

Treatment of adolescent rats with 1-benzylpiperazine: a preliminary study of subsequent behavioral effects

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Abstract

Adolescent male and female rats were intraperitoneally injected with either isotonic saline or 10 mg/kg/day 1-benzylpiperazine (BZP) from postnatal days 45 to 55. Approximately 17 days later, assessments were made of their responsiveness to a novel brightness change in a Y-maze, their ambulation, rearing, defecation and social interactions in an open field, and their dark to light emergence latencies and defecation in an emergence apparatus. Compared with saline controls, rats that had been previously treated with BZP entered the novel Y-maze arm less often, spent less time in it and entered both arms less often and spent less time in them, ambulated and reared less in the open field, reared proportionately less often in the center squares and spent less time in social interaction, and, in the emergence apparatus, emerged into the light more slowly and also defecated less often in the darkened start box. Female but not male rats also spent more time restraining their partner rat while socially interacting in the open field. With few exceptions, the results were consistent with BZP treatment during adolescence having led to heightened anxiety possibly because of interference with maturation of anxiety-associated forebrain mechanisms operated by 5-HT.

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1. Introduction

1-benzylpiperazine (BZP) is the active agent in a number of designer drugs or “party pills” that are ingested for their psychotropic effects which may be indistinguishable from those of the amphetamines [12,13]. Although in 2002 BZP was temporarily put into Schedule 1 of the United States Controlled Substances Act, in New Zealand it is still legal and freely obtainable from a variety of retail outlets by anyone over the age of 18. It is accordingly marketed as a “safer legal alternative” to illicit and poor quality methamphetamine based on the rationale that it is preferable for BZP to be ingested than amphetamines [11].

Over 30 years ago, it was reported that former amphetamine addicts were unable to distinguish between the effects of dexamphetamine and BZP, and that they preferred the subjective effects of the latter drug [13]. More recently, it was shown that rhesus monkeys will intravenously self-administer BZP at rates as high as they would for cocaine leading to the conclusion that

BZP has amphetamine-type abuse potential [19]. Such observations of BZP effects were consistent with similarities in the effects of BZP, amphetamine and methamphetamine on induction of contralateral circling behavior in rats that had undergone destruction of the nigrostriatal dopamine pathway by means of unilateral administration of 6-hydroxydopamine [45]. BZP has also been shown to increase motor activity in rats and produce stereotyped behaviour, in a similar fashion to what characterizes amphetamine drugs [9].

The primary central neurochemical effects of BZP are like those that typify amphetamines, namely facilitation of the action of dopamine (DA) and 5-hydroxytryptamine (5-HT) [8,9,27,45,50] through their non-exocytic release via interactions with 5-HT and especially DA transporters [8,9]. In fact, it was concluded [9] that increases in dialysate DA and 5-HT were reminiscent of earlier observations of methamphetamine's effects [7]. Overall, it would appear that there is little to distinguish BZP from the amphetamines in terms of both its behavioral and neurochemical effects.

Claims that BZP is “safer” than other amphetamine-type drugs can lead young people to ingest doses well in excess of the

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recommended range with some serious toxic consequences [22]. Although BZP is considerably less potent than methamphetamine, this difference can easily be accounted for by the common practice in New Zealand of taking significantly more than the recommended dose [2]. Methamphetamine abuse by humans is also known to produce cognitive and mood deficits that continue long after use of the drug has ceased [41,46,48]. It can also interfere with normal brain and behavioral development in rats especially when experienced 41 to 50 days after birth during what is known as the periadolescent stage i.e., P41-50 [51]. Developmentally, P41-50 is equivalent to adolescence in humans when experimentation with addictive drugs typically begins [39,47] at higher levels than for older adults [15]. In both rats and humans the adolescent brain is not fully mature and represents “a brain in transition” differing both anatomically and neurochemically from the adult brain especially in terms of prefrontal and limbic mechanisms and systems operated by DA, glutamate, GABA and 5-HT [49]. (As mentioned above, both DA and 5-HT are also affected by BZP.) It therefore follows that, during adolescence, the brain could be particularly vulnerable to any interference with its further development.

