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A comparison of 1-benzylpiperazine and methamphetamine in their acute effects on anxiety-related behavior of hooded rats

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ABSTRACT

Following i.p. treatment with saline, 10 or 20 mg/kg 1-benzylpiperazine, or 1 or 2 mg/kg methamphetamine, hooded rats were observed in an open field, a light–dark box and (24 h after exposure to the drugs) a Y maze with one novel and one familiar arm. Both drugs increased open-field rearing and ambulation, but only methamphetamine increased and decreased respectively occupancy of center squares and corners, while stereotyped head movements were increased by 20 mg/kg benzylpiperazine. Time spent in and entries of the light compartment of the light–dark box were decreased by benzylpiperazine but not methamphetamine, and entries of the novel Y-maze arm were decreased by methamphetamine for male rats only. Although most behavior emitted in the open field and light–dark box following treatment with methamphetamine could be ascribed to the drug's locomotor stimulant effect, increased stereotypy with the high dose probably interfered with this action for benzylpiperazine. However, both drugs may have led to some anxiety-related novelty avoidance in the Y maze. Overall, the patterns of results for the two drugs revealed more similarities than differences (with methamphetamine possibly being more effective than benzylpiperazine) and thus supported the view that, because of commonalities in their neurochemical effects, benzylpiperazine may have similar abuse and dependence risks to methamphetamine.

1. Introduction

In recent years, benzylpiperazine has been increasingly consumed in the form of "party pills" in order to experience a psychological "high" without the same perceived health risks and potential for dependence that characterize amphetamines, especially methamphetamine (STANZ, 2005), and other stimulants such as methylenedioxymethamphetamine (MDMA) or "Ecstasy" (Baumann et al., 2005). However, there is evidence of adverse consequences of benzylpiperazine consumption including toxic reactions (sometimes requiring medical intervention, Alansari and Hamilton, 2005; Gee et al., 2005; Nicholson, 2006) or possibly even death (Balmelli et al., 2001; Wikström et al., 2004), as well as a number of self-reported undesirable physical and psychological reactions to the drug, such as insomnia, stomach pains/nausea, headaches, mood swings, confusion and anxiety (Wilkins et al., 2007). And in spite of claims to the contrary (STANZ, 2005), it is likely that, because of its reinforcing properties in laboratory animals comparable to those of either cocaine (Fantegrossi et al., 2005; Meririnne et al., 2006) or methamphetamine (Brennan et al., 2007a), the abuse and dependence potential of benzylpiperazine is considerably higher than once believed (Brennan et al., 2007b; Johnstone et al., 2007). It has also been shown that benzylpiperazine and dexamphetamine can have very similar effects on human performance (Bye et al., 1973) and that, for former amphetamine addicts, its action can be indistinguishable from that of dexamphetamine with benzylpiperazine's subjective effects being preferred (Campbell et al., 1973).

Although little is known about the long-term consequences of benzylpiperazine use, there is some evidence that treating rats daily with benzylpiperazine during a developmental period equivalent to that of human adolescence may lead to increased anxiety (and possibly aggression for females only) in adulthood (Aitchison and Hughes, 2006). All of the outcomes described above for benzylpiperazine bear some resemblance to those reported for methamphetamine — for example, negative health consequences (Karch et al., 1999; Richards et al., 1999; Sommers et al., 2006), anxiety (Hayase et al., 2005), dependence potential (Meredith et al., 2005), and interference with normal development in adolescent rats (Vorhees et al., 2005). There are also similar behavioral effects shared by the two drugs, such as increased motor and stereotyped activity (Baumann et al., 2005; Brennan et al., 2007a) and turning behavior (Oberlander et al., 1979). However, the most compelling evidence for similarities between benzylpiperazine and methamphetamine is found in their effects on brain neurotransmitter systems for which the outcomes are sometimes almost indistinguishable. In particular, both drugs facilitate the action of dopamine and serotonin (Baumann et al., 2002, 2004, 2005; Hashimoto et al., 1992; Oberlander et al., 1979) via interactions with reuptake transporters for the two transmitters (Baumann et al., 2004,

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2005), hence their dopamine-related reinforcing properties (and dependence potential) and possibly serotonin-related anxiogenesis (Graeff, 2002).

