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The effects of midazolam on the acquisition and expression of fructose- and maltodextrin-based flavour preferences

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ABSTRACT

The effects of the benzodiazepine agonist midazolam on the acquisition and expression of flavour preferences were investigated. Rats (Experiment 1) were given one-bottle training with one flavoured solution (CS+) mixed with either fructose or maltodextrin and another solution (CS−) presented alone. Animals receiving 1 mg/kg midazolam during training consumed more CS− than did animals receiving vehicle injections although there was no drug effect on CS+ consumption. In two-bottle tests the CS+ was preferred to the CS− with the preference being larger in fructose trained animals. Midazolam (0.3–3 mg/kg) increased total intake but not CS+ preference. Training under midazolam reduced the CS+ preference when fructose, but not maltodextrin, was the reinforcer. In Experiment 2 training consumption was restricted to 10 ml/session. This removed the difference in CS+ preference between reinforcer types but otherwise the results were as in Experiment 1. The midazolam induced attenuation of fructose-based preferences might reflect an increase in CS− palatability during training which would reduce the difference between the reinforced and non-reinforced solutions. As maltodextrin supports preferences due to post-ingestive effects manipulation of palatability should be ineffective. Midazolam does not influence the expression of conditioned flavour preferences despite prior evidence that benzodiazepine agonists enhance palatability.

Animals can learn preferences for novel flavoured foods in a variety of ways. Perhaps the most well established of these are pairing the novel flavour with nutrients (e.g. Sclafani and Nissenbaum, 1988) or with non-nutritive substances with particularly palatable tastes such as saccharin (e.g. Holman, 1975). A fair amount is known about how flavour preferences are learnt at the behavioural level but relatively less is known about the underlying neurochemical basis of such conditioning although both the opioid and dopaminergic systems have received some attention. The opioid antagonist naloxone did not prevent either the acquisition or expression of flavour preferences based on palatability (Baker et al., 2004; Yu et al., 1999) or nutrients (Azzara et al., 2000) despite having a suppressive effect on consumption in general. This failure to prevent learning or expression of flavour preferences occurred despite the fact that opioid antagonists are well known to reduce consumption of palatable solutions (e.g. Cooper, 1983) and also appear to reduce the unconditioned palatability of such solutions (e.g. Parker et al., 1992). With respect to the dopaminergic systems the evidence is more mixed: D1 but not D2 antagonists blocked the acquisition of preferences conditioned by the gastric infusion of sucrose but had relatively little effect on the expression of such preferences (Azzara et al., 2001). In contrast, D1 and D2 antagonists blocked the expression of preferences conditioned by sham-fed sucrose but neither had any appreciable effect on acquisition (Yu et al., 2000a,b). However, when preferences were based on real-fed access to sucrose or fructose then D1 and D2 antagonists reduced both acquisition and expression of flavour preferences (Baker et al., 2003; Hsiao and Smith, 1995). Taken together these results suggest that dopamine antagonists can affect both the acquisition and expression of flavour preferences, but that the exact pattern of effects is moderated by the training procedure used.

One thing that all the existing studies of the pharmacology of flavour preferences have in common is that they have examined treatments thought to reduce the rewarding qualities of the relevant reinforcers. In contrast, there is a wealth of evidence that benzodiazepines can produce increased food intake as well as increased instrumental responding for food rewards and that they do so by enhancing the palatability or positive hedonic evaluation of such foods (for reviews see Berridge and Pecina, 1995; Cooper, 2005). In the light of the effects of dopamine antagonists, which affect both conditioned and unconditioned preferences, the fact that benzodiazepine agonists influence both consumption and palatability in unconditioned situations raises the possibility that they may also affect acquired preferences. In contrast, the fact that opioid antagonists reduce consumption without influencing the acquisition or expression of flavour preferences raises the possibility that there might be a similar dissociation in the effects of benzodiazepine agonists. Importantly, at least some of the effects of benzodiazepines on palatability are opioid dependent (e.g. Higgs and Cooper, 1997; Richardson et al., 2005). Therefore the current study examined the

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effects of the benzodiazepine agonist midazolam on the acquisition and expression of flavour preferences. Drug effects on the expression of acquired preferences were examined by training rats to associate the CS+ with the reinforcer and then treating them with the drug during two-bottle CS+ vs. CS− choice tests in the absence of the reinforcer. Drug effects on flavour preference acquisition were investigated by treating separate groups of rats with midazolam and saline throughout training and then comparing their performance during two-bottle CS+ vs. CS− choice tests in the absence of the reinforcer.

