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Responsiveness of 5-HT_{2C} receptors in repeatedly diazepam-injected rats: a behavioral and neurochemical study

Asma Khan, Darakhshan J. Haleem

Department of Biochemistry, Neurochemistry and Biochemical Neuropharmacology Research Unit, University of Karachi, Karachi-75270, Pakistan

Correspondence: Asma Khan, e-mail: asmakhan_ku@yahoo.com

Abstract:
The role of 5-hydroxytryptamine (serotonin; 5-$HT)_{2C}$ receptors in anxiety and the anxiolytic effects of drugs is well documented. In view of the withdrawal anxiety associated with repeated diazepam intake, the present study concerns the efficacy of 5-HT_{2C} receptors in rats treated with diazepam. Results show that diazepam injections at a dose of 2 mg/kg daily for two weeks increased weekly food intake and growth rate. Anxiolytic effects of the drug monitored in a light/dark activity box were not significant after single administration. One week and two weeks of administration elicited anxiolytic effects, which were smaller after two weeks of administration as compared to one week, suggesting the development of tolerance to the anxiolytic profile of diazepam. Moreover, three days' withdrawal from repeated administration elicited anxiogenic behavior in the light/dark activity box. The behavioral and neurochemical effects of 1-(m-chlorophenyl)piperazine (m-CPP) (3 mg/kg), a 5-HT_{2C} agonist, were monitored following withdrawal (three days) from two weeks of diazepam administration. Results showed that hypophagic as well as anxiogenic-like effects of m-CPP were not different from repeated saline or repeated diazepam-injected animals. Administration of m-CPP increased 5-HT metabolism in repeated saline as well as repeated diazepam-injected animals. However, m-CPP-induced increases in 5-HT metabolism were greater in repeated diazepam-injected animals. Results are discussed in the context of the role of 5-HT_{2C} receptors in the precipitation of withdrawal anxiety.

Key words:
light/dark activity, food intake, m-CPP, diazepam, 5-HT, 5-HT_{2C} receptors

Introduction

Benzodiazepines are the most commonly prescribed anxiolytic drugs, as they are effective against a wide spectrum of anxiety disorders. Benzodiazepine withdrawal in humans is associated with increased anxiety, insomnia, sensory disturbances and even seizures [36]. Similar symptoms have been observed in animals withdrawn from chronic benzodiazepine treatment [15].

Serotonin (5-HT) is one of the transmitters involved in the pathophysiology of depression and anxiety. The hypothesized role of 5-HT in the pathogenesis of anxiety stems from observations of 5-HT antagonists in operant models [39]. Furthermore, a significant correlation between a reduction in 5-HT turnover and the anxiolytic effects of benzodiazepines [23]

suggests that a reduction of serotonergic neurotransmission results in an anxiolytic-like effect. Conversely, increased serotonergic activity produces an anxiogenic response. Diazepam, a benzodiazepine, is often reported to produce its anxiolytic effects by decreasing central 5-HT neurotransmission [51]. Anxiety studies in animal models show that administration of diazepam increased entries and time spent in the open arm of an elevated plus maze. High (10, 32 mg/kg), but not low (3.2 mg/kg), doses of diazepam impaired motor coordination, as tested with the Rota-rod in rats and mice [28]; however, activity in an open field test decreased at low, moderate and high doses [6].

It is well established that long-term intake of diazepam produces tolerance and withdrawal anxiety upon abrupt discontinuation [16]. There is abundant experimental evidence that the median raphe nucleus-dorsal hippocampal 5-HT pathway mediates diazepam withdrawal-induced anxiety [3]. Withdrawal from chronic diazepam treatment has also been shown to induce an increase in 5-HT release in different brain regions, particularly the hippocampus [2].

Evidence suggests that the anxiogenic-like effects of 5-HT are mediated *via* the stimulation of postsynaptic 5-HT$_{2C}$ receptors. The 5-HT agonist m-CPP increases 5-HT release [7, 22] *via* the selective stimulation of postsynaptic 5-HT$_{2C}$ receptors [20]. One-time administration of the drug also elicits hypolocomotion [21, 32, 37, 47], hypophagia [25, 43, 44], impaired motor coordination [41] and other anxiety-like behaviors [19, 33] in experimental animals. Tolerance to the drug's hypolocomotive effects [18], but not to the hypophagic effects of m-CPP [48], is also reported following repeated administration of m-CPP.

In the present study, diazepam was injected at a dose of 2 mg/kg for two weeks to test whether tolerance to drug treatment occurred during days 7–14 of drug administration. It was hypothesized that repeated administration of diazepam would alter the responsiveness of 5-HT$_{2C}$ receptors, resulting in tolerance and withdrawal anxiety upon abrupt discontinuation. Therefore, m-CPP was injected at a dose that has been previously shown to elicit significant but submaximal anxiogenic-like and hypophagic effects [25, 31]. The behavioral and neurochemical effects produced by the selected dose of m-CPP were compared in repeatedly saline-injected and repeatedly diazepam-injected animals.