In view of the behavioral and neurochemical similarities between methamphetamine and BZP and also the longer-term consequences of treating P41-50 rats with the former drug [51], it is possible that exposure to BZP during adolescence might also modify subsequent behavioral development. If so, this could have implications for the abuse of “designer drugs” or “party pills” by teenagers, and for how such substances are classified in terms of drug regulations. Therefore, the present study aimed to assess subsequent behavioral outcomes following administration of BZP to rats during the equivalent of late human adolescence to early adulthood, namely P45-P55. Because, like methamphetamine, BZP is a 5-HT agonist [7,8] and because either increased or decreased anxiety is related to increased 5-HT activity in the forebrain and periaqueductal gray matter respectively [23], particular attention was paid to possible effects of the drug on later anxiety-related behavior. Both male and female rats were chosen as subjects in view of sex-related effects of other drugs on behavioral development [29,34,35,40].

2. Methods

2.1. Subjects

The subjects were 20 male and 20 female PVG/C hooded rats, bred in the Animal Facility of the Department of Psychology, University of Canterbury. From 30 days of age, all subjects were housed in 525 × 330 × 230 mm-high plastic cages in groups of three or four of the same sex. They were kept at an ambient temperature of 22 °C ± 2 °C (with 48% ± 10% humidity) on a 12 h light/dark cycle (lights on at 08.00 h). Standard laboratory food and water was freely available at all times.

All procedures for housing, treating and testing the rats were in accord with Parts 5 (Codes of Welfare) and 6 (Use of Animals in Research, Testing, and Teaching) of the New Zealand Animal Welfare Act (1999), and had been approved by the University of Canterbury’s Animal Ethics Committee.

2.2. BZP treatment

When the rats were 45 days old (P45), they were randomly allocated to a BZP treatment group and a control group both of which contained equal numbers of each sex. For the next 10 days, all BZP-treated subjects received a daily intraperitoneal injection of freshly prepared 1-benzylpiperazine (purchased from ABCR GmbH and Co, Karlsruhe, Germany) dissolved in 0.9% saline to produce a dose of 10 mg/kg. Because of suggestions that BZP has approximately a tenth of the potency of methamphetamine [13] this particular dose was chosen because it was ten times the dose of methamphetamine shown to have mild stimulant effects on hooded rats [36]. Control rats were administered intraperitoneal 0.9% saline for 10 consecutive days. The volume of each injection for both groups was 1 ml/kg.

2.3. Behavioral testing

All behavioral testing began approximately 17 days after the rats’ last injection during the period P72 to P95 when they were able to be defined as adults [3].

2.3.1. Responsiveness to brightness change

Every two or three days, all rats experienced a pair of acquisition and retention trials in a Y-maze procedure that measured their ability to locate an arm that had changed in brightness [31]. There is evidence that, in addition to assessing preferences for novelty, responsiveness to the changed arm may be a useful index of short-term memory [33,37].

The apparatus comprised an enclosed, unpainted wooden Y-maze that sat on a 700-mm-high table and was illuminated by dim (47 lx), overhead fluorescent lighting. The maze was 10 cm wide and 14 cm high, and consisted of a 30-cm stem and two 45-cm long arms with an angle of 120° between them. Each arm contained a removable black or white metal insert, which occupied the width, height and 40 cm of the length of the arms. The animals were individually placed in the first 15 cm of the stem and allowed to freely roam the entire maze for a 6-min acquisition trial. The rat was then removed and placed in a holding cage while the white and black inserts were replaced with two clean black inserts, and the entire maze washed with a solution of 20% Powerquats Blue disinfectant. It was then replaced in the first 15 cm of the stem for a 1-min retention trial.

During each retention trial the first arm entered, the total entries of each arm and the total time spent in each arm were recorded by means of a keyboard and PC. This enabled subsequent calculation of, (1) the percent entries of the novel arm, (2) the percent time spent in the novel arm, (3) the total entries/day of both arms, and (4) the total time/day spent in both arms. The rats were tested 4 times, with the novel arm being left for half the tests, and right for the other half.

2.3.2. Activity and social interactions

On days in between other forms of testing, pairs of unfamiliar rats from the same group were assessed twice for their social interactions, rearing and locomotor behavior thereby providing information about their possible anxiety status [6,21,53].

Lower levels of activity, the tendency to rear in the inner squares of an open field, and decreased social interactions between rats are all believed to be related to higher levels of anxiety. The animals were weighed on the day of each testing session to ensure that the body weights of each member of a pair did not differ by more than 10 g.

The apparatus consisted of a wooden 600×600-mm open field, 250 mm high, placed on a 700-mm high table in the same experimental room with the same level of illumination as for responsiveness to change testing. An infrared video camera was mounted 850 mm above the apparatus and connected to a video-recorder in a separate room.