It was noted above that the effects of D1 and D2 antagonists on conditioned flavour preferences varied as a factor of whether these preferences were conditioned with nutrients or palatable tastes as the relevant reinforcer. This dissociation alone indicates that any investigation of the pharmacology of flavour preference learning should address flavour–nutrient and flavour–flavour learning separately. There is also considerable behavioural evidence for differences between preferences based on palatability and those based on nutrients. For example palatability-based preferences are not formed when there is a delay between the cue flavour and the palatable reinforcer whereas nutrient-based preferences can be formed across a delay (e.g. Holman, 1975; Sclafani and Ackroff, 1994). In view of the evidence for differences between nutrient- and palatability-based preferences two different reinforcers were used here: fructose, which supports preferences based on its sweet taste but not its post-ingestive consequences (Sclafani and Ackroff, 1994; Sclafani et al., 1993, 1999) and maltodextrin, a hydrolyzed starch consisting mainly of polysaccharides with small quantities of maltose and glucose, which does support preferences based on its post-ingestive consequences (Elizalde and Sclafani, 1990; Sclafani and Nissenbaum, 1988). Although maltodextrin does taste palatable to rats (Sclafani, 1987) there are several reasons to think that it does not actually support preferences based on its palatable taste alone. Inhibiting the digestion of starch prevents polycose (a commercially available form of maltodextrin) from supporting learned flavour preferences (Elizalde and Sclafani, 1988). Under sham-feeding conditions sucrose, but not polycose, will condition a preference for the CS+ and also that in animals fed ad libitum, 2% sucrose, but not 2% polycose will condition a preference for the CS+ (Bonacchi et al., in press). Taken together these results suggest that in the absence of post-ingestive nutrient effects maltodextrin is ineffective as a reinforcer in flavour preference conditioning. The idea that maltodextrin and fructose reinforcers rely on different mechanisms to condition flavour preferences is supported by the fact that Dwyer and Quirk (2008) found a dissociation between the acquisition of context-conditional flavour preferences when fructose and maltodextrin were used as the reinforcers. Thus the current studies examined the effects of midazolam on the acquisition and expression of flavour preferences as a factor of whether the reinforcer was orally consumed fructose or maltodextrin (see Table 1).

1. Experiment 1

In order to reduce the possibility that associations between the effects of the drug and the cue flavours could affect the preference for the CS+ over the CS− drug-trained animals received midazolam prior to each training session with both the CS+ and CS− (the control animals received saline injections). Free consumption of both the CS+ and CS− was allowed during training to confirm that treatment with midazolam does increase consumption in the current circumstances. Finally, because preferences based on palatability are not formed when there is a delay between presentation of the cue flavour and presentation of the reinforcer, the reinforcing solution (either fructose or maltodextrin) was presented in simultaneous compound with the CS+ flavour. Presenting the CS+ flavour in simultaneous compound with the reinforcing solution during training does mean that there will be a difference in its taste compared to the tests when the CS+ is presented alone. Although such a change between training and test can influence flavour preference learning in some situations it certainly does not prevent the observation of learnt flavour preferences both in my laboratory and elsewhere (e.g. Dwyer, 2005; Harris et al., 2004; Sclafani and Ackroff, 1994).

2. Method

2.1. Subjects, apparatus and drugs

All experimental procedures were conducted in accordance with the UK Animals Scientific Procedures Act 1986. Thirty-two naive male hooded Lister rats supplied by OLAC, Bicestor, UK were used in Experiment 1. All were housed in pairs under a 12 h/12 h light/dark cycle and had free feeding weights in the range 283–314 g at the start of the experiment. Prior to training the rats were placed on a restricted feeding schedule and their weight was allowed to drop to between 85 and 90% of their free feeding weights over a period of seven days. During training and testing the rats' food ration was adjusted to maintain their weights between these limits. This food ration was given in the home cages 1 h after the daily experimental session had been completed.

