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ORIGINAL INVESTIGATION

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Comparative behavioural and neurochemical studies with a psychomotor stimulant, an hallucinogen and 3,4-methylenedioxy analogues of amphetamine

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Abstract Spontaneous behaviours were assessed in freely moving rats after treatment with equimolar doses of drugs that share a basic amphetamine structure. The drugs used included a psychomotor stimulant [(+)-amphetamine (AMPH)], an hallucinogen [*para*-methoxyamphetamine (PMA)] and the entactogens 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxy-*N*-ethylamphetamine (MDE). A detailed analysis of the frequency and duration of 30 different behaviours and the temporal organization of the behaviours was conducted in addition to measuring motor activity with an automated device. Levels of the biogenic amines and their acid metabolites in discrete brain regions and brain drug levels were also obtained. The automated motor activity measures discriminated among entactogens, the stimulant and the hallucinogen, but failed to distinguish between the hallucinogen and vehicle. Principal components analysis and cluster analysis of the frequencies and durations of the behaviours did not improve the classification of the drugs over the automated motor activity measures. Only the cluster analysis of the transitions between individual behaviours succeeded in differentiating the drug classes from each other and from vehicle treatment. All the behavioural measures classified one entactogen (MDE) as an hallucinogen. Cortical 5-hydroxytryptamine (5-HT) measures grouped MDE with the other entactogens but did not distinguish AMPH from vehicle. However, striatal dopamine measures differentiated AMPH from vehicle treatment. Variations in the durations of behavioural effects across drugs were associated with large differences in drug levels 3 h after injection. Although the neurochemical data provided a classification system that most closely parallels human subjective effects of these drugs, both the neurochemical and the behavioural measures supported the existence of an entactogen class distinct from a psychomotor stimulant and an hallucinogen.

Key words Rats · Entactogens · Amphetamine · MDMA · MDA · MDE · PMA

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Introduction

It has been suggested that the methylenedioxy analogues of amphetamine, 3,4-methylenedioxyamphetamine (MDA; "Love") and 3,4-methylenedioxymethamphetamine (MDMA; "Ecstasy") represent a novel class of drugs, labelled entactogens, distinct from psychomotor stimulants and hallucinogens (Nichols et al. 1986; Nichols and Oberlender 1990). Hermle et al. (1993) supported the hypothesis that 3,4-methylenedioxy-N-ethylamphetamine (MDE; "Eve") also belonged to the novel entactogenic class, describing a partially controllable state of enhanced insight, empathy and peaceful feeling after MDE ingestion in a double blind placebo controlled study with human volunteers.

Attempts to develop suitable animal models and behavioral paradigms with which to study recreationally used drugs have met with many interpretive and methodological problems, especially when comparing the effects among the different classes of compounds. Much has been written about the effects of stimulants on spontaneous behavior in rats (e.g. Moore 1978; Robbins et al. 1990). The effects of MDMA on spontaneous behaviour in freely moving rats have also been examined, using photobeam interruption counts as a measure of locomotor activity and a 4-point scale to assess "serotonin syndrome" behaviours [forepaw treading, head weaving, low body posture, piloerection and salivation (Spanos and Yamamoto 1989)]. However, it has been more difficult to examine the effects of hallucinogens on spontaneous behaviours in

animals. Hallucinations, defined as sensory experiences not based on reality, are not necessarily accompanied by consistently observable behaviours (Glennon 1992). Jacobs et al. (1976) proposed the term "hallucinatory behaviours" to describe any behaviour that is distinctively induced by a known hallucinogen. Such behaviours, including limb flicks and "wet dog shakes", occur at such low frequency that statistical analyses often fail to show significance. In recent reviews of the various animal models used in research into hallucinogens (disruption of conditioned avoidance responding, head twitch, hyperthermia, "serotonin syndrome", drug discrimination and startle response), Glennon (1990, 1992) concluded that there is no one model that alone can consistently predict hallucinogenic properties.

Martin-Iverson (1991) developed a computerized methodology to collect and analyze behavioural data from freely moving rats in order to characterize behavioural effects of chronic AMPH treatments. The measured behaviours include those described as "hallucination-like" (Jacobs et al. 1976; Ellison 1991). It may also be important to include the sequences of behaviour because dopamine (DA) systems which are known to be affected by AMPH are involved in the temporal coordination and selection of contextually appropriate behaviours (Norton 1968, 1973; Evenden and Robbins 1983). Thus, AMPH affects not only the performance of individual behaviours, but also the organization of behavioural patterns. Hallucinogens might also disrupt the organization of behavioural patterns, with sensory misperceptions playing a role in the animal's behavioural response, possibly via alterations in 5-HT neurotransmission (Jacobs 1984).

The methodology of Martin-Iverson (1991) was applied to determine the effects of equimolar doses of racemic MDA, MDMA, and MDE in contrast to AMPH and the hallucinogenic amphetamine analog, *para*-methoxyamphetamine (PMA). The representative stimulant and hallucinogen were chosen on the basis of their similarity in core structure to the methylenedioxy compounds. The dose of 32 μmol/kg was chosen so that the dose used would be similar to that reported in other studies of the individual compounds (Shulgin et al. 1969; Menon et al. 1976; Shulgin 1978; Shulgin and Nichols 1978). Although differing behavioural effects between the (+)-MDMA and (−)-MDMA in rodents and between (+)-MDA and (−)-MDA in rodents and human volunteers have been reported (Shulgin 1978; Nichols 1986; McKenna et al. 1991), the racemates of the methylenedioxy compounds and PMA were used in this study to resemble more closely the situation when the drugs are taken recreationally. If the effects of the methylenedioxy analogues as a group could be differentiated from the other class representatives, it would add supportive evidence for the creation of the entactogen class. It could also provide another experimental methodology for assessing hallucinogenic compounds.