In spite of the acknowledged similarities between benzylpiperazine and methamphetamine in some of their effects, there have been very few behavioral comparisons made between them within a single investigation. The present study therefore sought to address this deficiency by comparing the two drugs in their acute effects on several measures of activity and unconditioned choice in rats. Since both compounds have been reported to have anxiogenic properties (Rawson et al., 2002; Wilkins et al., 2007), special attention was paid to this possibility. Specifically, ambulatory and rearing activity in an open field was recorded, along with occupation of the center and corners of the apparatus. Lower levels of ambulation, rearing and center occupancy and higher levels of the corner occupancy are frequently regarded as indices of higher anxiety (Archer, 1973; Belzung, 1999; Brain and Marrow, 1999; Hall, 1934). Tendencies to enter and spend time in the light half of a light–dark box were also recorded since lower levels of both these measures are viewed as indicative of higher anxiety (Hascoët et al., 2001; Sanchez, 1996). In addition, preferences for the novel arm of a Y maze were measured 24 h after drug administration because such preferences can reflect levels of anxiety-related novelty avoidance along with memory-based ability to recognize novelty (Hughes, 2007a). Methamphetamine is reported to affect memory in a bimodal fashion (Shoblock et al., 2003) and increase anxiety, although such effects more typically follow chronic treatment with high doses (Clemens et al., 2007). Although there have been no controlled studies of the effects of acute benzylpiperazine on anxiety and memory, clinical reports suggest that heightened anxiety and memory loss can accompany high doses of the drug (e.g., Theron et al., 2007).

Because most previous research dealing with benzylpiperazine and methamphetamine effects on laboratory animals has involved males exclusively, both sexes were investigated in case there were some sex-related effects (as has been shown for a number of other psychotropic drugs, Hughes, 2007b). It was expected that any sex differences in responsiveness to the drugs would appear as significant drug dose × sex ANOVA interactions.

2. Methods

2.1. Subjects

The subjects were 30 male and 30 female PVG/C hooded rats approximately 5 months old bred in the Animal Facility of the University of Canterbury. They were housed in groups of 3 or 4 same-sexed animals in a room with an ambient temperature of 22 (±2°) °C, 48% ± 10% humidity, freely available food and water, and 12 h light:12 h dark lighting (with the light phase beginning at 08:00 h). All animal maintenance and experimental procedures conformed to requirements of Part 5 (Codes of Welfare) and Part 6 (Use of Animals in Research, Testing, and Teaching) of the New Zealand Animal Welfare Act (1999), and had been approved by the Animal Ethics Committee of the University of Canterbury.

2.2. Apparatus

Three sets of apparatus were used — an open field, a light–dark box and a Y-maze with removable arm inserts. They were all present in the same experimental room and each one sat on a 700-mm-high table. Even illumination was provided for all apparatus by dim, overhead fluorescent lighting.

2.2.1. Open field

The open field consisted of a wooden 600 × 600-mm wooden arena, 250 mm high, and painted black. The floor of the field was divided into a 16 equal-sized squares by means of a grid of white painted lines. An infrared video camera was suspended 850 mm above the floor of the apparatus and connected to a video recorder.

2.2.2. Light–dark box

The clear-varnished wooden light–dark box comprised two 300-mm-long × 200-mm-wide × 300-mm-high compartments separated from each other by a wooden partition containing a 100-mm × 100-mm opening through which a rat was able to freely move between the compartments. This opening could be closed by means of a removable horizontal slide. One compartment was covered by a hinged wooden lid that restricted the amount of light entering it (the dark side), and the other was covered by a hinged clear Perspex lid.

2.2.3. Y maze

The clear-varnished wooden Y maze consisted of two 45-cm-long arms set at an angle of 120 °C to each other, and a 30-cm-long stem. The arms and stem were 10 cm wide and 14 cm high and were covered by a hinged, clear Perspex lid except for the first 15 cm of the stem (the start area) that was covered by a hinged wooden lid. Removable black or white metal inserts were also provided for the arms. Each insert covered the walls and floor of an arm except for the first 5 cm.

2.3. Drugs

Benzylpiperazine had been purchased from ABCR Gmbh & Co, Karlesruhe, Germany in the form of a 1-benzylpiperazine solution and was diluted with 0.9% saline to give “High” and “Low” doses of 10 and 20 mg/kg. Methamphetamine had been donated as a pure crystal form of the drug by Environmental Science & Research Ltd (ESR, Wellington, New Zealand). It was crushed and then dissolved in 0.9% saline to give “High” and “Low” doses of 1 and 2 mg/kg. The doses of both drugs were based on previous research that demonstrated their behavioral effectiveness in rats (Brennan et al., 2007a; Hughes and Greig, 1976) and the suggestion that amphetamines have approximately ten times the potency of benzylpiperazine (Campbell et al., 1973).

2.4. General procedure

The rats were randomly assigned to a saline control group or one of the two benzylpiperazine or methamphetamine groups. All groups contained equal numbers of each sex. Twenty min prior to testing in the open field, each rat was injected (1 ml/kg, i.p.) with its appropriate drug and dose. Immediately after its open-field test, the rat experienced a test in the light–dark box. On the next day, each rat experienced an acquisition trial in the Y maze 20 min after drug administration which was followed 24 h afterwards by a retention trial. The acquisition and retention trial procedure was repeated one week later. All apparatus was thoroughly cleaned with a 20% solution of Powerquat Blue disinfectant before introduction of a new rat.