Training and testing took place in a separate experimental room that contained sixteen acrylic drinking boxes, 32×15×12 cm, with smooth flat flooring of the same material as the walls and wire mesh lids. Two 50-ml drinking bottles with a metal spouts could be inserted 8 cm apart at one end of each box. Consumption was assessed by weighing the bottles before and after each session. The cue flavours used in the experiment were deionised water flavoured with 0.05% (w/v) unsweetened grape and cherry Kool Aid (Kraft Foods USA, Rye Brook, NY, USA) and 0.1% (w/v) sodium saccharin (Sigma-Aldrich Chemie, Steinheim, Germany) was added to all cue flavours. The reinforcing solutions were 8% (w/v) maltodextrin (C*Dry MD 01904, Cerestar-UK, Manchester, UK. Note, this contains approximately 2% mono and di sugars with the remainder being polysaccharides) and 8% (w/v) fructose (Cerestar-UK, Manchester, UK). Midazolam was supplied by Hameln Pharmaceuticals (Gloucester, UK) as a 5 mg/ml solution. This was diluted with isotonic saline to concentrations of 0.3 mg/ml, 1 mg/ml, and 3 mg/ml.

2.2. Procedure

2.2.1. Preference training

All rats received three sessions of preliminary training, during each of which they were placed in the drinking cages for 30 min with unlimited access to 0.1% saccharin. Animals were run in two batches of 16, the first starting at 10 am and the second at 11 am. Following this pre-training the CS+ flavour was presented in simultaneous compound with the reinforcer on training days 1, 3, 5, and 7. On days 2, 4, 6, and 8 the CS− flavour was presented alone. On days 1, 4, 5, and 8 the bottle was presented on the left of the cage. On the other days it was on the right. The identity of the CS+ and CS− was counterbalanced between rats. For half of the animals the reinforcer was maltodextrin and for the remainder is was fructose. All sessions were 30 min long and rats were allowed unlimited access to the cue flavours. During this training period half of the animals trained with each reinforcer had received an intraperitoneal injection of 1 mg/kg midazolam 20 min before the start of each session with the remainder receiving saline injections at 1 ml/kg. There were thus four groups of eight animals in Experiment 1: maltodextrin control, maltodextrin drug-train, fructose control, and fructose drug-train.

2.2.2. Test

After a four-day interval following training all rats were tested for their preference for the CS+ after the administration of vehicle, 0.3, 1 and 3 mg/kg midazolam with injections 20 min before fluid access.

Table 1
Design of Experiments 1 and 2

Group	Train	Test
Drug train	4×CS+ and 4×CS− all 20 min after 1 mg/kg midazolam	CS+ v CS− in extinction, all tested under vehicle and midazolam (0.3–3 mg/kg).
Vehicle train	4×CS+ and 4×CS− all 20 min after vehicle	

Note: CSs are Kool Aid flavours, + is reinforcer (i.e. fructose or maltodextrin). Free access to the CS+ and CS− solutions was given throughout the training phase of Experiment 1. In Experiment 2 consumption was capped to 10 ml per session.

The order of administration was counterbalanced using a Latin square design. During each of the four test sessions animals had unlimited to access to both the CS+ and CS− and the identity of the solution presented on the left and right of the cage alternated over test days. There were two rest days between tests to allow drug clearance.