Materials and Methods

Animals

Locally bred albino Wistar rats weighing 200–220 g were obtained from Aga Khan University, Karachi. Animals were individually housed in cages-covered with sawdust flooring with free access to cubes of standard rodent diet and tap water 3 days before starting the experiment. At the start and during the experiment, weighed amounts of food were given to the animals. All experiments were performed according to a protocol approved by the local Animal Care Committee. The temperature of the room was maintained at 20 ± 1°C.

Drugs and injections

Diazepam (F. Hoffmann–La Roche Ltd., Basel Switzerland), purchased as injectable ampoules of 5 mg/ml, was injected intraperitoneally (*ip*) at a dose of 2 mg/kg. m-CPP purchased from Sigma (USA) was dissolved in saline (0.9% NaCl) at a dose of 3 mg/kg. Control animals were injected with saline (0.9% NaCl) in a volume of 1 ml/kg.

Experimental protocol

Twenty-four male albino Wistar rats were randomly divided into two equal groups of 12 each: control and test. Baseline values for the two groups of rats were monitored in the light/dark activity paradigm one day before starting the experiment. Control animals received vehicle (1 ml/kg), while experimental animals received diazepam (2 mg/kg) once daily for two weeks. During the treatment, rat movements were monitored following day 1, day 7 and day 14, 1 h after the injection of saline or diazepam. Food intake and body weight were monitored weekly.

Two days after drug withdrawal, animals were further divided into four groups of six each: *(i)* saline plus saline (1 ml/kg); *(ii)* saline plus m-CPP (3 mg/kg); *(iii)* diazepam plus saline (1 ml/kg); *(iv)* diazepam plus m-CPP (3 mg/kg). On day 16, food was removed from the cages. On day 17, animals were injected with either saline or m-CPP. Activity in the light/dark box was monitored 30 min after the saline or m-CPP injection. After testing in the light/dark box, animals were returned to their home cages. Weighed amounts

of food were placed in the food buckets, and food intake was monitored at 2 and 4 h after m-CPP or saline injection. The animals were decapitated 4 h after the m-CPP injection to collect hippocampus for neurochemical estimations of 5-HT and 5-HIAA by HPLC-EC.

Behavioral analysis

Food intakes

Food intake was monitored by giving rats a weighed amount of food and weighing the food remaining in cage hoppers.

Growth rate

Body weights of rats were monitored weekly during treatment. Growth rate was calculated in terms of percentage of initial body weight.

Light/dark activity

The apparatus used in this investigation was a two-compartment box. The compartments are of equal size (26 × 26 × 26 cm), with a place of access (12 × 12 cm) between the compartments, each different in its sensory properties. One of the compartments was dark (made up of a black plastic wall), and the other was white (made up of transparent plastic). A rat injected with saline or diazepam was introduced to the apparatus by placing it in the light compartment. Time spent in the light compartment and the number of entries into the light box was monitored for 5 min. The apparatus was placed in the light room.

Neurochemical analysis

HPLC-EC determination of 5-HT and 5-HIAA (5-hydroxyindole acetic acid)

A 5μ shim-pack ODS separation column of 4.5 mm internal diameter and 15 cm length was used. The mobile phase was 0.1 M sodium phosphate buffer (pH 2.9) containing 14% HPLC grade methanol, 0.023% OSS, 0.005% EDTA. Electrochemical detection was done at an operating potential of 0.8 V (glassy carbon electrode *vs.* Ag/AgCl reference electrode).

Statistical analysis

The effects of diazepam administration on food intake, growth rate and light/dark activity were analyzed by two-way ANOVA (repeated measure design). Neurochemical and behavioral data on the effects of m-CPP were analyzed by two-way ANOVA. *Post-hoc* comparisons were done with the Newman-Keuls test; $p < 0.05$ were considered significant.

Results

Figure 1 shows the effects of two-weeks of diazepam administration on weekly food intake and growth rate. A two-way ANOVA (repeated measure design) (df = 1,22) showed significant effects of diazepam on food intake ($F = 31.87$, $p < 0.01$) and growth rate ($F = 10.41$, $p < 0.01$). Effects of weekly measures for food intake ($F = 2.67$, $p > 0.01$) and growth rate

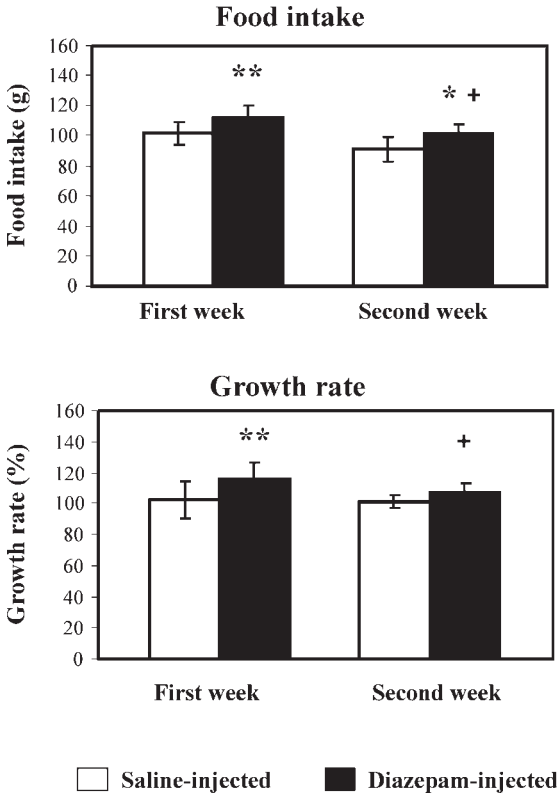

Fig. 1. Effects of two weeks of administration of diazepam on weekly food intake and growth rates. Values are the means ± SD (n = 12). Significant differences by Newman-Keuls test. * $p < 0.05$, ** $p < 0.01$ from repeatedly saline-injected rats, + $p < 0.05$ from first week diazepam-injected rats, following two-way ANOVA (repeated measure design)