2.4.1. Open-field procedure

Each rat was placed in the center of the field and its behavior was video recorded for exactly 5 min. At a later stage, the video tape of its behavior was viewed and the following responses noted:

1. the total number of white lines crossed by the hind legs (ambulation)
2. the total number of times it reared up on its hind legs (rearing)
3. every 3 s, whether the rat was occupying one or more of the four center squares (occupancy of center squares) or one of the corners of the apparatus (occupancy of corners).

It has been known for some considerable time that amphetamines can induce stereotyped behavior at the expense of increased locomotion (Creese and Iversen, 1974; Peters et al., 1978). There is also evidence of increased stereotypy following exposure to both benzylpiperazine and methamphetamine (Brennan et al., 2007a).

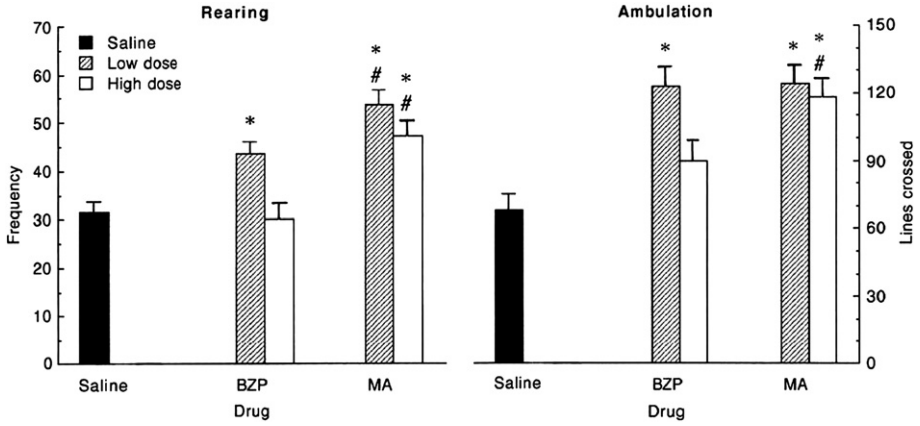

Fig. 1. Mean±S.E.M. frequencies of open-field rearing and ambulation following treatment with saline (n=12), 10 mg/kg benzylpiperazine (BZP, n=12, low dose) or 1 mg/kg methamphetamine (MA, n=12, low dose), or 20 mg/kg benzylpiperazine (BZP, n=12, high dose) or 2 mg/kg methamphetamine (MA, n=12, high dose). *Significantly different from the saline group. #Significantly different from the other drug group for the same dose level.

Therefore, each rat's video tape was replayed in order to record the incidence of repetitive head movements that are indicative of stereotypy in rats administered dopaminergic stimulants (White et al., 1998). Every 3 s for 5 min it was noted if the rat was sitting stationary and engaging in rapid horizontal or vertical head movements while sniffing. The total number of such occasions comprised the rat's stereotypy score.

2.4.2. Light–dark-box procedure

Immediately following each rat's open-field test, it was placed in the dark compartment of the light–dark box with the slide separating the two compartments in place. Approximately 30 s later, the slide was withdrawn and the rat was allowed free access to both compartments for exactly 5 min. The total time spent in and entries of the light side were recorded.

2.4.3. Y-maze procedure

The following day each rat was injected with the same drug and dose and, 20 min later, placed in the stem of the Y maze (with one arm containing a black insert and the other containing a white insert) for a 6-min acquisition trial. It was then removed and both inserts were replaced with clean black inserts. This ensured that any subsequent choices of the changed previously white arm were not merely due to avoidance of any odor cues left by the rat in the unchanged arm during acquisition. Twenty four h later, the rat was returned to the stem for a 3-min retention trial (in the absence of any drug action) during which the time spent in and the repeated entries of each arm were recorded. From these data it was possible to subsequently calculate the percent time spent in and the percent entries of the arm that had changed from white to black (the novel arm), as well as the total time/day spent in and the total entries/day of both arms. Then one week later, the rat received another injection of the same drug and dose 20 min before it experienced a second acquisition trial with the positions of the black and white arms reversed from what they had been previously. After 24 h this was followed by a second retention trial with the white arm changed to black.

2.5. Statistical analysis

All data were presented as means±S.E.Ms. For all responses recorded in the Y maze, these were computed from averages for the two retention trials. Each measure was subjected to a 3 (drug dose)×2 (sex) ANOVA for the two drugs separately. The same saline comparison group was used for each drug. When significant dose

