation of the principal component solution was performed using only components that were significant according to cross-validation [36] All data are presented as means±SEM p-Values below 0 05 were regarded as significant Cross-validation ratio <1 indicates significant principal component [36] For a discussion on the use of different multivariate statistical methods see Wold *et al* [37] and Ståhle and Wold [28] The computer programmes for the statistical analysis were written by ourselves in C-basic (ANOVA, MANOVA, Dunnett's test and T^2 test) and pascal (PCA using the NIPALS algorithm [35] with cross-validation) The programmes have been validated against textbook examples [23] and the PCA programme has been checked against a commercially available programme package (SIMCA, see Wold *et al* [37]

RESULTS

Behaviour During a Single 90-Min Trial in the Holeboard

During a 90-min session in the holeboard rats habituate

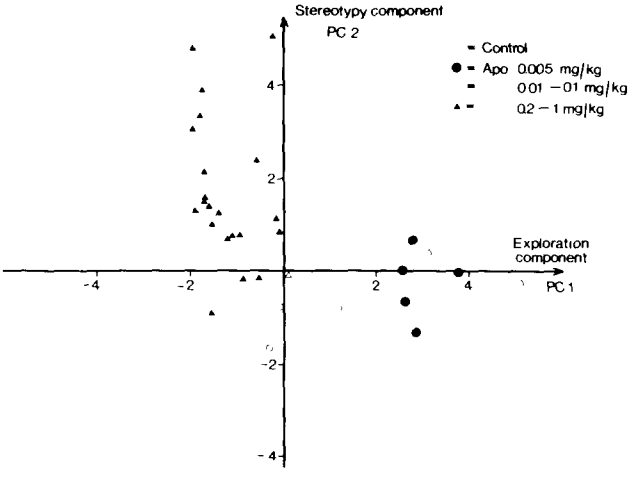

FIG 3 Component scores of each individual in the APO dose response curve PC1 and PC2 are the first and the second principal components

TABLE 1

MEAN ± SEM FOR ALL VARIABLES AND RATIOS FOR CONTROLS AND APO 0 005–1 0 mg/kg

		ACT	FL	TL	HC	HT	CC	CT	RC	RT	HAB	FL/TL	HT/HC	CT/CC
saline	n=13													
mean		1300	35	61	75	68	93	124	14	21	0 63	0 58	0 87	1 29
SEM		102	4	6	7	9	9	22	2	3	0 08	0 03	0 07	0 20
APO 0 005	n=5													
mean		1352	31	53	88	72	108	115	12	17	0 82	0 58	0 82	1 03
SEM		96	3	2	4	8	15	26	2	3	0 07	0 05	0 08	0 16
APO 0 01	n=7													
mean		649	13	28	41	33	81	285	6	10	0 45	0 49	0 80	7 97
SEM		103	3	6	8	7	15	70	1	1	0 05	0 06	0 05	4 17
APO 0 05	n=8*													
mean		569	9	20	37	31	50	197	6	11	0 74	0 50	0 85	4 89
SEM		84	1	4	5	5	7	63	1	3	0 33	0 04	0 08	1 98
APO 0 1	n=7*													
mean		395	3	9	26	19	37	323	7	14	0 58	0 45	0 71	9 39
SEM		65	1	3	5	4	5	63	1	3	0 18	0 12	0 08	3 56
APO 0 2	n=7*													
mean		708	15	19	45	22	56	95	34	61	0 82	0 76	0 48	1 61
SEM		44	3	3	5	4	9	22	15	33	0 13	0 05	0 07	0 18
APO 0 5	n=7*													
mean		673	9	14	23	7	43	37	46	77	0 10	0 66	0 29	0 90
SEM		71	1	1	4	2	8	7	18	33	0 05	0 07	0 07	0 08
APO 1	n=7*													
mean		662	9	12	18	3	77	59	37	49	0 83	0 73	0 15	0 92
SEM		84	2	2	2	1	39	24	13	18	0 14	0 05	0 03	0 11
ANOVA F(7,53)		15 1	16 6	19 1	16 7	14 3	2 4	5 6	3 2	2 5	1 3	0 7	14 8	3 1
p-value <		0 0001	0 0001	0 0001	0 0001	0 0001	0 03	0 0002	0 007	0 03	0 3	0 4	0 0001	0 008