Unfamiliar same-sexed pairs of rats from the same treatment group, matched for body weight, were placed facing each other into the center of the open field, and their behavior was then video-recorded for 5 min. The apparatus was subsequently washed with a 20% Powerquat Blue solution before another pair of rats was tested.

The videotapes were later replayed and the following activity measures were recorded for each rat: (1) frequencies of rearing in the outer 12 squares (outer rearing), (2) frequencies of rearing in the inner 4 squares (inner rearing), (3) the number of lines crossed (ambulation). For the social interaction measure, each pair of rats was treated as a unit because the behavior of one member affects that of the other [21]. Levels of social interactive behavior were determined by calculating the time the pairs of rats spent sniffing, grooming, mounting, crawling over, crawling under or following each other.

In the course of analyzing the video tapes, there was a strong indication that one member of each pair of BZP-treated rats (especially females) often restrained its partner by grasping the skin of its neck with its teeth, or by holding it down on the floor of the apparatus with its forelimbs. Although this behavior initially looked like an aggressive response, on further inspection it preceded what appeared to be an investigation of a spot of non-toxic dye that had been put on its partner's neck (to enable the observer to distinguish between the two rats). Such sequences of behavior seemed to be much less characteristic of control rats. Therefore, the tapes were replayed and the time spent in these restraining encounters was recorded for each pair. The number of fecal boluses (defecation) deposited by each pair was also recorded as a measure of emotionality [53].

2.3.3. Emergence from the dark into the light

After either a responsiveness to change or social interaction test, the rats' latencies to emerge from a darkened start box into an illuminated arena were recorded by means of a hand-held stop watch for a total of 6 trials with an interval of two or three days between each. This test involves a conflict between rodents' tendency to explore a novel environment and their fear of bright light [26]. Thus, longer emergence latencies are regarded as reflecting higher levels of emotionality or anxiety in rats [4,34].

The apparatus was constructed from wood and comprised a 200×150×200-mm-high unlit black painted start box, which opened (via a sliding door) to a 500×400×200-mm-high illuminated arena. The floor of the arena consisted of translucent white Perspex that was illuminated from underneath by

Table 1

Mean (±S.E.M.) values/day of each response and total fecal boluses, total social interactions and total restraining encounters recorded in the three behavioral tests for control and BZP-treated rats

	Treatment group	
	Control	BZP
<i>Responsiveness to brightness change</i>		
Percent entries of novel arm	58.64 (±3.01) ^a	47.39 (±4.17)*
Percent time spent in novel arm	56.57 (±2.86) ^a	46.02 (±4.60)*
Entries of both arms	1.29 (±0.10)	0.90 (±0.11)*
Time spent (s) in both arms	28.59 (±1.95)	17.89 (±1.45)*
<i>Activity and social interactions</i>		
Ambulation	104.78 (±4.61)	108.28 (±3.75)
Rearing in outer squares	40.00 (±1.07)	36.45 (±1.20)*
Rearing in inner squares	6.43 (±0.64)	3.58 (±0.46)*
Total social interactions (s)	120.97 (±4.09)	103.14 (±4.99)*
Total restraining encounters (s)	2.77 (±1.12)	7.14 (±2.24)
Total fecal boluses	1.50 (±0.51)	1.55 (±0.53)
<i>Emergence from the dark into the light</i>		
Emergence latency (s)	47.57 (±10.03)	109.64 (±15.99)*
Total fecal boluses	1.80 (±0.47)	0.65 (±0.24)*

*Difference between the control and BZP-treated groups significant (ANOVA, see text). ^aSignificantly greater than a chance expectancy of 50%, $p < 0.05$, one-sample t -test, $df = 19$.

two 16-w fluorescent tubes that produced a light level of 172 lx. The roof of the arena was made of fine wire mesh. The apparatus sat on a 700-mm-high table in the same room as for other behavioral tests.

Each rat was placed in the darkened start box and, 60 s later, the sliding door to the illuminated arena was opened and the rat's latency to fully emerge (all 4 paws) recorded. If it did not emerge within 5 min, the trial was terminated and the rat assigned a latency of 300 s. Numbers of fecal boluses deposited in the start box were also counted and then the apparatus was washed with a 20% solution of Powerquat Blue before another rat's trial.

3. Results

Unfortunately, no empirical data were collected relating to BZP's acute effects on behavior during the treatment phase of the study. However, from casual observations of the treated rats, it is possible that, contrary to control animals, some individuals displayed amphetamine-like stereotypical behavior [9] in the form of intense grooming, but further research is needed to verify this.