(F = 4.26, p > 0.01) were not significant. Interaction between weekly measurements and the drug for food intake (F = 3.32, p > 0.01) and growth rate (F = 2.60, p > 0.01) were also not significant. *Post-hoc* analysis showed that diazepam administration increased food intake and growth rate during the first week of treatment. Increase in food intake but not growth rate was observed during the second week of treatment. Mean values of food intake and growth rate after the second week were less than values from the first week in diazepam-injected animals.

Figure 2 shows the effects of diazepam on the activity of rats in a light/dark box. The data on number of entries analyzed by two-way ANOVA (repeated measure design) showed significant effects of diazepam (F = 219.473 dF = 1,22; p < 0.01), repeated monitoring (F = 37.066 dF = 3,66; p < 0.01) and interaction between the two factors (F = 52.17 dF = 3,66; p < 0.01). *Post-hoc* analysis showed that diazepam administration increased entries into the light compartment following single, one-week and two-week administration. Effects on entries following one week and two weeks of repeated administration were greater than those following single administration of diazepam, while entries into the light compartment were fewer after two weeks as compared to one week of drug administration.

The data on time spent in the light compartment were analyzed by two-way ANOVA (repeated measure design) and showed significant effects of diazepam (F = 234.91 dF = 1,22; p < 0.01), repeated monitoring (F = 54.13 dF = 3,66; p < 0.01) and interaction between the two factors (F = 73.21 dF = 3,66; p < 0.01). *Post-hoc* analysis showed that time spent in the light compartment increased following one week and two weeks of repeated administration but not following single administration. Effects on time spent in light compartment following one week and two weeks of repeated administration were greater than following single administration of diazepam. Mean values of time spent in the light compartment following administration for two weeks were smaller than for one week.

Figure 3 shows the effects of administering m-CPP to repeatedly saline-injected and repeatedly diazepam-injected animals on entries into and time spent in the light compartment of a light/dark activity box. Two-way ANOVA (dF = 1,20) performed on the numbers of entries into the light compartment shows that the effects of diazepam (F = 4.21, p > 0.01) were not significant. Effects of m-CPP (F = 43.80, p < 0.01)

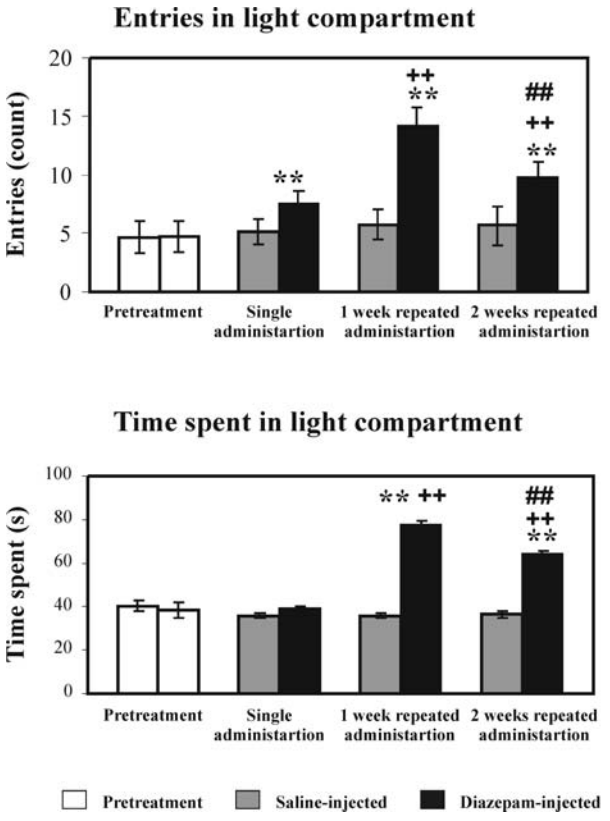

Fig. 2. Effects of diazepam on the activity of rats in a light/dark box following single, one-week and two-week repeated administration. Values are the means ± SD (n = 12) 1 h after saline or diazepam injection. Significant differences by Newman-Keuls test. ** p < 0.01 from saline-injected rats, $^{++}$ p < 0.01 from single diazepam-injected rats, $^{\#\#}$ p < 0.01 from one-week diazepam-injected rats, following two-way ANOVA (repeated measure design)

were significant; interaction between diazepam and m-CPP (F = 0.0052, p > 0.01) was not. *Post-hoc* analysis showed that administration of m-CPP decreased entries into the light compartment in saline-injected (46.7%) as well as diazepam-injected (57.44%) animals.