*Denotes significant difference from controls as tested by the T^2 test F-values refer to univariate ANOVA These F-values are included for descriptive purposes ACT—activity, FL—forward locomotion, TL—total locomotion, HC—hole count, HT—hole time, CC—corner count, CT—corner time, RC—rearing count, RT—rearing time, HAB—habituation

with respect to most measured variables (Fig 2a–c) When the first and the last 5-min periods are compared, there is a highly significant decrease in all holeboard variables except CORNER TIME (see below) Direct observation of the animals reveals initial locomotion, sniffing, head-dipping into the holes and rearing Grooming behaviour was occasionally observed and later they became progressively inactive The CORNER TIME variable was dependent on whether an inactive rat chose to be quiescent in the corner or outside the corner (Fig 2d) Hence CORNER TIME was either very high or very low at the end of the 90 min session seen as a larger variance at 90 min than at 5 min, $F(1,20)=5\ 96$, $p<0\ 05$

Apomorphine Dose-Response

There was a clear effect of APO due to dose (MANOVA, $F(70,280)=4\ 25$, $p<0\ 0001$) The contribution was strongest from the ACTIVITY, LOCOMOTION and the HOLE variables The only variable not significantly affected was HABITUATION For details see Table 1 T^2 tests detected highly significant effects 0 05–1 mg/kg compared to controls The profile of behaviour in rats given 0 05 or 0 1 mg/kg APO resembled that of normal animals (Table 1) and when observed they appeared less active though still normal Doses of 0 2 mg/kg and higher produced stereotypies which showed up as changes in the LOCOMOTION and HOLE ratios (Table 1) On observation the animals showed intense sniffing, stereotyped locomotion and the behaviour was less directed towards the holes The tails were raised and sometimes broke the rearing photobeam causing false REARING to occur in the data

Principal Component Analysis

In the analysis of the dose response relationship of APO the original ten variables and FORWARD LOCOMOTION/TOTAL LOCOMOTION, HOLE TIME/HOLE COUNT and CORNER TIME/CORNER COUNT were included All treatment groups were used in this analysis Two distinct and stable components, accounting for 37% and 24% of the total variance respectively, were found according to cross validation (ratios 0 854 and 0 872 respectively) The third component was not significant (ratio 1 14) Only these two components separated groups significantly (Fig 5a and b) The first component separates the control animals from the

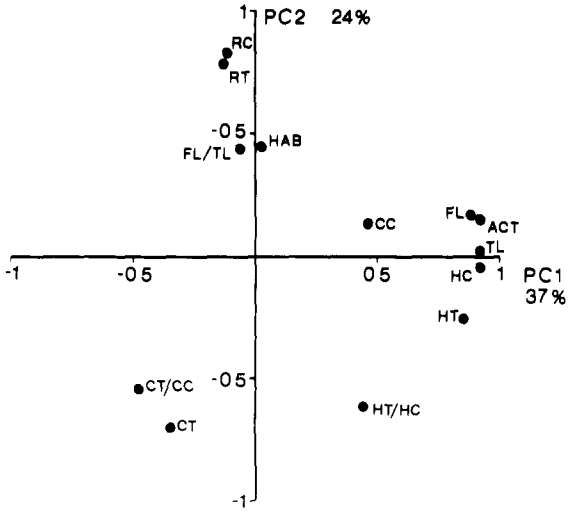

FIG 4 Correlations (loadings) of the original variables with the the first two principal components of the APO dose response relationship PC1 and PC2 are the loading vectors of the first and the second principal components respectively ACT—activity, FL—forward locomotion, TL—total locomotion, HC—hole count, HT—hole time, CC—corner count, CT—corner time, RC—rearing count, RT—rearing time, HAB—habituation

TABLE 2

LOADINGS OF THE ORIGINAL VARIABLES ON THE FIRST PRINCIPAL COMPONENT OF APO 0–0 1 mg/kg (LEFT COLUMN) AND THE FIRST COMPONENT FOUND WHEN THE SALINE CONTROLS WERE ANALYSED SEPARATELY (RIGHT COLUMN)

	Principal Component 1 APO 0–0 1 mg/kg	Principal Component 1 Saline
Activity	0 97	0 90
Forward Locomotion	0 92	0 88
Total Locomotion	0 93	0 83
Hole Count	0 91	0 46
Hole Time	0 79	0 22
Corner Count	0 69	0 15
Corner Time	0 62	0 64
Rearing Count	0 83	0 89
Rearing Time	0 75	0 83
Habituation	0 42	0 20
% variance explained	64	45