Regional levels of DA, 5-HT and noradrenaline (NA) and acid metabolites were also examined using a high performance liquid chromatography (HPLC) with electrochemical detection. Drug levels in whole brain were obtained using gas chromatography with nitrogen-phosphorus detection.

Materials and methods

Subjects and apparatus

Male Sprague-Dawley rats (250–400 g) were randomly assigned to drug or vehicle treatment groups ($n = 12$). Each animal was used only once. The light/dark cycle was reversed so that the animals were in their active phase during the testing period. Animals were individually placed into 1 of 48 stainless steel cages [24 (width) × 26 (length) cm^2], with Plexiglas sides and a wire-mesh floor for 10 days prior to drug treatment to acclimatize them to the cages and the reversed light/dark cycle. In front of each cage was an infrared-sensitive CCD video camera (Canon Ci-20R) mounted to view one cage. Each camera was equipped with a 12-mm auto-iris lens Canon C 61205) and an infrared light source (IR-20W) that does not provide light in the visible spectrum. The video signal from each camera was forwarded to a video monitor (Hitachi VM-900), a time data generator (Pelco TDG200DT), and an extended video cassettle recorder (NED PV-1200A), all of which were kept in a room adjacent to the testing room. The cages were also equipped with infrared photocell beams. All procedures involving rats were conducted according to Canadian Council on Animal Care guidelines.

Drugs

(+)-AMPH sulfate was provided courtesy of SmithKline Beecham Pharma. The hydrochloride salts of the methylenedioxy compounds, (±)-MDMA, (±)-MDA and (±)-MDE, and of (±)-PMA were provided by Health and Welfare Canada. Each of the drugs was administered at a dose of 32 μmol/kg (based on free base weight) such that each rat received 2 ml/kg of drug solution.

Behavioural procedure

On the test day, an equimolar dose of one of the five drugs (32 μmol/kg dissolved in saline) of saline was administered via acute intraperitoneal injection. The rats were videotaped with infrared sensitive cameras using a time-sampling procedure: 2.5-min observation periods every 30 min for 1.5 h prior to and 3 h post-injection, with a total post-drug sampling time of 15 min. Photobeam interruption counts were collected at 30-min intervals for the 1.5 h prior to and the 3 h post-drug administration.

Neurochemistry procedures

All animals were killed by guillotine decapitation immediately following their last taped observation period (at 3 h post-drug). After midline sagittal splitting of the brain, dissections were carried out on one of the halves to obtain hippocampal, hypothalamic, striatal and cortical tissues. The other half of the brain was kept for drug level analysis. An equal proportion of right and left halves were used for the neurochemical and the drug level determinations. All tissues were stored at −80° C until assayed. The HPLC analyses were carried out using the method described by Baker et al. (1987) and were performed using a Waters (Milford, Moss. USA) WISP

710B sample injector system and a model 510 pump, a Waters M460 detector and a Waters 740 data module integrator. The compounds of interest were measured using a glassy carbon electrode set at 0.88 V versus an Ag/AgCl reference electrode. The flow rate was set at 0.8 ml/min through a Phenomenex C18 column (4.6 mm × 250 mm; 5 μm particle size, Torrance Calif. USA) coupled to a precolumn. The mobile phase consisted of $NaH_2PO_4 \cdot H_2O$ (55 mM), sodium octyl sulfate (0.85 mM), disodium EDTA (0.37 mM) and acetonitrile (10%). The mobile phase was filtered through a type HA filter (0.45 μm, Millipore) before being degassed and adjusted to a pH of 3 using phosphoric acid.

The concentrations of monoamines and acid metabolites were determined by comparing peak areas for the brain samples to authentic standards processed in parallel with each assay (range 5–100 ng).

Drug levels were obtained using a modification of the gas chromatographic assay procedure developed by Hegadoren et al. (1993) for analysis of MDMA and MDA.

Data analysis

Videotape records were analyzed by an observer blind to the drug conditions of the animals and the purpose of the experiment. Frequency, duration and sequences of 30 different behaviours were scored with the Basic Experimental Behavioural Observation Program (BEBOP) (see Table 1). The clock in this program was linked to the time/date signal on the videotapes for timing behaviours. A key was pressed at the initiation and again at the termination of a bout of a behavior, allowing determination of bout durations. Behavioural categories were not necessarily mutually exclusive. For example, locomote, sniff, head movement and snout contact with cage surface may all occur simultaneously. However, they are not likely to be initiated simultaneously. The program allowed for editing of scoring, such that a tape could be rewound and replayed at normal or slow speeds until the observer was confident that the behaviors were accurately scored. To establish level of intra-observer reliability, selected tapes were analyzed twice for the frequencies and duration of behaviours and frequencies of transitions and compared using Hoyt's reliability analysis.