3. Results and discussion

3.1. Training consumption

Mean consumption per session for both CS+ and CS− during training is shown in Table 2. Except for the preference and acceptance ratios described below all of the statistical analyses were performed without any transformation of the data. Training consumption was analysed with a mixed ANOVA with between subjects factors of reinforcer type (fructose or maltodextrin) and drug training (control or drug trained) and a within subjects factor of CS. There were significant effects of CS [$F(1,28)=442.46$, $p<0.001$], reinforcer [$F(1,28)=47.27$, $p<0.001$], and drug training [$F(1,28)=14.51$, $p<0.001$]. This confirmed that rats consumed more of the CS+ than the CS−, that overall consumption was lower in the groups with fructose as the reinforcer and that overall consumption was higher in drug-trained rats. There was a significant CS×drug-train interaction [$F(1,28)=16.28$, $p<0.001$] and a simple main effects analysis revealed that consumption of the CS− was higher in drug trained than control groups [$F(1,28)=34.59$, $p<0.001$] but that there was no corresponding difference in consumption of the CS+ [$F<1$]. There was a significant CS×reinforcer interaction [$F(1,28)=20.82$, $p<0.001$]. Although consumption of the CS+ was greater than that of CS− in all groups this difference was greater in the rats trained with maltodextrin as the reinforcer. There was no significant reinforcer×drug-train [$F(1,28)=2.30$, $p=0.140$] or CS×reinforcer×drug-train interaction [$F(1,28)=2.93$, $p=0.098$]. The acceptance percentage for the CS+ was calculated as 100× consumption of the CS+ divided by total consumption (see Table 2). Analysis of these data revealed an effect of drug training [$F(1,28)=26.82$, $p<0.001$] but no effect of US type [$F(1,28)=2.21$, $p=0.158$] or interaction [$F(1,28)=2.22$, $p=0.149$]. This is consistent with the analysis of total training consumption in indicating that the acceptance percentage for the CS+ was reduced in drug trained rats as a result of the extra CS− consumption. In summary, consumption was higher in the groups for which maltodextrin was the reinforcer and 1 mg/kg midazolam had a facilitatory effect on CS− consumption but not CS+ consumption (although any effect on CS+ sessions may have been masked by ceiling effects).

3.2. Test consumption

Mean consumption of both CS+ and CS− during testing is shown in Fig. 1 for all doses of midazolam. Test consumption was analysed with a mixed ANOVA with between subjects factors of reinforcer type (maltodextrin or fructose) and drug training (control or drug trained) and a within subjects factors of CS and drug dose (vehicle, 0.3, 1 and 3 mg/kg). There were significant effects of CS [$F(1,28)=281.48$, $p<0.001$] and drug dose [$F(3,84)=14.44$, $p<0.001$] but not reinforcer or drug training [$Fs<1$]. This confirmed that rats consumed more of the CS+ than the CS−, but that there was no difference in overall consumption between groups with maltodextrin and fructose as the reinforcer nor any overall effect of drug training on test consumption. A contrast analysis of the effect of drug dose revealed that consumption was higher at all doses of the midazolam than it was after vehicle treatment [lowest $F(1,28)=11.27$, $p=0.002$]. There was a significant CS×reinforcer interaction [$F(1,28)=6.25$, $p=0.019$]. Although consumption of the CS+ was greater than that of CS− in all groups this difference was greater in the rats trained with fructose as the reinforcer. There was a CS×drug dose interaction [$F(3,84)=2.97$, $p=0.037$] and a contrast analysis revealed that the magnitude of the difference in CS+ and CS− consumption was higher at after all doses of midazolam than it was after saline control [lowest $F(1,28)=5.97$, $p=0.021$]. There was a significant CS×reinforcer×drug-train interaction [$F(1,28)=4.49$, $p=0.043$]: When fructose was the reinforcer the magnitude of the difference between CS+ and CS− consumption was lower in drug-trained rats than it was in control rats [$F(1,28)=8.97$, $p=0.006$] but this was not the case when maltodextrin was the reinforcer [$F<1$]. There were no other significant interactions [highest $F(1,28)=2.91$, $p=0.099$ for the CS×drug-train interaction].

The percentage preference for the CS+ during test is also shown in Fig. 1. Analysis of these data revealed a significant effect of reinforcer type [$F(1,28)=9.10$, $p=0.005$] which confirms the analysis of raw consumption in indicating that the preference for the CS+ was higher overall in the fructose trained animals. There was also a significant interaction between reinforcer type and drug training [$F(1,28)=6.80$, $p=0.014$]. Simple effect analyses revealed that there was a significant effect of drug training when fructose was the reinforcer [$F(1,28)=9.17$, $p=0.005$] but not when the reinforcer was maltodextrin [$F<1$]. This confirms that that the size of the CS+ preference was reduced by training under the influence of midazolam when the reinforcer was fructose but not when it was maltodextrin. There was no effect of dose [$F<1$] indicating that midazolam did not affect the relative preference for the CS+ over the CS−. The fact that the analysis of raw consumption indicated that midazolam resulted in a greater difference in consumption between the CS+ and CS− simply reflects the fact that the greater consumption overall in the midazolam-treated animals on test would be most obvious in the solution for which they had an existing preference. There were no