The data on time spent in the light compartment analyzed by two-way ANOVA (dF = 1,20) demonstrate significant effects of diazepam (F = 11.8, p < 0.01) and m-CPP (F = 90.613, p < 0.01). Interaction between the two factors (F = 2.08, p > 0.01) was not significant. *Post-hoc* analysis showed that m-CPP decreased the time spent in the light compartment in saline-injected as well as diazepam-injected rats. Diazepam plus saline-injected animals spent less time in the light compartment than saline plus saline-injected animals, while diazepam plus m-CPP- (76.4%) injected animals spent less time in the light than saline plus m-CPP- (49.2%) injected animals.

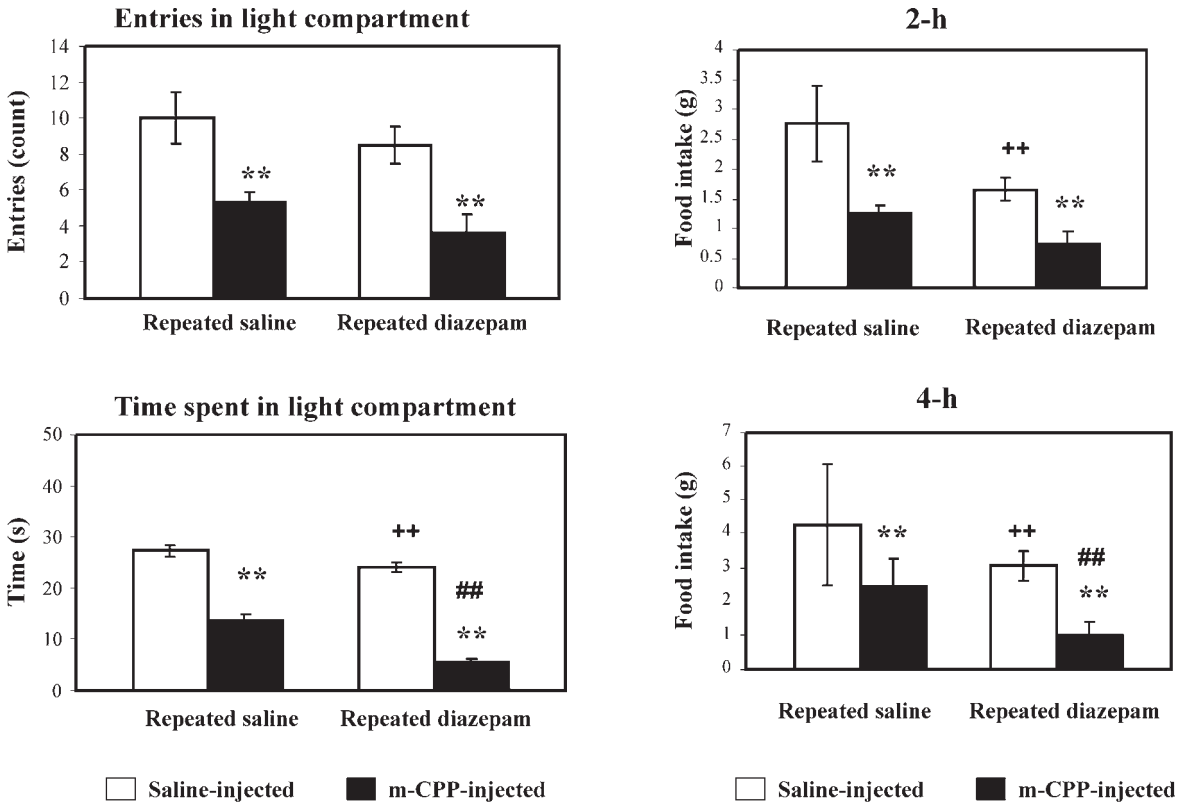

Fig. 3. Effects of m-CPP on entries into and time spent in light compartment of light/dark activity box in repeatedly saline-injected or repeatedly diazepam-injected animals. Values are the means ± SD (n = 6). Significant differences by Newman-Keuls test. ** $p < 0.01$ from respective saline-injected animals, $^{++}$ $p < 0.01$ from saline plus saline-injected animals, $^{\#\#}$ $p < 0.01$ from saline plus m-CPP-injected animals, following two-way ANOVA

Fig. 4. Effects of m-CPP on 2-h and 4-h food intake in repeatedly saline-injected or repeatedly diazepam-injected animals. Values are the means ± SD (n = 6). Significant differences by Newman-Keuls test. ** $p < 0.01$ from respective saline-injected rats, $^{++}$ $p < 0.01$ from saline plus saline-injected animals and $^{\#\#}$ $p < 0.01$ saline plus m-CPP-injected animals, following two-way ANOVA

Figure 4 shows the effects of m-CPP on 2-h and 4-h food intake (post-injection) in repeatedly saline-injected and diazepam-injected animals. The data on 2-h food intake analyzed by two-way ANOVA (dF = 1,20) showed significant effects of diazepam ($F = 8.34$, $p < 0.01$) and m-CPP ($F = 18.64$, $p < 0.01$). The interaction between diazepam and m-CPP ($F = 1.213$, $p > 0.01$) was not significant. *Post-hoc* analysis showed that administration of m-CPP decreased food intake in repeatedly saline-injected and repeatedly diazepam-injected animals. Diazepam plus saline-injected animals exhibited smaller values of food intake than saline plus saline-injected animals.