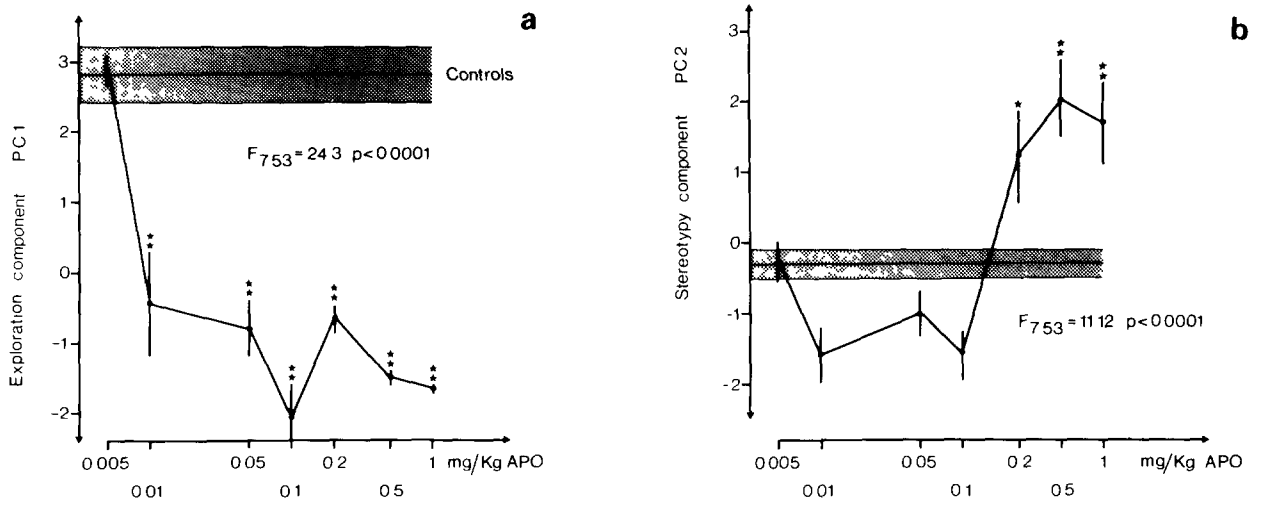

FIG 5 Dose response curve for APO on the first two principal components of the second set of data (including ratios) The F-values refer to univariate ANOVA tests made on each component separately * is $p<0$ 05 and ** is $p<0$ 01 compared to saline controls in Dunnett's multiple comparison procedure PC1 in (a) is the first principal component, PC2 in (b) is the second principal component

APO treated (Fig 3) All APO treated groups (except the group treated with 0 005 mg/kg) had significantly lower scores (Fig 5a) on this component (ANOVA, $F(7,53)=24$ 3, $p<0$ 0001) ACTIVITY and the LOCOMOTION and HOLE variables had the highest loadings on this component (Fig 4) Saline treated subjects and subjects given APO 0–0 1 mg/kg were analysed separately using only the originally measured variables It was found that the loadings of the first principal component of the two analyses closely resembled one another (Table 2)

The second component of the complete APO dose response relationship separates animals given 0 2–1.0 mg/kg APO from the other treatment groups (Fig 3) Only these three groups had higher scores (Fig 5b) on the second component than controls (ANOVA, $F(7,53)=11$ 12, $p<0$ 0001) REARING, FORWARD LOCOMOTION/TOTAL LOCOMOTION, HABITUATION and HOLE TIME/HOLE COUNT have the highest loadings on this component The animals with high scores on this component are those exhibiting stereotyped locomotion (an increased LOCOMOTION ratio) and shorter bouts of visits to the holes (a decreased HOLE ratio) Varimax rotation of the first two principal components did not affect the loadings (data not shown)

DISCUSSION

The aim of this study has been to create a behavioural model suitable for separating and measuring *qualitatively* different patterns of behaviour induced by dopamine receptor agonists, using multivariate automatic recording of behaviour and multivariate statistical methods The problem arises from the fact that the commonly available "activity boxes" may record an equal number of counts during normal exploratory behaviour and during drug induced stereotyped behaviour (see ACTIVITY in Table 1) in spite of the fact that the behaviours are clearly separable by the observer

By using an automatic holeboard [20] capable of separating nine different behavioural variables we have tried to capture enough components of normal exploratory behaviour as well as stereotyped behaviour to permit a quantitative determination of the qualitative differences The effects of APO in different dose levels was used to examine the methods

The results of the initial experiment—where the animal was allowed to explore the holeboard for 90 min—indicated that the first 10 min was a suitable test period The animals did intensely explore the environment during this period which was reflected in high values for all measured variables, except CORNER TIME (Fig 2) Low exploration during the last periods if the 90 min habituation was reflected by low levels of all variables except CORNER TIME which was very high or very low, depending upon if the animal settled in a corner or not

The decrease in ACTIVITY from the first to the second five-minute period formed the basis for expressing HABITUATION as the counts during the second five-minutes divided by the counts during the first five-minute period