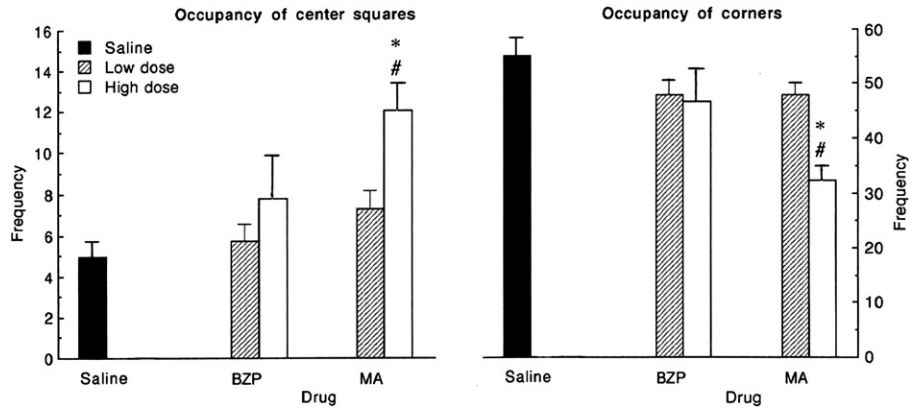

Fig. 2. Mean±S.E.M. frequencies of occupancy of center squares and corners of the open field following treatment with saline (n=12), 10 mg/kg benzylpiperazine (BZP, n=12, low dose) or 1 mg/kg methamphetamine (MA, n=12, low dose), or 20 mg/kg benzylpiperazine (BZP, n=12, high dose) or 2 mg/kg methamphetamine (MA, n=12, high dose). *Significantly different from the saline group. #Significantly different from the other drug group for the same dose level.

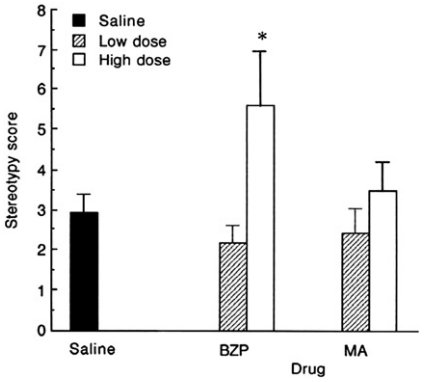

Fig. 3. Mean ± S.E.M. stereotypy scores recorded in the open field following treatment with saline (n = 12), 10 mg/kg benzylpiperazine (BZP, n = 12, low dose) or 1 mg/kg methamphetamine (MA, n = 12, low dose), or 20 mg/kg benzylpiperazine (BZP, n = 12, high dose) or 2 mg/kg methamphetamine (MA, n = 12, high dose). *Significantly different from the saline group.

effects occurred, post hoc comparisons were made by means of Fischer's PLSD tests. Any comparisons between the two drugs at either dose level were carried out by means of t-tests (df = 22).

3. Results

3.1. Open-field behavior

3.1.1. Rearing and ambulation

As outlined in Fig. 1, both benzylpiperazine and methamphetamine significantly affected rearing [benzylpiperazine, $F(2,30) = 7.01$, $P < 0.005$; methamphetamine, $F(2,30) = 14.53$, $P < 0.0001$] and ambulation [benzylpiperazine, $F(2,30) = 13.90$, $P < 0.0001$; methamphetamine, $F(2,30) = 15.30$, $P < 0.0001$].

Whereas benzylpiperazine significantly increased the two responses with 10 but not 20 mg/kg, they were significantly increased by both 1 and 2 mg/kg methamphetamine. At both dose levels of each drug, rats treated with methamphetamine reared significantly more often than those treated with benzylpiperazine. However, a similar difference in ambulation was only apparent at the higher dose level.

The sex difference in rearing was not significant for rats in either drug group [benzylpiperazine, females, mean ± S.E.M. = 36.67 ± 3.11, males = 33.39 ± 2.10, $F(1,30) = 1.00$, $P > 0.3$; methamphetamine, females = 45.22 ± 3.32, males = 43.00 ± 3.22, $F(1,30) = 0.41$, $P > 0.5$]. However, ambulation was significantly higher for females (108.67 ± 9.06) than for males (78.78 ± 6.67) in the benzylpiperazine group [$F(1,30) = 12.00$, $P < 0.002$]. Although in the same direction, the sex difference in ambulation failed to reach significance for rats in the methamphetamine group [females = 111.28 ± 9.03, males = 95.44 ± 8.44, $F(1,30) = 3.06$, $P > 0.09$].

3.1.2. Occupancy of center squares and corners

While benzylpiperazine had no significant effect on either occupancy of the center squares [benzylpiperazine, $F(2,30) = 1.18$, $P > 0.3$] or corners of the apparatus [$F(2,30) = 1.26$, $P > 0.2$], both responses were significantly affected by methamphetamine [center squares, $F(2,30) = 11.16$, $P < 0.0002$; corners, $F(2,30) = 18.93$, $P > 0.0001$, see Fig. 2].

The methamphetamine effects arose from higher occupancy of the center squares and lower occupancy of corners following treatment with 2 (but not 1) mg/kg. Rats treated with 2 mg/kg methamphetamine occupied the center squares more often and the corners less often than rats treated with 20 mg/kg benzylpiperazine.