The data on 4-h food intake analyzed by two-way ANOVA (dF = 1,20) showed significant effects of diazepam ($F = 11.33$, $p < 0.01$) and m-CPP ($F = 23.82$, $p < 0.01$). Interaction between diazepam and m-CPP ($F = 0.0867$, $p > 0.01$) was not significant. *Post-hoc* analysis showed that administration of m-CPP decreased food intake of repeatedly saline-injected and repeatedly diazepam-injected animals. Four-hour food intake was smaller in diazepam plus saline-injected animals and diazepam plus m-CPP-injected animals than in saline plus saline-injected animals and saline plus m-CPP-injected animals, respectively.

Figure 5 shows the effects of m-CPP on 5-HT and 5-HIAA levels in the hippocampus of repeatedly saline-injected and repeatedly diazepam-injected rats. The data on 5-HT levels analyzed by two-way ANOVA (dF = 1,20) revealed significant effects of diazepam ($F = 49.16$, $p < 0.01$) and m-CPP ($F = 91.043$, $p < 0.01$), as well as a significant interaction between diazepam and m-CPP ($F = 36.26$, $p < 0.01$) in rats. *Post-hoc* analysis showed that administration of m-CPP increased 5-HT concentration in both saline-injected and diazepam-injected rats. The levels of 5-HT were higher in diazepam plus m-CPP-injected rats than in saline plus m-CPP-injected rats.

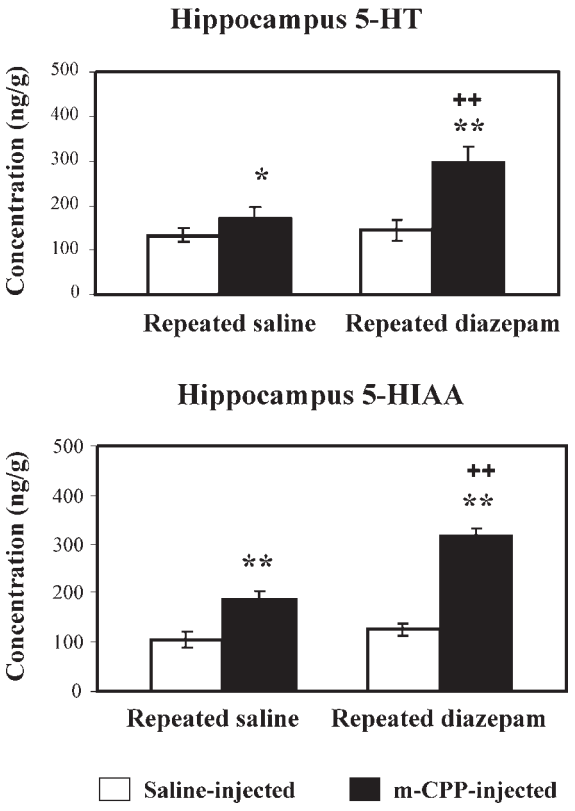

Fig. 5. Effects of m-CPP on 5-HT and 5-HIAA levels in the hippocampi of repeatedly saline-injected and repeatedly diazepam-injected animals. Values are the means ± SD (n = 6). Significant differences by Newman-Keuls test. * $p < 0.05$, ** $p < 0.01$ from saline plus saline-injected animals, $^{++}$ $p < 0.01$ from saline plus m-CPP-injected animals, following two-way ANOVA

The data on 5-HIAA levels analyzed by two-way ANOVA (dF = 1,20) revealed significant effects of diazepam ($F = 92.94$, $p < 0.01$) and m-CPP ($F = 320.17$, $p < 0.01$) and also a significant interaction between the two factors ($F = 48.23$, $p < 0.01$). *Post-hoc* analysis showed that administration of m-CPP increased 5-HIAA levels in saline-injected as well as diazepam-injected rats. The levels of 5-HIAA were greater in diazepam plus saline-injected animals than in saline plus m-CPP-injected animals.

Discussion

The present study shows that withdrawal from repeated administration of diazepam elicited anxiety, as animals exhibited fewer entries into and less time spent in the light compartment of a light/dark box. This suggests that withdrawal from repeated administration of diazepam elicits anxiety. Other authors have also reported that withdrawal from repeated administration of diazepam elicits withdrawal anxiety in social interaction and elevated plus maze tests [8]. Although administration of diazepam at a dose of 2 mg/kg did not decrease the number of entries into the open arm of an elevated plus maze [34], extensive evidence demonstrates that the anxiolytic effects of diazepam in a light/dark box are not due to drug-induced sedation [38].

Acute administration of diazepam at doses 0.25–1 mg/kg and 3.2 mg/kg has also been shown to produce anxiolytic effects in the light/dark box [45]. We find that single administration of diazepam at a dose of 2 mg/kg did not produce anxiolytic effects in this behavioral test. However, repeated administration for one week increased entries into and exploration of the lit compartment in the light/dark activity box, consistent with the anxiolytic effects of the drug. Previously, it has been shown that a single prior exposure to the elevated plus maze results in one-trial tolerance [17]. Tolerance following a single administration could be due to this phenomenon. However, the anxiolytic effects following one week of repeated administration were greater than those observed after single administration (Fig. 2). A decrease in the anxiolytic effects of the drug after one or two weeks of administration suggesting the development of tolerance to the anxiolytic profile diazepam [16].