A score/day was calculated for each rat on each behavioral measure recorded except for fecal boluses deposited during the emergence and social interaction tests, social interaction and restraining encounters. In these cases, totals were determined. Effects of BZP treatment and sex on all measures are outlined in Table 1. Data for each measure were subjected to separate 2 (BZP treatment) × 2 (sex) ANOVAs.

3.1. Responsiveness to brightness change

Compared with control subjects, BZP-treated rats entered the novel arm significantly less often, $F(1,36) = 4.63$, $p < 0.04$, and

spent marginally less time in it, $F(1,36)=3.74$, $p=0.061$. They also entered both arms significantly less often, $F(1,36)=7.15$, $p<0.02$, and spent less time in both than control rats, $F(1,36)=24.09$, $p<0.0001$. Although control rats chose to enter and spend longer in the novel arm at levels significantly greater than expected by chance, BZP-treated subjects showed no similar preference for this arm. Both arms were entered significantly more often by females, mean (\pm S.E.M.)= 1.26 (± 0.12), than by males, 0.93 (± 0.09), $F(1,36)=5.43$, $p<0.03$. No other sex differences or interactions were significant.

3.2. Activity and social interactions

BZP-treated rats reared significantly less often than control subjects when they occupied both the outer, $F(1,36)=4.79$, $p<0.04$, and inner squares of the open field, $F(1,36)=12.77$, $p<0.001$. Because it was clear in Table 1 that all rats spent more time in the outer than in the inner squares, the percentages of differences between rearing frequencies in the two areas were calculated for individual rats, and then subjected to the same ANOVA applied to other measures. For control rats, the number of rears that occurred in the inner squares was an average (\pm S.E.M.) of 83.92 (± 1.51) percent less than those that occurred in the outer squares, compared with 90.21 (± 1.27) percent for BZP-treated animals. This difference between the two groups was significant, $F(1,36)=9.82$, $p<0.004$. Although treatment with BZP did not affect the measure of ambulation, female rats crossed significantly more lines, mean (\pm S.E.M.)= 119.63 (± 2.32), than males, 93.43 (± 3.50), $F(1,36)=37.65$, $p<0.0001$.

The time spent engaged in social interactions was significantly shorter for BZP-treated rats, than for control subjects, $F(1,36)=7.83$, $p<0.009$. Although BZP treatment did not significantly affect the time spent restraining a partner rat, the effect was suggestive, $F(1,36)=3.12$, $p=0.085$, as was the interaction between the treatment and sex, $F(1,36)=2.91$, $p=0.097$. Therefore, the BZP effect was examined for males and females separately. As shown by t -tests, the difference between male control, mean (\pm S.E.M.)= 4.32 (± 2.12), and BZP-treated rats, 4.48 (± 2.06), was not significant. However, this difference was significant for females, control= 1.21 (± 0.56), BZP= 9.81 (± 3.93), $t(18)=2.17$, $p<0.045$.

While the sex differences in social interactions and restraining encounters were not significant, male rats deposited marginally more fecal boluses in the open field, 2.20 (± 0.55), than females, 0.85 (± 0.44), $F(1,36)=3.59$, $p=0.066$. No other sex differences or interactions were significant.

3.3. Emergence from the dark into the light

BZP-treated rats took significantly longer to emerge into the illuminated arena than control animals, $F(1,36)=11.04$, $p<0.002$, and deposited fewer fecal boluses in the darkened start box, $F(1,36)=5.87$, $p<0.02$. While male rats defecated more often, mean (\pm S.E.M.)= 2.00 (± 0.44) than females, 0.45 (± 0.25), $F(1,36)=10.66$, $p<0.002$, there was no significant sex difference in emergence latencies, nor were any interactions significant.

4. Discussion

Contrary to control rats, those that had been treated with BZP during adolescence showed no significant tendency to enter and spend time in the novel Y-maze arm in the responsiveness to brightness change test. This suggested that either they were unable to remember which of the two arms had been previously white, or they had no preference for exploring a novel versus familiar environment. If the former, it could be argued that the treatment had interfered with encoding, consolidation or retrieval of information. But, if the latter, they may have been less inclined to respond to novel stimuli possibly because of neophobia arising from heightened emotional reactivity (or “anxiety”), as can occur with anxiogenic experiences in other situations [1,30,43].

Because the BZP-treated rats also entered both arms less often and spent less time in them than control subjects, it is unlikely that acquisition or memory deficits were responsible for the results since, in responsiveness to change settings, these responses are more indicative of activity levels (that could reflect anxiety) rather than the operation of higher order processes [32,37]. And as activity is inversely related to anxiety [4], higher anxiety in BZP-treated animals is the best way of accounting for all the behavior observed in the Y-maze.