The was no significant sex difference in center squares occupancy for rats in either the benzylpiperazine [females = 5.94 ± 0.68, males = 6.33 ± 1.51, $F(1,30) = 0.01$, $P > 0.8$] or methamphetamine group [females = 8.56 ± 1.06, males = 7.61 ± 1.16, $F(1,30) = 0.56$, $P > 0.4$]. However, females in both drug groups occupied corners significantly more often than males [benzylpiperazine, females = 55.06 ± 2.45, males = 44.78 ± 4.16, $F(1,30) = 4.31$, $P < 0.05$; methamphetamine, females = 48.33 ± 3.02, males = 41.94 ± 1.51, $F(1,30) = 4.22$, $P < 0.05$].

3.1.3. Stereotypy scores

Stereotypy was significantly affected by benzylpiperazine [$F(2,30) = 6.75$, $P < 0.004$], but not by methamphetamine [$F(2,30) = 0.78$. $P > 0.4$]. As shown in Fig. 3, the benzylpiperazine effect was due to a significant increase in stereotypy scores for rats treated with the higher dose.

The sex difference for this measure was not significant for rats in either drug group [benzylpiperazine, females = 2.33 ± 0.57, males = 4.11 ± 0.98, $F(1,30) = 3.23$, $P > 0.08$; methamphetamine, females = 3.22 ± 0.58, males = 2.67 ± 0.40, $F(1,30) = 0.62$, $P > 0.4$].

3.2. Time in and entries of the light side of the light–dark box

Although both measures of light–dark preference were significantly affected by benzylpiperazine [time, $F(2,30) = 8.46$, $p < 0.005$; entries, $F(2,30) = 11.87$, $P < 0.0002$], this did not apply to methamphetamine [time, $F(2,30) = 0.10$, $P > 0.9$; entries, $F(2,30) = 0.82$, $P > 0.4$]. As is evident in Fig. 4, the benzylpiperazine effect was due to significant decreases in both measures following treatment with 20 but not 10 mg/kg. Consequently, rats treated with this higher dose spent

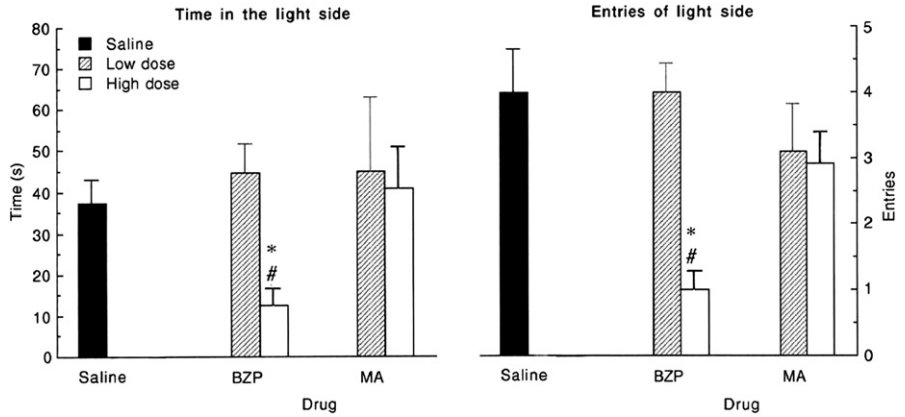

Fig. 4. Mean ± S.E.M. time spent in and number of entries of the light side of the light–dark box following treatment with saline (n = 12), 10 mg/kg benzylpiperazine (BZP, n = 12, low dose) or 1 mg/kg methamphetamine (MA, n = 12, low dose), or 20 mg/kg benzylpiperazine (BZP, n = 12, high dose) or 2 mg/kg methamphetamine (MA, n = 12, high dose). *Significantly different from the saline group. #Significantly different from the other drug group for the same dose level.

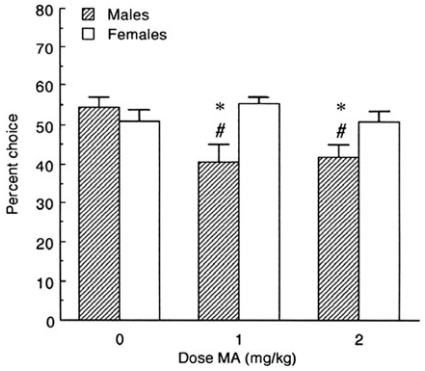

Fig. 5. Mean±S.E.M. percent entries of the novel Y-maze arm by male rats following treatment with saline (0 mg/kg, $n=6$) or 1 mg/kg methamphetamine (MA, $n=6$) or 2 mg/kg methamphetamine (MA, $n=6$), and female rats following treatment with saline (0 mg/kg, $n=6$) or 1 mg/kg methamphetamine (MA, $n=6$) or 2 mg/kg methamphetamine (MA, $n=6$). *Significantly different from the saline group. #Significant sex difference.

significantly less time in and made fewer entries of the light side than rats treated with 2 mg/kg methamphetamine.