It has been well established that activation of the serotonergic system results in the suppression of feeding behavior [10, 12, 26]. The hypothalamus is a region of the brain known to have a role in the regulation of appetite [30]. The facilitation of food intake by benzodiazepine has been shown by a number of studies to be due in part to direct effects on appetite and palatability [9, 13, 46]. The hyperphagic effects of 2 mg/kg diazepam that occurred over a period of two weeks in the present study (Fig. 1) are explainable in terms of a decrease in 5-HT metabolism, particularly in the hypothalamus [25], resulting in decreased availability of 5-HT at local hypophagic sites.

Administration of m-CPP has also been shown to decrease the number of entries and time spent in the lit compartment of the light/dark activity box [11, 24], suggesting that the drug may elicit anxiogenic behavior. A single dose of 5-HT$_{2A/2C}$ antagonists mianserin and ritanserin effectively ameliorated withdrawal

anxiety produced by two weeks of diazepam administration (4 mg/kg) in rats [8]. The present study shows that anxiogenic effects of m-CPP in the light/dark activity test were not different in repeatedly saline-injected and repeatedly diazepam-treated animals.

The 5-HT_{2C} receptor subtype has also been implicated extensively in the regulation of ingestive behavior. Research on this receptor shows that increases in the functional activity of 5-HT at postsynaptic 5-HT_{2C} sites elicited satiety in experimental animals [35]. In the present work, administration of m-CPP decreased food intake 2 and 4 h post-injection (Fig. 4). Other authors have also reported decreased food intake (hypophagia) following the administration of m-CPP [18, 29]. The present study shows that the hypophagic effects of m-CPP did not differ in repeatedly saline-injected or repeatedly diazepam-injected animals, suggesting that the responsiveness of hypophagic 5-HT_{2C} receptors is also not altered in diazepam-induced withdrawal anxiety.

The hippocampus is involved in the induction of anxiety symptoms [4]; 5-HT_{2C} receptors are highly expressed in the hippocampus [1]. Pharmacological evidence suggests that administration of 5-HT_{1A} agonists and benzodiazepines decreased serotonergic activity in whole brain and various brain regions (including hippocampus) to elicit anxiolytic effects [40, 42, 49]. Previous reports from our laboratory have shown that repeated administration of diazepam – decreased 5-HT metabolism [27] in the hippocampus by inhibiting the activity of tryptophan hydroxylase [5], possibly due to the activation of somatodendritic 5-HT_{1A} receptors. The present study shows that, after withdrawal from repeated administration of diazepam, 5-HT metabolism was normal in the hippocampus.

Administration of m-CPP has been shown to suppress 5-HT turnover and to enhance extracellular levels of 5-HT [14] by inhibiting reuptake of 5-HT into the synaptosomes [50]. In the present study, m-CPP-induced 5-HT metabolism was greater in the hippocampus of repeatedly diazepam-treated animals than in repeatedly saline-treated animals (Fig. 5). This suggests that a greater increase in m-CPP-induced 5-HT efflux is associated with diazepam-induced withdrawal anxiety.

In conclusion, the present study shows that administration of diazepam for only two weeks may result in withdrawal anxiety that is not associated with greater availability of 5-HT in the hippocampus or greater anxiogenic response of post-synaptic 5-HT_{2C} receptors. It is not likely that the anxiolytic effects of diazepam and diazepam-induced withdrawal anxiety are both due to previous trial tolerance or amnesic effects of the drug. Responsiveness of hypophagic 5-HT_{2C} receptors is also not altered. It is suggested that increased stimulation-induced 5-HT efflux in the hippocampus may have a role in withdrawal anxiety or in the tolerance accompanying the anxiolytic profile of diazepam and other benzodiazepines.

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References:

1. Abramowski D, Rigo M, Duc D, Hoyer D, Staufenbiel M: Localization of the 5-hydroxytryptamine$_{2C}$ receptor protein in human and rat brain using specific antisera. Neuropharmacology, 1995, 34, 1635–1645.
2. Andrews N, File SE: Increased 5-HT release mediates the anxiogenic response during benzodiazepine withdrawal: a review of supporting neurochemical and behavioral evidence. Psychopharmacology (Berl), 1993, 112, 21–25.
3. Andrews N, File SE, Fernandes C, Gonzalez LE, Barnes NM: Evidence of the median raphe nucleus-dorsal hippocampal pathway mediates diazepam withdrawal-induced anxiety. Psychopharmacology (Berl), 1997, 130, 228–234.
4. Andrews N, Gonzalez LE, File SE: 5-HT_{1A} receptors in the median raphe nucleus and dorsal hippocampus may mediate anxiolytic and anxiogenic behaviors respectively. Eur J Pharmacol, 1994, 264, 259–264.
5. Batool F, Haleem DJ: Neurochemical and behavioural effects of diazepam: evidence from animal models. Pak J Pharm Sci, 1997, 10, 1–8.
6. Batool F, Parveen T, Haleem DJ: Enhancement of brain regional tryptophan concentration following administration of diazepam: a dose related study. Pak J Sci Ind Res, 1995, 38, 267–270.
7. Baumann MH, Ayestas MA, Dersch CM, Rothman RB: 1-(m-chlorophenyl) piperazine (m-CPP) dissociates in vivo release from long term serotonin depletion in rat brain. Neuropsychopharmacology, 2001, 24, 492–501.
8. Begg DP, Hallam KT, Norman TR: Attenuation of benzodiazepine withdrawal anxiety in the rat by serotonin antagonists. Behav Brain Res, 2005, 161, 286–290.
9. Berridge KC, Pecina S: Benzodiazepines, appetite and taste palatability. Neurosci Biobehav Rev, 1995, 19, 121–131.
10. Bickerdike MJ: 5-HT_{2C} receptor agonists as potential drugs for the treatment of obesity. Curr Top Med Chem, 2003, 3, 885–897.