Statistical analyses of the photobeam interruption counts and the frequencies and durations of behaviours were completed using ANOVA followed by multiple F-test comparisons ($\alpha = 0.05$). Principal components analysis was conducted on the totals of both the frequencies and the durations of the 17 behaviours that were significantly affected by drug treatment. Seven factors were identified as accounting for 81% of the variance in the behaviours. These components were computed by summing the products of the correlation coefficients and the behavioural measures that contributed to the factor, and then were subjected to ANOVA with drug as the independent factor. In two cases (factors 2 and 7), there was a lack of homogeneity of variances across groups. Therefore, the square roots of the values of the factors were used for the ANOVA.

Behavioural sequences were analyzed by the method of adjusted residuals, comparing the actual observed behavioural transitions with the random expected transition, given the frequency of initiations of behaviour (Haberman 1973). Computer software for these analyses was developed by one of the authors (M. T. Martin-Iverson). Multiple correlations in terms of the ratios of the observed and expected transitions across treatment groups were determined.

Cluster analyses were carried out on the frequencies and durations of the behaviours significantly affected by drug treatment and on the ratios of the observed and expected transitions across treatment groups to identify more fully the homogeneity among the drug groups. Agglomerative hierarchical clusterings were completed using the commonly used distance measure, the squared Euclidean distance, which is the sum of the squared differences over all of the variables being considered. Squaring the differences gives more weight to greater differences. The Euclidean distances are plotted by rescaling the actual distances to numbers between 0 and 25 and are represented in the form of dendograms.

Statistical analyses on the neurochemistry were conducted using ANOVA followed by the multiple F test ($\alpha = 0.05$). 5-HT turnover was expressed as the ratio of levels of its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) to 5-HT. DA turnover was defined as the ratio of the levels of the sum of the two DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) to DA. All ANOVAs, principal components analyses and cluster analyses were carried out using commercial software (SPSSPC).

Table 1. Basic Experimental Behavioural Observation Program (BEBOP) coded behaviours and their operational definitions

1. *Wet-dog shake:* abrupt shaking/shivering predominantly of the shoulders.
2. *Eating:* gnawing of food pellets.
3. *Backwards walking:* moving in reverse, using all four limbs with a result of altering the animal's position in the cage.
4. *Stretch:* extension of the forequarters with the forelimbs stretched out in advance of the animal.
5. *Yawn:* mouth gaping widely.
6. *Hindlimb scratch:* scratching of the body or head with one of the hindlimbs.
7. *Pause:* a momentary cessation of all active behaviours (e.g. sniffing, walking), but not of postural behaviours.
8. *Sniff:* rapid flaring and contracting of the nostrils accompanied by movements of the whiskers.
9. *Drink:* licking the water spout.
10. *Limb flick:* a rapid twitch of one of the limbs.
11. *Groom:* face, head or body stroking with the forelimbs, or licking of any part of the body.
12. *Head movements:* head weaving (lateral movements) or bobbing (vertical movements).
13. *Jump:* a leap upwards into the air such that all four limbs leave the cage floor at some point.
14. *Lick:* the tongue can be observed making contact with some surface other than the animal's own body.
15. *Locomote:* movement with all four limbs that alters the animal's position in the cage.
16. *Sleep:* eyes are closed, breathing is even and slow.
17. *Retrieve food:* reaching into the food bin with forepaws or mouth and bringing out a food pallet.
18. *Snout contact with a cage surface:* self-explanatory.
19. *Postural adjustment:* a movement of the trunk of the body that alters the posture of the animal excluding major transitions of posture (i.e. excluding changes between stand, sit, lie, locomote, rear, reverse locomote, jump). For example, a shift of the body while lying down so that a slightly different area of the body is in contact with the cage floor.
20. *Gnaw:* chewing on something in the cage other than food.
21. *Mouth movements:* jaws opening and closing in empty air (excludes gnawing, licking, eating and drinking).
22. *Can't tell:* the animal faces away from the camera, occluding the view so no adequate assessment of the behaviour can be determined.
23. *Rear:* the animal is standing with its hindlimbs extended to the floor and the forelimbs are in the air or resting on a wall.
24. *Lie:* the animal is lying down such that its legs are retracted close to the body or stretched out.
25. *Sit:* the animal is on its haunches, without supporting its body with its forelimbs.
26. *Stand:* all four limbs are extended and apparently supporting the weight of the animal.
27. *Twitch:* a sudden quick contraction of some part of the trunk or head.
28. *Scrabble:* pawing at the floor or wall.
29. *Clockwise rotation:* turn to the right of at least 90°.
30. *Counter-clockwise rotation:* turn to the left of at least 90°.

Results

Intra-observer reliability

The results of Hoyt's reliability analysis indicated that reliability coefficients (r^2) were 0.996 for frequencies (SE of measurements = ± 1.02), 0.9996 for durations (SE of measurement = 3.07) and 0.960 for sequences (SE of measurement = 1.2)

Locomotor activity

Locomotor activity, as measured by photobeam interruption counts, is shown in Fig. 1. There were no significant differences among the six treatment groups in the 1.5-h predrug time period [$F(1,66) = 1.60$, $P > 0.15$]. By 90 min post-drug administration, three distinct patterns emerged and continued throughout the remaining time periods. As expected, AMPH resulted in the largest increase, which peaked at 120 min post-drug, and remained above vehicle counts throughout the entire study period. Both MDMA and MDA caused a rapid increase over the first 30 min, then fell to almost vehicle levels by 180 min. Neither PMA nor MDE produced any significant change from vehicle at any of the time periods.