It is likely that the lower frequencies of rearing in both the inner and outer squares of the open field shown by BZP-treated rats were due to anxiety-induced reductions in vertical exploration [4]. Compared with control animals, these rats also reared proportionately less often in the inner than in the outer squares. As rats are fearful about occupying the center of an open field and prefer to remain near its walls [4,28], it seems likely that rearing of BZP-treated rats reflected their higher levels of anxiety. This possibility is strongly supported by the significantly less time they spent in social interaction [21,53].

The female-only increase in restraining encounters by BZP treatment is difficult to account for. It may have arisen from attempts to investigate the spot of marking dye on one of the partner’s neck especially since head sniffing of a conspecific is regarded as an example of olfactory social investigation [10]. If so, then why BZP treatment should have enhanced it in females cannot yet be explained. However, the restraining behavior might have been equivalent to “submitting” which is defined as an aggressive response [20] and, in this case, could have been secondary to anxiety. If so, then it is possible that BZP-mediated modification of either DA or 5-HT systems may have been involved since both have been implicated in aggressive behavior [17]. Whatever the exact nature of the response, more research is obviously required to determine if the rather tenuous BZP effect observed in the present study is reliable. If it were proved to be so, it would then be necessary to establish what factors mediate it, what its function is and why it was affected by BZP treatment during adolescence for females but not for males.

The possibility that treatment with BZP had led to higher levels of anxiety was further supported by the longer emergence latencies of treated rats in the emergence test, because longer latencies are widely accepted as indicative of higher anxiety [5].

But the fact that BZP-treated rats also defecated less often in the darkened start box than control subjects argues against them being more anxious [5]. When acutely administered as an anti-parasitic agent for treating intestinal worms, piperazine decreases defecation for physiological rather than emotional reasons [44]. However, it seems unlikely that any similar effect of BZP would still be active more than 2 weeks after the last dose of the drug. As no comparable finding characterized defecation in the social interaction test and as most other results of the study supported heightened BZP-induced anxiety, it is possible that this particular outcome was merely an aberration. Clearly, further research could substantiate this.

The greater number of entries of both arms of the Y-maze, the greater number of lines crossed in the open field and the lower frequency of defecation in the darkened start box of the emergence apparatus shown by female than by male rats is entirely consistent with many other reports of their higher activity [5] and their lower anxiety or fearfulness [24].

It seems very likely that most of the significant behavioral effects of BZP treatment during adolescence observed in the present study arose from the treated rats' higher levels of anxiety. If so, this could have arisen from interference with the development of anxiety-related forebrain mechanisms operated by 5-HT, in a similar fashion to what characterizes long-term effects of 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine [16,25], and selective serotonin reuptake inhibitors [18]. As has been shown for fluoxetine [52], this interference might be in the form of enduring BZP-mediated increases in 5-HT transporter density especially in the frontal cortex where 5-HT activity is associated with anxiety [23]. Alternatively, since BZP is predominantly a DA releaser [8,9] at least some of the results (such as less rearing and slower dark–light emergence) could have been due to changes in motor activity arising from DA deficits. However, on balance, the results favored higher anxiety following the BZP treatment.

Apart from the possibility that BZP affected restraining encounters for females only in the social interaction test, there were no sex differences in responsiveness to the treatment. However, it should be kept in mind that the social interaction test may not be a valid test of anxiety for female rats [38]. Nevertheless, the absence of sex-related effects in the other tests tends to suggest that, overall, females were affected in a similar fashion to males in terms of BZP-mediated increases in anxiety.

While the results of the present study demonstrated that exposure to 10 mg/kg/day BZP during adolescence can have longer-term outcomes in rats, there is clearly a need to assess the effects of such exposure during other developmental periods. This is particularly important in view of findings that other similar drugs with serotonergic and dopaminergic agonist properties, such as methamphetamine, MDMA and methylphenidate can influence later behavior when administered before or after adolescence [14,16,42]. In future research, there is also a need to include more than one dose as well as to vary periods of administration during any specific developmental stage, along with measures of the drug's acute effects during treatment and neurochemical analyses of changes in 5-HT, DA and other neurotransmitter systems in appropriate brain areas. However,

in spite of its limitations, the present study provides some compelling preliminary results that, by supporting similarities between BZP and amphetamine effects, raise further doubts about the desirability of social use of the drug by adolescents.

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