11. Bilkei-Gorzo A, Gyertyan I, Levay G: m-CPP induced anxiety in light dark box in rats – a new method for screening anxiolytic activity. Psychopharmacology (Berl), 1998, 36, 291–298.
12. Blundell JE: Is there a role of serotonin (5-hydroxytryptamine) in feeding? Int J Obes, 1977, 1, 15–42.
13. Cooper SJ: Benzodiazepine as appetite-enhancig compound. Appetite, 1980, 1, 7–19.
14. Eriksson E, Engberg G, Bing O, Nissbrandt H: effects of m-CPP on the extracellular concentrations of serotonin and dopamine in rat brain. Neuropsychopharmacology, 1999, 20, 287–296.
15. File E: The history of benzodiazepine dependence: a review of animal studies. Neurosci Behav Rev, 1990, 14, 135–146.
16. File SE: Tolerance to the behavioral actions of benzodiazepines. Neurosci Biobehav Rev, 1985, 9, 113–121.
17. File SE, Mabbutt PS, Hitchcott PK: Characterisation of the phenomenon of one-trial tolerance to the anxiolytic effect of chlorodiazepoxide in the elevated plus-maze. Psychopharmacology (Berl), 1990, 102, 98–101.
18. Fone KCF, Austin RA, Topham IA, Kennett GA, Punhani T: Effects of chronic m-CPP on locomotion, hypophagia, plasma corticosterone and 5-HT$_{2C}$ receptor levels in the rat. Br J Pharmacol, 1998, 123, 1707–1715.
19. Fone KCF, Shalders K, Fox ZD, Arthur R, Marsden CA: Increased 5-HT$_{2C}$ receptor responsiveness occurs on rearing rats in social isolation. Psychopharmacology (Berl), 1996, 123, 346–352.
20. Gibson EL, Barnfield AM, Curzon G: Dissociation of effects of chronic diazepam treatment and withdrawal on hippocampal dialysate 5-HT and m-CPP-induced anxiety in rats. Behav Pharmacol, 1996, 7, 185–193.
21. Gleason SD, Shannon HE: *Meta*-chlorophenylpiperazine induced changes in locomotor activity are mediated by 5-HT$_1$ as well as 5-HT$_{2C}$ receptors in mice. Eur J Pharmacol, 1998, 341, 135–138.
22. Godbout R, Chaput Y, Blier P, de Montigny C: Tandospirone and its metabolite, 1-(2-pyridinyl)-piperazine. Effects of acute and long term administration of tandospirone on serotonin neurotransmission. Neuropharmacology, 1991, 30, 679–690.
23. Goldberg ME, Manian AA, Efron DH: A comparative study of certain pharmacological responses following acute and chronic administration of chlorodiazepoxide. Life Sci, 1969, 6, 481–491.
24. Griebel G, Misslin R, Pawlowski M, Vogel E: 1-M-chloropiperazine enhances neophobic and anxious behavior in mice. Neuroreport, 1991, 2, 627–629.
25. Haleem DJ: Function specific supersensitivity of m-chlorophenylpiperazine-induced serotonergic neurotransmission in female compared to male rats. Life Sci, 1993, 52, PL279–284.
26. Haleem DJ: Serotonin appetite and anorexia nervosa. JCPSP, 1996, 3, 167–172.
27. Haleem DJ, Batool F: Regionally specific effects of diazepam on brain serotonin metabolism in rats: sustained effects following repeated administration. Life Sci, 1996, 59, PL239–246.
28. Harada K, Aota M, Inoue T, Matsuda R, Mihara T, Yamaji T, Ishibashi K, Matsuoka N: Anxiolytic activity of a novel potent serotonin 5-HT$_{2C}$ receptor antagonist FR260010: a comparision with diazepam and buspirone. Eur J Pharmacol, 2006, 553, 171–184.
29. Hewitt KN, Lee MD, Dourish CT, Clifton PG: Serotonin 2C receptor agonists and the behavioral satiety sequence in mice. Pharmacol Biochem Behav, 2002, 71, 691–700.
30. Hikiji K, Inoue K, Iwasaki S, Ichihara K, Kiriike N: Local perfusion of m-CPP into ventromedial hypothalamic nucleus, but not in lateral hypothalamic area and frontal cortex, inhibits food intake in rats. Psychopharmacology (Berl), 2004, 174, 190–196.
31. Inam QA, Haleem MA, Haleem DJ: Neurochemical and behavioral effects of 1-(3-chlorophenyl) piperazine (m-CPP) in sugar rich diet treated rats. Pak J Biochem Mol Biol, 2006, 39, 67–72.
32. Kennett GA, Curzon G: Evidence that m-CPP may have behavioral effects mediated by central 5-HT$_{1C}$ receptors. Br J Pharmacol, 1988, 94, 137–147.
33. Kennett GA, Wood MD, Bright F, Trial B, Riley G, Holland V, Avenell KY et al.: SB-242084, a selective and brain penetrant 5-HT$_{2C}$ antagonist. Neuropharmacology, 1997, 6, 609–620.
34. Khan A, Haleem DJ: Tolerance in the anxiolytic profile following repeated administration of diazepam but not buspirone is associated with a decrease in the responsiveness of postsynaptic 5-HT$_{1A}$ receptors. Acta Biol Hung, 2007, 58, 345–357.
35. Kitchener SJ, Dourish CT: An examination of the behavioural specificity of hypophagia induced by 5-HT$_{1B}$ and 5-HT$_2$ receptor agonist using postprandial satiety sequence in rats. Psychopharmacology, 1994, 113, 369–377.
36. Ladewig D: Dependence liability of the benzodiazepines. Drug Alcohol Depend, 1984, 13, 139–149.
37. Lucki I, Ward HR, Frazer A: Effects of 1-(m-chlorophenyl) piperazine and 1-(m-trifuromethylphenyl) piperazine on locomotor activity. J Pharmacol Exp Ther, 1989, 249, 155–164.
38. Mehta AK, Ticku MK: Ethanol- and diazepam-withdrawal hyperlocomotion is not due to 5-HT$_3$ receptor stimulation. Pharmacol Biochem Behav, 1993, 45, 755–757.
39. Robichaud RC, Slede KL: The effects of p-chlorophenylalanine on experimentally induced conflict in rat. Life Sci, 1969, 8, 965–969.
40. Samad N, Batool F, Haleem DJ: Neurochemical and behavioral effects of 8-OH-DPAT following exposure to restraint stress in rats. Pharmacol Rep, 2007, 59, 173–180.
41. Samad N, Haleem MA, Haleem DJ: Behavioral effects of 1-(m-chlorophenyl)piperazine (m-CPP) in a rat model of tardive dyskinesia. Pak J Pharm Sci, 2008, 21, 262–268.
42. Samad N, Perveen T, Haider S, Haleem MA, Haleem DJ: Inhibition of restraint-induced neuroendocrine and serotonergic responses by buspirone in rats. Pharmacol Rep, 2006, 58, 636–642.
43. Samanin R, Mennini T, Ferrais A, Bendotti C, Borsini F, Garattini S: M-chloropiperazine a central serotonin agonist causing powerful anorexia in rats. Naunyn-Schmiedebergs Arch Pharmacol, 1979, 308, 159–163.