Sex differences in neither measure were significant for rats in the benzylpiperazine drug group [time, females=32.94±6.80 s, males=30.11±4.33 s, $F(1,30)=0.18$, $P>0.6$; entries, females=3.06±0.58, males=2.94±0.45, $F(1,30)=0.04$, $P>0.8$]. Similarly, there were no significant sex differences for methamphetamine-treated rats [time, females=44.18±11.67 s, males=37.86±7.83 s, $F(1,30)=0.20$, $P>0.6$; entries, females=3.78±0.52, males=2.89±0.50, $F(1,30)=1.44$, $P>0.2$].

3.3. Y-maze behavior

3.3.1. Percent time spent in and entries of the novel arm

For rats treated with benzylpiperazine, there were no significant drug effects on either time spent in the novel arm (saline=53.00±2.43%, 10 mg/kg=48.68±1.05%, 20 mg/kg=49.02±3.77%, $F(2,30)=0.75$, $P>0.4$), or entries of this arm (saline=52.59±2.00%, 10 mg/kg=51.60±1.75%, 20 mg/kg=51.08±2.47%, $F(2,30)=0.11$, $P>0.8$). There was also no significant methamphetamine effect on time spent in the novel arm [saline=53.00±2.43%, 1 mg/kg=43.13±4.89%, 2 mg/kg=43.56±2.68%, $F(2,30)=2.82$, $P>0.07$]. However, although the methamphetamine main effect was not significant for entries of the novel arm [$F(2,30)=2.07$, $P>0.1$], there was a significant drug dose×sex interaction for this measure, outlined in Fig. 5 [$F(2,30)=4.61$, $P<0.02$]. This interaction revealed significant reductions in novel arm entries by both doses for males (but not females) to the point that a significant preference for the less novel arm was evident with 2 mg/kg [one-sample $t(5)=2.57$, $P=0.05$].

There was no significant sex difference amongst benzylpiperazine-treated rats for either time spent in [females=50.61±1.89%, males=49.87±2.46%, $F(1,30)=0.05$, $P>0.8$] or entries of the novel arm [females=50.80±1.43%, males=52.65±1.87%, $F(1,30)=0.55$, $P>0.4$]. Although a similar outcome occurred for time spent in the novel arm by methamphetamine-treated rats [females=40.05±2.23%, males=44.08±3.56%, $F(1,30)=1.68$, $P>0.2$], there was a significant sex effect for novel arm entries [$F(1,30)=7.21$, $P<0.02$]. However, as shown by the drug dose×sex interaction for this measure described above, the sex difference only applied to rats treated with 1 and 2 mg/kg methamphetamine. In both cases, because they were uniquely affected by the drug, males made proportionately fewer entries of the novel arm than females (see Fig. 5).

3.3.2. Total time/day spent in and total entries/day of both arms

The total time/day spent in both Y-maze arms outlined in Fig. 6 was not significantly affected by either benzylpiperazine [$F(2,30)=1.78$, $P>0.1$] or methamphetamine [$F(2,30)=1.69$, $P>0.2$].

However, as also outlined in Fig. 6, both drugs significantly affected total entries/day of both arms [benzylpiperazine, $F(2,30)=3.44$, $P<0.05$; methamphetamine, $F(2,30)=9.00$, $P<0.001$]. For benzylpiperazine-treated rats, this comprised a significant decrease with 20 (but not 10) mg/kg, whereas both 1 and 2 mg/kg methamphetamine decreased the response.

There were significant sex differences favoring females for both measures amongst rats in each drug group i.e., benzylpiperazine: time — females=81.85±2.30 s, males=69.37±2.43 s, $F(1,30)=6.63$, $P<0.02$, entries — females=8.22±0.32, males=5.69±0.45, $F(1,30)=23.95$, $P<0.0001$; methamphetamine: time — females=77.75±4.08 s, males=64.59±4.60 s, $F(1,30)=4.68$, $P<0.05$, entries — females=6.97±0.0.50, males=4.39±0.48, $F(1,30)=19.83$, $P<0.0001$.

3.4. Comparative summary of all drug effects

Table 1 provides a summary of all the drug-related results described above.

From inspection of the table, it is clear that both drugs had similar patterns of effects on a number of measures although they varied in the extent to which these effects were statistically significant. In general, methamphetamine appeared to be more effective than benzylpiperazine, as indicated by differences between saline- and drug-treated animals and between effects of the two drugs at each