Table 2
Mean consumption per session of CS+ and CS− (with SEM), along with the acceptance percentage for the CS+, during flavour preference training for Experiments 1 and 2

		Consumption (g)	SEM	CS+ acceptance percentage	SEM
Experiment 1					
Fructose					
Control	CS+	14.2	0.7	70.2	2.4
	CS−	6.2	0.8		
Drug train	CS+	14.4	0.6	63.3	1.8
	CS−	8.4	0.6		
Maltodextrin					
Control	CS+	19.8	0.7	75.7	2.0
	CS−	6.4	0.6		
Drug train	CS+	20.2	0.9	63.2	0.9
	CS−	11.7	0.6		
Experiment 2					
Fructose					
Control	CS+	9.5	0.2	58.2	3.6
	CS−	7.1	0.9		
Drug train	CS+	9.9	0.2	55.2	1.9
	CS−	8.1	0.5		
Maltodextrin					
Control	CS+	9.4	0.2	55.8	3.0
	CS−	7.7	0.9		
Drug train	CS+	9.9	0.1	55.6	2.2
	CS−	8.2	0.7		

Note: Fructose and maltodextrin refer to the reinforcing solution mixed with the CS+ during training.

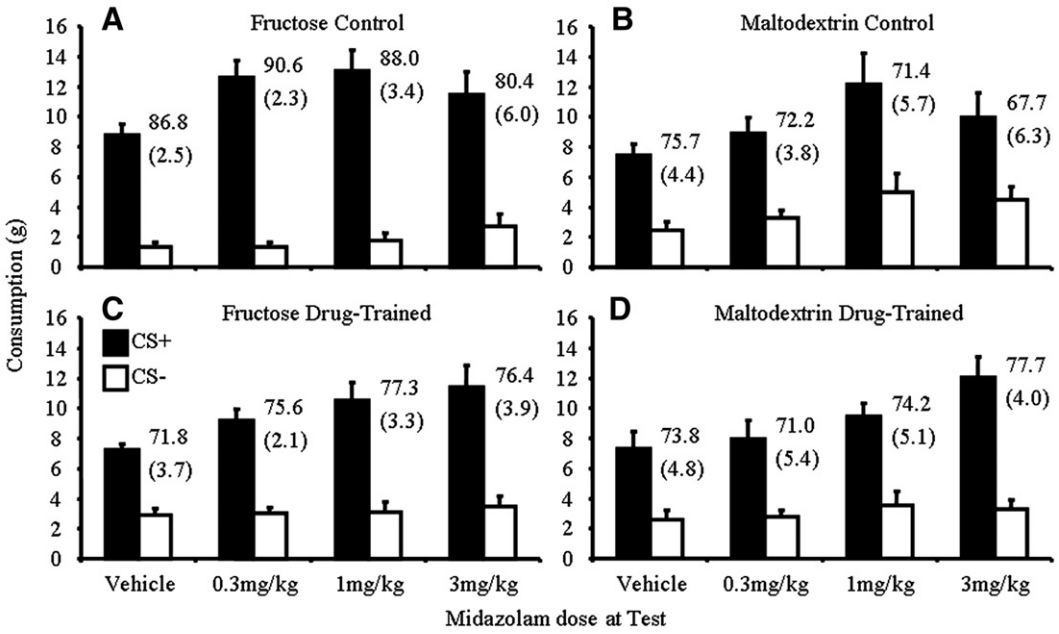

Fig. 1. Reports the mean consumption (with SEM) from Experiment 1 as a function of both cue type (black bars=CS+, white bars=CS−) and midazolam dose at test: Panels A and B show the data from animals given vehicle injections during training while Panels C and D show the data from animals given 1 mg/kg midazolam during training. Panels A and C show the data from animals trained with fructose as the US while Panels B and D show the data from animals trained with maltodextrin as the US. Numbers beside each pair of bars report the mean percentage preference for the CS+ (with SEM).

other significant main effects or interactions [highest $F(1,28)=2.80$, $p=0.105$ for the reinforcer by drug-train interaction].