44. Schuhler S, Clark A, Joseph W, Patel A, Lehnen K, Startford E, Horan TL et al.: Involvement of 5-HT receptors in the regulation of food intake in Siberian Hamsters. J Neuroendocrinol, 2005, 17, 276–285.
45. Shimada T, Matsumoto K, Osanai M, Matsuda H, Terasawa K, Watanabe H: The modified light/dark transition test in mice: evaluation of classic and putative anxiolytic and anxiogenic drugs. Gen Pharamacol, 1995, 26, 205–210.
46. Soderpalm AHV, Berridge KC: Food intake after diazepam, morphine or muscimol: microinjections in the nucleus accumbens shell. Pharmacol Biochem Behav, 2000, 66, 429–434.
47. Takashaki H, Takada Y, Urano T, Takada A: Dissociation of systemic and hippocampal modulation of rat locomotor activity by 5-HT_{2C} receptors. Neurosci Res, 2001, 40, 97–103.
48. Vickers SP, Benwell KR, Porter RH, Bickerdike MJ, Kennett GA, Dourish CT: Comparative effects of continuous infusion of mCPP, Ro 60-0175 and d-fenfluramine on food intake, water intake, body weight and locomotor activity in rats. Br J Pharmacol, 2000, 130, 1305–1314.
49. Wisłowska-Stanek, Hamed A, Lehner M, Bidziński A, Turzyńska D, Sobolewska A, Walkowiak J, Płaźnik A: Effects of midazolam and buspirone on *in vivo* concentration of amino acids and monoamine metabolites in the rat hippocampus. Pharmacol Rep, 2008, 60, 209–218.
50. Wolf WA, Kuhn DM: The 5-HT transporter is an additional site of action for the 5-HT agonists RU24969 and TFMPP. Neurochem Int, 1991, 19, 39–44.
51. Wright IK, Upton N, Marsden CA: Effect of established and putative anxiolytics on extracellular 5-HT and 5-HIAA in the ventral hippocampus of rats during behaviour on the elevated X-maze. Psychopharmacology (Berl), 1992, 109, 338–346.

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