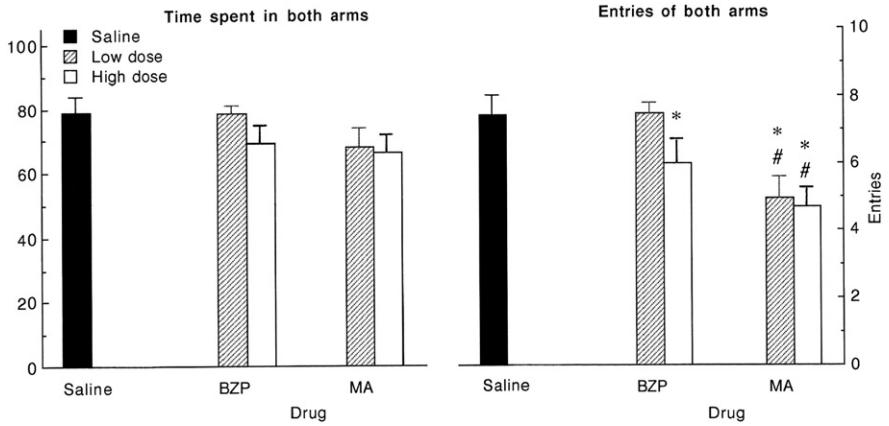

Fig. 6. Mean±S.E.M. time spent in and numbers of entries of both Y-maze arms following treatment with saline ($n=12$), 10 mg/kg benzylpiperazine (BZP, $n=12$, low dose) or 1 mg/kg methamphetamine (MA, $n=12$, low dose), or 20 mg/kg benzylpiperazine (BZP, $n=12$, high dose) or 2 mg/kg methamphetamine (MA, $n=12$, high dose). *Significantly different from the saline group. #Significantly different from the other drug group for the same dose level.

Table 1
Summary of the behavioral effects of benzylpiperazine and methamphetamine

Apparatus and measure	Behavioral effect	
	Benzylpiperazine	Methamphetamine
Open field:		
Rearing	*Increased* by *lower* dose	*Increased* by *both* doses
Ambulation	*Increased* by *lower* dose	*Increased* by *both* doses
Occupancy of center squares	No effect	*Increased* by *higher* dose
Occupancy of corners	No effect	*Decreased* by *higher* dose
Stereotypy scores	*Increased* by *higher* dose	No effect
Light–dark box:		
Time in the light side	*Decreased* by *higher* dose	No effect
Entries of the light side	*Decreased* by *higher* dose	No effect
Y maze:		
% time in the novel arm	No effect	No effect
% entries of the novel arm	No effect	*Decreased* by *both* doses for *males* only
Total time spent/day in both arms	No effect	No effect
Total entries/day of both arms	*Decreased* by *higher* dose	*Decreased* by *both* doses

dose level (especially the higher level). However, for stereotypy in the open field and time spent in and entries of the light side in the light–dark box, benzylpiperazine at the higher dose was more effective than either dose of methamphetamine.

4. Discussion

The results of this study showed that, overall, the behavioral effects of benzylpiperazine and methamphetamine were qualitatively very alike. This is not particularly surprising given the similarities between them in their central neurochemical actions, especially with respect to changes in dopaminergic and serotonergic activity (Baumann et al., 2002, 2004, 2005; Oberlander et al., 1979). In particular, benzylpiperazine is a substrate for the dopamine transporter and, in a similar fashion to methamphetamine, it promotes non-exocytotic release of the transmitter (Baumann et al., 2004, 2005). Similarities between the two drugs have also been described in the dose-related hyperactivity and stereotypy that occurs with both, as well as behavioral sensitization and cross-sensitization to methamphetamine produced by chronic benzylpiperazine (Brennan et al., 2007a). In fact, because of benzylpiperazine's ability to initiate dopamine-mediated self-administration (Brennan et al., 2007b) and contrary to popular belief (Bowden, 2004) , it may well prove to be a drug of abuse with dependence potential (Johnstone et al., 2007) not unlike methamphetamine and other monoamine stimulants. Although the present results suggest that benzylpiperazine may have had lower efficacy than methamphetamine, it is possible that the particular doses used (10 and 20 mg/kg) may not have been sufficiently high enough to represent doses of 1 and 2 mg/kg methamphetamine. However, with the same doses of each drug, Brennan et al. (2007a) observed even greater similarities between their effects on ambulation and stereotypy than was evident for the particular responses recorded in the present study. Nevertheless, the possibility of differences in efficacy being responsible for the results obtained in the present study should be addressed by utilizing wider dose ranges for each drug.

The effects of both doses of methamphetamine and the lower dose of benzylpiperazine on open-field rearing and ambulation reflected increased motor activity consistent with previous observations (Brennan et al. 2007a; Schindler et al., 2002). The failure for 20 mg/kg benzylpiperazine to exert a similar effect was almost inevitably due to the increased stereotypy associated with this dose, even though Brennan et al., (2007a) observed increases in locomotion as well as stereotypy with the same dose.

The increased occupancy of center squares and decreased occupancy of corners of the open field by rats treated with 2.0 mg/kg methamphetamine might seem to have been due to lower rather than higher anxiety. However, these results were more likely to have been an artifact of increased ambulation arising from the drug's motor stimulant effects, an outcome that could have been prevented for higher dose benzylpiperazine-treated rats by their increased stereotypy. The failure for methamphetamine to affect either time spent in or entries of the light side of the light–dark box in contrast to the higher dose of benzylpiperazine, might appear as heightened anxiety for rats treated with the latter but not former drug. Methamphetamine (2.5 and 5.0 mg/kg i.p.) has also been shown to have no effect on either the latency to emerge from a small darkened box into an illuminated arena or the time spent out of the box (Clemens et al., 2004). Rather than the results for benzylpiperazine-treated subjects reflecting increased anxiety, it is more likely that the rats were manifesting a higher level of stereotypy (evident in the open field) which interfered with the locomotor activity required to move between the two sides of the box. Unfortunately, stereotypy was not measured in this apparatus.