Principal components

Factor 1 is labelled "large body movements", since it included locomote, rear and clockwise rotation. No significant drug effects were seen [$F(1,66) = 0.35$, $P > 0.5$]. Factor 2, labelled "stimulus-induced behaviours", included jumping, retrieval of food and scratching and is shown in Fig. 2. MDE was the only drug that did not significantly decrease these behaviours

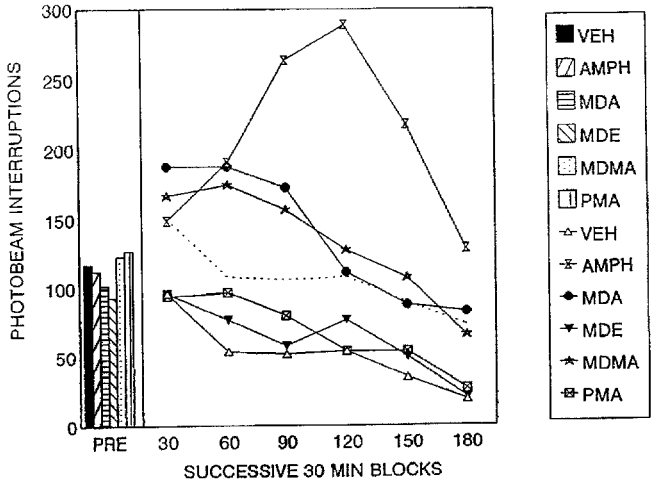

Fig. 1 Photobeam interruption counts. *Bars* on *left* represent total counts for the 1.5 h prior to drug treatment. *Dotted line* represents minimum counts required to achieve statistical significance ($P<0.05$)

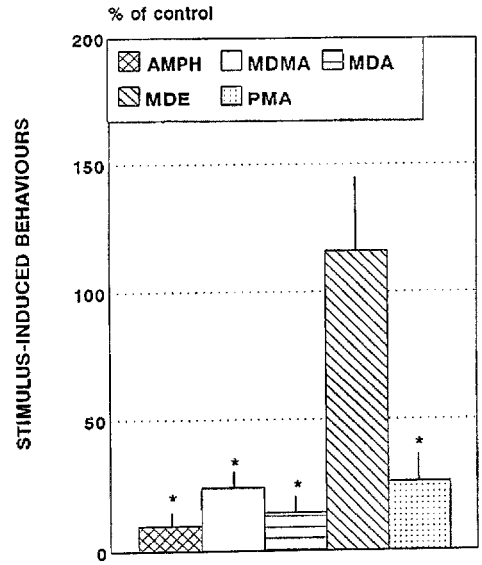

Fig. 2 Stimulus-induced behaviours expressed as mean % ± SEM of control. * Indicates significant difference, $P < 0.05$

[$F(1,66) = 10.5$, $P < 0.001$]. Factor 3, labelled "olfactory exploration", included sniffing, head movements, snout contact with a cage surface and standing (Fig. 3). The same pattern of drug groupings was seen as in the photobeam count data, with AMPH > MDMA = MDA > MDE = PMA. Factor 4 involved "yawning behaviours" (yawning, mouth movements, lying down and stretching) (Fig. 4). AMPH produced the greatest decrease, MDMA and MDA also caused significant decreases and MDE and PMA had no significant effects [$F(1,66) = 5.7$, $P < 0.001$]. Factor 5, which was limited to grooming only, was not affected by drug treatment [$F(1,66) = 1.9$, $P > 0.1$]. Factor 6, labelled "postural adjustment" (Fig. 5), included body adjust-

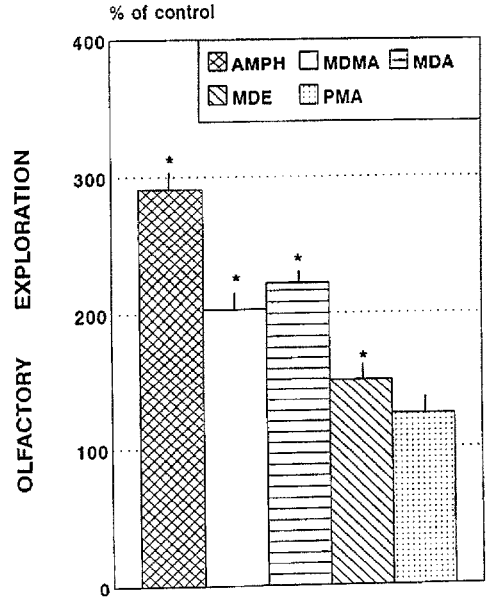

Fig. 3 Olfactory exploration behaviours expressed as mean % ± SEM of control. * Indicates significant difference, $P < 0.05$