APO in the low dose-range (0 01–0 1 mg/kg) caused a quantitative reduction in the amount of exploration with increasing doses although the behaviour qualitatively resembles that of untreated animals during normal exploration The level of some components, e g , ACTIVITY, FORWARD LOCOMOTION and HOLE COUNT are dose dependently reduced, whilst two of the ratios calculated (FORWARD LOCOMOTION/TOTAL LOCOMOTION and HOLE TIME/HOLE COUNT) are not different from saline controls (Table 1) Thus the animals vary their locomotion and explore holes in the same fashion as untreated rats. The fact that the animals are less active but otherwise normal is shown by the principal component analysis since the loadings of the first component found, when subjects given APO 0–0 1 mg/kg are analysed, closely resembles the loadings of the first component of the control animals analysed separately (Table 2) We have therefore interpreted the first principal component as an exploration component The T^2 test was less powerful than the principal component analysis in finding group differences since the difference between APO 0 01 mg/kg and the controls was not detected

Higher doses of APO (0 2–1 0 mg/kg) induce profound qualitative changes, the most obvious differences being the almost complete lack of thorough exploration of the holes (a very low HOLE TIME/HOLE COUNT ratio) and a stereotyped locomotor behaviour [27] picked up as an increase in FORWARD LOCOMOTION/TOTAL LOCOMOTION This group of animals do not habituate in a normal way (HABITUATION close to 1) The principal component analysis reveals this pattern as shown by the fact that the highest loadings on the second component (Fig 4), which separated animals given APO 0 2–1 mg/kg from the other treatment groups, come from HABITUATION, the LOCOMOTION and HOLE ratios and the REARING variables This group of animals had high component scores on the second component and they exhibited a stereotyped behaviour and we therefore interpret it as a stereotypy component The finding that the varimax rotation of the first two principal components did not change the loadings considerably, justifies the direct interpretations of the principal components The T^2 test was as effective as the principal component analysis in finding group differences in this dose range

Thus two distinct behavioural syndromes are produced related to the dose APO administered The lower doses produce a dose-dependent decrease of exploration, but the behaviour is qualitatively similar to normal exploration High doses induce stereotyped patterns of behaviour, qualitatively different from normal exploration The break point between these two behavioural patterns seem to occur between APO 0 1 and 0 2 mg/kg Although this break point is evident in all variables and ratios except HOLE TIME (Table 1) it is clear that no single variable by itself is sufficiently discriminative to separate subjects receiving saline, low doses of APO or high doses of APO from each other Neither do the traditional MANOVA and T^2 test show this since no appropriate multiple comparison method is available In this connection the principal component analysis appears to be a powerful method for the detection of such breakpoints when new effects or different patterns appear Principal component analysis in combination with ANOVA and, e g , Dunnett's test made on the component scores appears to be a more powerful method than the MANOVA with the T^2 test Gage *et al* [13] used a similar approach using discriminant function analysis The present approach has the advantages that the PCA is not assuming a multivariate normal distribution (provided that cross validation is used to determine the number of significant components [36], see also Ståhle [28]) and the PCA is independent of grouping PCA can also be used when the number of variables (p) exceed the number of subjects (n), the opposite ($n>>p$) is a requirement for discriminant function analysis However, when the requirements for discriminant function analysis are met it may perform at least as well as PCA [37] For a more detailed discussion of these methods see Ståhle and Wold [28] Thus, this statistical method makes possible the separation of, e g , ACTIVITY levels that are qualitatively different This is most obvious for the doses 0 01 mg/kg and 0 2 mg/kg which cannot be separated by the ACTIVITY measurement but easily discriminated when the ratios FORWARD LOCOMOTION/TOTAL LOCOMOTION, HOLE TIME/HOLE COUNT and HABITUATION (Fig 4) are considered by means of the principal component analysis where the higher doses of APO form a distinct component of stereotypy

Low doses of APO are thought to selectively stimulate DA autoreceptors [5, 18, 30, 33] while high doses also involve postsynaptic receptors, which is the basis for the induction of stereotyped behaviour [3, 6, 10, 19] When the present findings are compared to results from the measurement of dopamine (DA)-release after various doses of APO [31,38] there is also an evident difference between the effects of APO given in low and high doses The low dose range inhibits DA-release to about 50% while DA-release is almost completely inhibited after APO 0 5 mg/kg and above It seems theoretically possible that after low doses of APO,

DA-release is decreased but some physiological synaptic modulation maintained, while after high doses of APO stimulation of the receptors by endogenous DA is prevented and replaced by a tonic stimulation of the exogenous agonist This may constitute a reason for the appearance of the abnormal stereotyped behaviour A similar conclusion was suggested by Wachtel *et al* [32]—who stated—on the basis of experiments with alfa-methylparatyrosine and reserpine, that normal behaviour is dependent on release of dopamine by nerve impulses

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