While both doses of methamphetamine reduced the number of entries of (but not time spent in) the novel Y-maze arm for male (but not female) rats only 24 h after exposure to the drug, neither response was affected by benzylpiperazine. As their novel arm entries were decreased to the level that the familiar arm was preferred with the higher dose, this suggests anxiety-related novelty avoidance (Hughes, 2007a) which may have developed through an association between the acquisition trial experience 24 h earlier, and possible aversive properties of the drug (as can occur with other drugs, Hughes, 1982). Amphetamines are known to have aversive properties in other situations (Berger, 1972) and acute methamphetamine can reduce preferences for novelty to the extent that neophobia-related familiarity is preferred (Hughes and Greig, 1976; Misslin and Ropartz, 1981). And since the rats were tested 24 h after their exposure to the drug, it is unlikely that the effects of methamphetamine on the male rats' entries of the novel arm were due to increased stereotypy (which of course was unaffected by this drug in the open field). It therefore seems possible that the nature of this response for males arose from an earlier association between their acquisition trial experiences and some aversive effects of methamphetamine. Alternatively, as recognition of novelty obviously involves memory, the result may have been due to memory impairment. However, this is unlikely because, by showing a preference for the familiar alternative, the animals were clearly able to discriminate between the two arms in terms of their novelty/familiarity characteristics thus indicating that their memory was intact. Besides, neither the dose range nor the number of injections of drug would have been sufficient to attain the level of methamphetamine-induced neurotoxicity required for interference with central memory mechanisms (Belcher et al., 2005; Bisagno et al., 2002).

The reduction in entries of both Y-maze arms following treatment 24 h beforehand with the higher dose of benzylpiperazine and both doses of methamphetamine may have arisen from anxiety-related novelty avoidance (in a similar fashion to what was suggested above for effects of methamphetamine on repeated entries of the novel arm for male rats only). Since it was obviously the most familiar area of the apparatus because of remaining acquisition trial odors and other unchanged stimuli, remaining in the stem of the Y maze would have enabled an anxious rat to avoid the comparatively higher degree of novelty of either the novel or the familiar arm. And again the 24 h interval between exposure to the drugs and testing makes it unlikely that drug-induced stereotypy was responsible for these particular results. Overall, the effects on Y-maze behavior were generally similar for the two drugs but, in contrast to what occurred in the light–dark box, the effects for methamphetamine appeared greater. In spite of this and other minor differences, it is reasonable to assume that, for both

drugs, the overall patterns of behavioral effects in each type of apparatus were similar. While results generated in the open field and the light–dark box are not easily accountable for by anxiogenic properties of benzylpiperazine and methamphetamine, it is more difficult to dismiss involvement of anxiety in the Y maze. This is because drug-induced stereotypy was unlikely to be operating and thus interfering with the locomotor stimulant effects of either compound. Clearly, more research is required to resolve this conflict.

There were significant sex differences (favoring females) in open-field ambulation for rats treated with benzylpiperazine, and in open-field corner occupancy and time spent in and entries of both Y-maze arms for rats in either drug group. All of these differences were generally consistent with many earlier observations of higher levels of motor activity in female than in male rats (Archer, 1975). However, because of a significant drug dose×sex interaction, the overall sex difference (favoring females) in percent entries of the novel arm for rats in the methamphetamine group proved to only apply to those subjects that had experienced the drug. Apart from this example, there were no other sex-related effects of either drug.

The main conclusion to be drawn from the present study is that benzylpiperazine and methamphetamine proved to be very similar in their effects on the particular forms of behavior that were recorded. Although there was little convincing evidence for anxiogenic effects of either drug, there was some suggestion that earlier associations between their action and acquisition trial experiences in the Y maze might have affected the rats' later preferences for novelty (which may or may not have been anxiety related). In this respect, it should be kept in mind that both drugs have been reported to induce anxiety in humans (Rawson et al., 2002; Wilkins et al., 2007). The similarities in the behavioral effects of the two drugs along with evidence of similarities in their central neurochemical effects (Baumann et al., 2002, 2004, 2005; Oberlander et al., 1979) support the likelihood that benzylpiperazine and methamphetamine could present similar risks for abuse and dependence. Such risks along with reports of benzylpiperazine leading to seizures in rats (Baumann et al., 2005) and humans (Wood et al., 2007) plus evidence of other toxic reactions (Alansari and Hamilton, 2005; Gee et al., 2005; Nicholson, 2006; Staak, 2007) make it quite clear that the drug is not a safe alternative to MA.

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