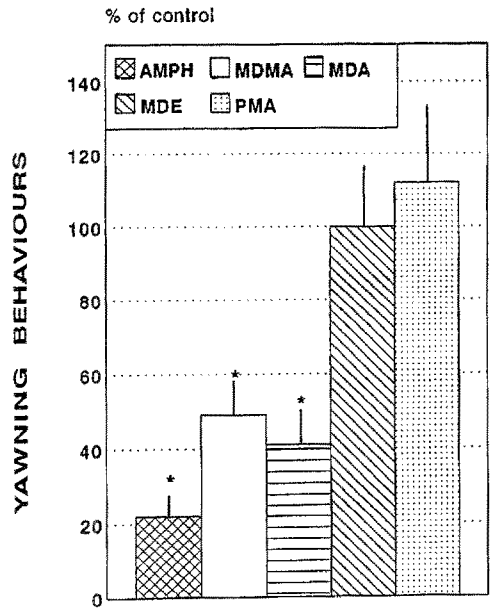

Fig. 4 Yawning behaviours expressed as mean % ± SEM of control. * Indicates significant difference, $P < 0.05$

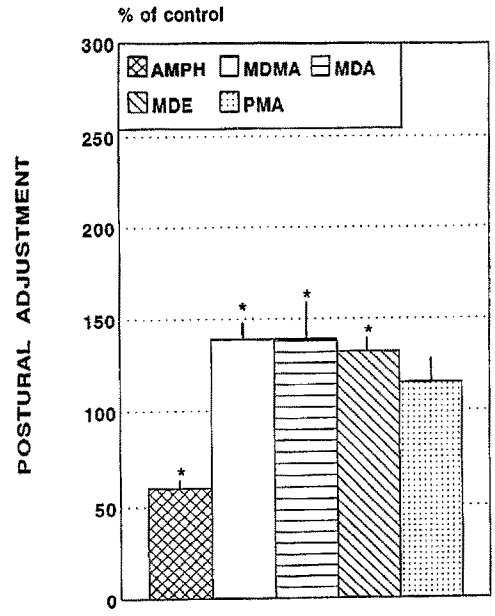

Fig. 5 Postural adjustment behaviours expressed as mean % ± SEM of control. * Indicates significant difference, $P < 0.05$

ments and clockwise rotation. Here, the difference between AMPH and the other compounds was not one of potency, but of the direction of drug effect: AMPH significantly decreased these behaviours; MDMA, MDA and MDE all resulted in increases; and PMA had no significant effect [$F(1,66) = 8.7$, $P < 0.001$]. Factor 7 involved sleep. All drug treatments caused a significant decrease in sleep [$F(1,66) = 5.72$, $P < 0.001$]. Administration of AMPH, MDMA and MDA caused a complete loss of sleep, while treatment with MDE or PMA had a less potent effect (decreased to 38% and 32% of control, respectively).

Transitional analyses

The significant transitions between sets of two behaviours for all the treatment groups are listed in Table 2. The transition from contact to head movement was the most frequently observed transition in the vehicle-, MDMA-, MDE- and PMA-treated groups, while locomote to stand was the most frequently observed in the AMPH- and MDA-treated groups. The observed frequency of the transition from stand to sniff was significantly different from that expected by chance in only the AMPH-, MDMA- and MDA-treated groups. The opposite pattern was seen in the transition from lie to body adjustments, where only vehicle, MDE and PMA produced significant differences. Table 3 summarizes the multiple correlations between all the ratios of observed/expected transitions and the treatment groups. The highest degree of similarity existed between PMA and MDE. The pattern of drug grouping was consistent with the photobeam interruption counts and the principal components analysis of the frequencies and durations of the individual behaviours: AMPH

Table 2. Frequencies of observed transitions significantly different from those expected from random, using method of adjusted residuals (ratio = observed/expected). N.S. = not significant

Transition	VEH	AMPH	MDMA	MDA	MDE	PMA
Contact → Head movement	122/48.5	99/38.6	142/66.4	147/59.5	151/70.2	139/66.5
Locomote → Stand	101/21.8	199/50.7	114/18.5	173/31.5	148/27.6	129/21.7
Stand → Contact	98/48.4	145/90.1	78/51.9	105/63.4	106/56.2	80/46.1
Sniff → Head movement	97/32.8	N.S.	N.S.	77/30.3	107/43.1	114/46.6
Stand → Locomote	58/22.9	166/55.3	60/21.1	80/34.3	79/29.7	65/23.2
Sniff → Contact	48/27.5	73/30.1	52/35.5	54/30.6	63/35.6	52/33.1
Rear → Contact	46/12.7	35/21.3	20/12.7	32/16.2	36/10.9	32/9.2
Stand → Rear	24/14.4	N.S.	N.S.	27/16.2	20/9.7	18/8.4
Body adjust → Body adjust	23/16.7	N.S.	72/35.6	57/25.1	61/46.7	90/47.7
Head movement → Mouth movement	20/14.1	2/2.8	13/8.5	12/5.6	25/17	27/15.5
Mouth movement → Sniff	19/ 7.8	17/2.1	18/4.4	14/2.5	31/8.1	24/7.3
Stretch → Yawn	18/ 0.3	1/0	2/0	3/0	10/0.2	11/0.2
Lie → Body adjust	15/ 5.9	N.S.	N.S.	N.S.	17/10.6	20/12.2
Contact → Locomote	N.S.	73/49.1	N.S.	44/32.5	48/30.7	33/23.8
Stand → Sniff	N.S.	45/29.1	47/30.9	43/28.1	N.S.	N.S.

Table 3. Percent variation in common in terms of the ratios of observed/expected transitions across treatment groups, results of multiple correlations (% variation = correlation value^2 × 100

	PMA	MDE	MDA	MDMA	AMPH	VEH
VEH	92	90	30	16	3	
AMPH	11	16	64	55		
MDMA	40	41	91			
MDA	55	58				
MDE	99					

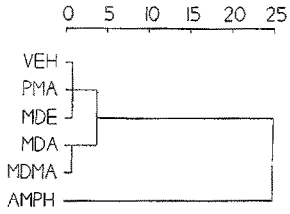

Fig. 6 Dendogram displaying the clustering of the treatment groups by the frequencies and durations significantly affected by drug treatment. *Vertical lines* denote joined clusters. The position of the line on the scale indicates the relative distance at which the clusters were joined

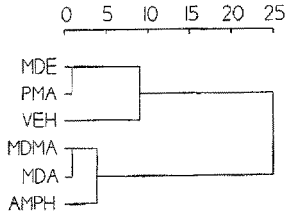

Fig. 7 Dendogram displaying the clustering of the treatment groups by the ratios of the observed and expected transitions between individual behaviours. *Vertical lines* denote joined clusters. The position of the line on the scale indicates the relative distance at which the clusters were joined

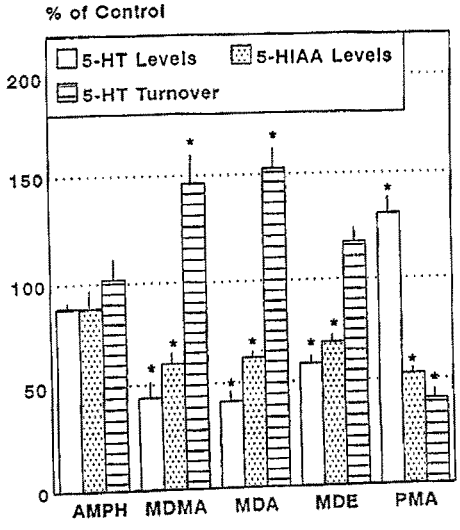

Fig. 8 5-HT parameters in rat cortex 3-h after acute IP injection of equimolar doses (32 μmol/kg) of the drugs. Values are expressed as % of control. * Indicates significant difference ($P<0.05$)

constituted a distinct group; MDMA and MDA another; and MDE, PMA and vehicle a third group.

Cluster analyses

The dendogram of the clustering of the treatment groups by the frequencies and durations of behaviours is shown in Fig. 6. From the ratios of the distances, the pattern of drug groupings is consistent with the locomotor activity data, with AMPH as a distinct cluster, MDMA and MDA closely related and PMA and MDE closely related but not distinguishable from vehicle. However, the dendrogram of the clustering of the treatment groups by the ratios of the observed and expected transitions, as seen in Fig. 7, shows that PMA and MDE are more closely related to each other and only distantly related to vehicle. AMPH remains a distinct cluster and MDMA and MDA remain another cluster with some degree of similarity to AMPH.

Neurochemistry

Regions analyzed for levels of biogenic amines and their acid metabolites included the hypothalamus, hippocampus, striatum and the cortex. In general, the data supported a change in the pattern of drug groupings from that derived from the behavioural data, with MDE-treated animals sharing more similarity in effects with MDMA and MDA than with PMA. Data from the AMPH- and PMA-treated animals supported AMPH and PMA remaining as distinct drug groupings.

Figure 8 shows 5-HT parameters in the cortex. AMPH produced no changes. MDMA, MDA and MDE produced significant decreases in the levels of 5-HT and 5-HIAA and increases in 5-HT turnover, although the increase in turnover following MDE did not achieve statistical significance. PMA also produced decreased 5-HIAA levels, but this was accompanied by increases in 5-HT levels and decreased 5-HT turnover. A similar pattern of 5-HT/5-HIAA changes was also seen in the striatum and the hippocampus. There were no significant drug effects on NA levels in the hypothalamus. Figure 9 depicts the changes in NA levels in the other three regions. MDMA and MDE had no effect. AMPH and MDA produced significant decreases in NA levels in the cortex, while in the

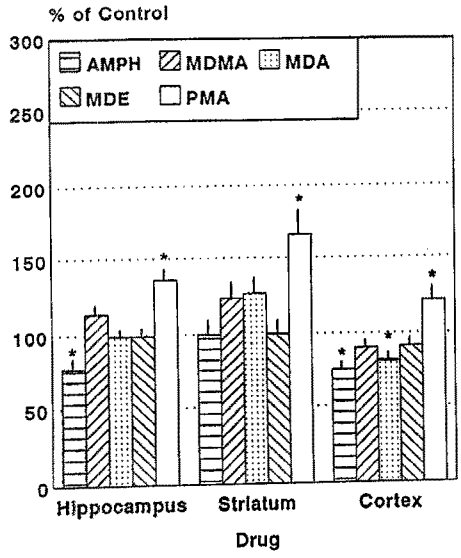

Fig. 9 Noradrenaline levels in three brain regions 3-h after acute IP injection of equimolar doses (32 μmol/kg) of the drugs. Values are expressed as % of control. * Indicates significant difference ($P < 0.05$)

hippocampus decreases were only seen in the AMPH-treated animals. PMA caused a significant increase in all three regions.

Figure 10 outlines the changes seen in DA parameters in the striatum. Some regional differences, both in the degree and direction of change are seen. Overall, DA levels were not affected by drug treatment, with the exception that animals treated with MDA showed a significant increase in DA levels in the hypothalamus. MDE was the only drug that failed to produce decreased levels of DOPAC and HVA and a decrease in DA turnover. Similar drug effects on DA parameters were seen in the cortex: DA turnover was decreased by all drug treatments except MDE; DOPAC levels were decreased after MDMA, MDA and PMA administration; and HVA levels were decreased by MDMA and PMA (data not shown). AMPH increased HVA levels in the cortex and MDE increased DOPAC levels in the cortex, hypothalamus and hippocampus (data not shown).

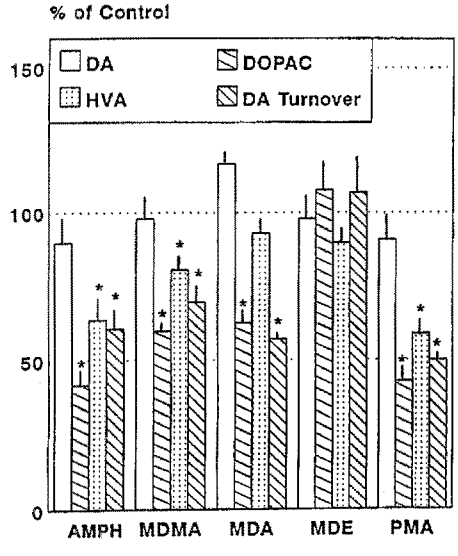

Fig. 10 Dopamine parameters in rat striatum 3-h after acute IP injection of equimolar doses (32 μmol/kg) of the drugs. Values are expressed as % of control. * Indicates significant difference ($P < 0.05$)

Drug levels

The drug levels in whole brain at 3-h post-injection are shown in Fig. 11. There are large differences in the levels of the various drugs, with MDA > MDMA > AMPH > MDE > PMA.

Discussion

The behavioural study was undertaken to examine the effects on spontaneous behaviour in freely moving rats of drugs with a similar structure but functionally separated into the putative entactogens, a stimulant and a hallucinogen. It was hypothesized that a detailed analysis of the duration and frequency of individual behaviours, the sequences of these behaviours and the neurochemical effects of the drugs could provide a basis to differentiate among the three drug classes and vehicle treatment. Data from the photobeam interruption counts supported the division into three distinct drug groupings: the first being AMPH, the second MDMA and MDA and the third PMA, MDE and vehicle. The results support the literature in terms of similar but less potent stimulatory effects of MDMA

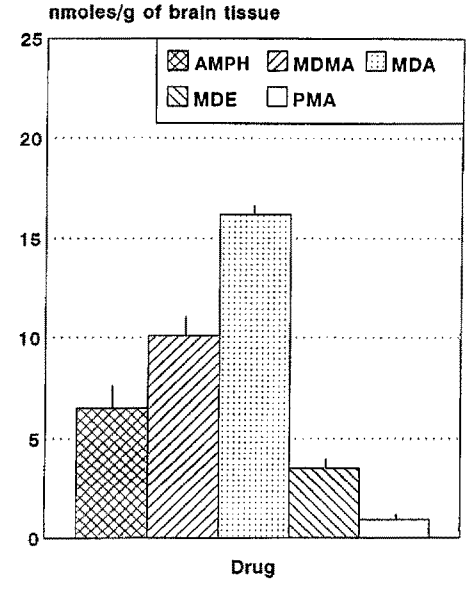

Fig. 11 Drug levels in whole brain 3-h post-injection of equimolar doses (32 μmol/kg) of the drugs. Values are expressed as nmol/g brain tissue. *Bars* represent ± SEM

and MDA than is seen with AMPH (Thiessen and Cook 1973; Glennon and Young 1984a,b; Matthews et al. 1989; Spanos and Yamamoto 1989). Although Hermle et al. (1993) reported increased psychomotor drive in human volunteers after oral administration of MDE, no information was found in the literature regarding MDE effects on locomotor activity in rats. The lack of effect on locomotor activity seen with the dose of PMA used in this study supports the findings of Hitzemann et al. (1971) that doses of 30 mg/kg of PMA were required to increase psychomotor activity. However, Menon et al. (1976) showed that PMA produced increased locomotor activity at doses of 5 and 10 mg/kg. This discrepancy might arise from procedural differences. Menon and his colleagues measured locomotor activity in a social context, in that the rats were paired. In addition, their animals were in locomotor boxes for the test period only, whereas our animals were in the test boxes for 10 days prior to the experiment. Locomotor activity measured during the animals' dark cycle in this study could be different from values obtained during the more quiescent day cycle. The timing of the study by Menon et al. is not reported. Principal components analysis and cluster analysis of the frequency and duration measures of behaviours did not provide any further discriminative data than that from the photobeam interruption counts. Only the cluster analysis of transition frequencies as a ratio of expected frequencies differentiated vehicle from the hallucinogen and MDE. In this case, four groupings were found: (1) MDMA and MDA (2) PMA and MDE (3) AMPH and (4) vehicle. In addition, this analysis revealed that AMPH was more closely related to MDMA and MDA than to vehicle or the hallucinogen. Thus, the cluster analysis of the behavioural transitions produced a classification scheme of the drugs closest to human subject reports. The one discrepancy was that MDE was consistently classified with the hallucinogen and not with the entactogens. As indicated earlier, dose is an important factor in determining overall behavioural effects of these compounds. The administered dose of 32 μmol/kg represents a relatively large dose of AMPH and PMA in rats (5.9 mg/kg and 5.3 mg/kg, respectively) but a fairly modest dose of the other compounds (MDMA 6.2 mg/kg, MDA 5.8 mg/kg and MDE 6.7 mg/kg). The possibility remains that a more accurate drug classification requires behavioural transition analysis coupled with dose-response curves.

The neurochemical effects of the different drugs provided further discriminative data that differentiated among the drug classes but not consistently between the individual drugs and vehicle treatment. The NA levels in the hippocampus could be used to discriminate among AMPH, PMA and the methylenedioxy compounds, but the methylenedioxy compounds could not be differentiated from vehicle treatment. The cortical 5-HT measures also discriminated among AMPH, PMA and the methylenedioxy compounds but failed to differentiate between AMPH and vehicle treatment. Striatal DA measures were not helpful in discriminating among the drugs. AMPH, MDMA, MDA and PMA all caused significant decreases in the levels of DOPAC and DA turnover. MDE administration did not alter any of the DA measures from those obtained in the vehicle-treatment animals. Gibb et al. (1990) investigated the effects of MDMA and MDA (10 mg/kg) on striatal DA and found a time-dependent increase in DA. The lack of agreement of their findings and our results might be due to dose and/or time dependency of the DA increase.

The neurochemical data support the literature regarding changes in 5-HT parameters after administration of the compounds of interest. Both MDMA and MDA are known to inhibit tryptophan hydroxylase, thus decreasing the synthesis of 5-HT, and this action, in combination with their 5-HT releasing and uptake inhibiting actions, produce decreases in 5-HT and 5-HIAA levels and increased 5-HT turnover (Mokler et al. 1987; Johnson et al. 1989; Schmidt and Taylor 1990). PMA has also been shown to cause 5-HT release and inhibit its reuptake (Menon et al. 1976; Tseng et al. 1976). However, coupled with its selective inhibition of monoamine oxidase A (MAO-A)-mediated 5-HT metabolism (Green and Halt 1980), PMA produced an increase in 5-HT levels and decreased 5-HIAA levels and 5-HT turnover. The MAO-A inhibitory activity may also account for the elevated NA levels seen in the brains of animals treated with PMA.

The neurochemical results from the MDE-treated animals are in keeping with previously published reports both in terms of the similarity of the neurochemical changes with those seen after MDMA and MDA administration and in relative potency (Johnson et al. 1987). The lack of agreement between the neurochemical effects and the behavioural effects in relation to classifying MDE could be at least partially due to the differences in the time frames of the sets of data. The neurochemical data represent a single "snapshot" of the levels of the biogenic amines and their metabolites 3 h after drug administration. The behavioural data represent the total of behaviour outputs over the entire 3 h time period. Thus, the relationship between the neurochemical effects and the peak behavioural effects could not be examined within the experimental design used here, It has been shown that MDE has a shorter duration of action than MDMA and MDA (Shulgin 1978). Perhaps the shorter time course increases the difficulties in relating ongoing behaviour to endstage neurochemical measures.

The limitation of the stimulant and hallucinogen representatives to single examples also needs to be expanded to stimulants and hallucinogens with different structures. The choices made in this experiment were guided by structural similarity: all the drugs

used in the present experiment shared the same basic skeleton. Although Shulgin et al. (1969), on the basis of extensive clinical studies, reported PMA to be hallucinogenic, drug discrimination studies in rodents and studies in chronic spinal dog have produced equivocal results (Martin et al. 1978; Winter 1984). We were interested in testing the hypothesis that this methodology could separate drugs according to their subjective effects in humans and thus felt that PMA was an appropriate choice as a structurally similar compound. However, the analysis must be generalizable to other drug structures before confidence in this method can be firmly established.

The large differences in the brain levels of the various drugs support the literature on the relative durations of behavioural effects in humans (MDA > MDMA and AMPH > MDE and PMA) (Shulgin 1978; Shulgin and Nichols 1978; Segal and Schuckit 1983; Nichols et al. 1986). MDA levels remained higher at 3 h than those of MDMA, supporting earlier metabolic studies showing that MDA, as a metabolite of MDMA, is cleared from the brain at a slower rate than is seen with the parent compound, MDMA (Hegadoren et al. 1993). The level of PMA is the lowest of the drugs studied, a result similar to that observed by Sherry-McKenna and Baker (personal communication) when comparing brain levels of the amphetamine analogues tranylcypromine and paramethoxytranylcypromine.

In conclusion, the results of the present study suggest that analysis of the effects of the drugs on the temporal organization of behaviour in rats more closely parallels observed subjective effects reported by humans than does measurement of frequency and duration of behaviours. This analysis successfully classified MDMA with MDA, and differentiated them from a stimulant and an hallucinogen. Furthermore, the hallucinogen was successfully differentiated from vehicle treatment. Further examination of dose-response relationships is required to clarify whether MDE is perhaps a "hybrid", sharing properties of more than one class, or is an entactogen devoid of any stimulatory activity.

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