In summary, there was a general preference for the CS+ over the CS− in all groups although this was higher in those trained with fructose as the reinforcer. During test midazolam had general facilitatory effect on consumption. This general increase in consumption produced by midazolam acted to increase the consumption of the CS+ more than that of the CS−. However, this does not appear to have produced a greater preference for the CS+ as there was no effect of midazolam on the percentage preference for the CS+. The effects of midazolam during training depended on the nature of the reinforcer: when fructose was the reinforcer giving midazolam during training produced an increase in CS− consumption during test but no such effect was seen with maltodextrin as the reinforcer. This extra CS− consumption reduced the percentage preference for the CS+ in the fructose trained animals, while when maltodextrin was the reinforcer giving midazolam during training had little effect on the preference for the CS+ on test. In addition, consumption of the CS+ fructose was lower than the CS+ maltodextrin during training. This may reflect the fact that the CS+ maltodextrin contained saccharin and the combination of sweet and maltodextrin flavours is especially rewarding for rats (e.g. Ackroff et al., 1993; Sclafani et al., 1998).

4. Experiment 2

In Experiment 1 free consumption of both the CS+ and CS− was allowed during training. This resulted in differential exposure to the cue solutions prior to test with greater overall consumption in the animals trained with maltodextrin compared to those trained with fructose as well as greater consumption in the drug-trained animals as compared to the control animals. Unsurprisingly there was also greater consumption of the CS+ compared to the CS− and the size of this difference varied as a factor of drug exposure and reinforcer type. Thus it is at least possible that the effects of drug training and/or reinforcer type observed in Experiment 1 were in fact caused by differential exposure to the cue solutions rather than directly caused by the relevant manipulations themselves. In order to reduce the differences cue exposure during training only 10 ml of solution was presented to the animals on each training day in Experiment 2.

5. Method

All details of Experiment 2 were the same as described for Experiment 1 with one exception: During the training phase of Experiment 2 access to the CS+ and CS− solutions was capped by providing only 10 ml of solution on each training session. The rats used had free feeding weights in the range 281–310 g at the start of the experiment. One animal from Group Fructose Control was removed from the analysis because it did not consume any fluids during the final test session.

6. Results and discussion

6.1. Training consumption

Mean consumption per session for both CS+ and CS− during training is shown in Table 2: Inspection of that table reveals that rats consumed essentially all of the 10 ml available of the CS+ on each session regardless of condition but that consumption of the CS− was not maximal across training. Training consumption was analysed as in Experiment 1. There was a significant effect of CS [$F(1,27)=21.22$, $p<0.001$] and the effect of drug training approached significance [$F(1,27)=3.11$, $p=0.089$]. This confirmed that rats consumed more of the CS+ than the CS−. Possibly as a result of the consumption cap there was only a trend towards greater overall consumption in the drug-trained groups. There were no other significant main effects or interactions [all Fs<1]. The acceptance percentage for the CS+ was calculated as in Experiment 1 and these data are shown in Table 2. There were no significant effects of drug training, US type or interaction between them [all Fs<1]. In summary, capping the amount of solution available during training to 10 ml/session reduced, but did not totally prevent, the differential consumption of CS+ and CS− during training. However, this treatment did remove the differences between animals trained with fructose and maltodextrin as the reinforcer in overall consumption and greatly reduced the tendency

for the drug-trained animals to consume more during the training period (Table 2).

6.2. Test consumption

Mean consumption of both CS+ and CS− during testing is shown in Fig. 2 for all doses of midazolam. Test consumption was analysed as in Experiment 1. There were significant effects of CS [$F(1,27)=261.90$, $p<0.001$] and drug dose [$F(3,81)=59.64$, $p<0.001$] but not of drug training [$F<1$]. The effect of reinforcer approached the standard level of significance [$F(1,27)=3.92$, $p=0.058$]. This confirmed that rats consumed more of the CS+ than the CS−, but that there was no overall effect of drug training on test consumption. Unlike in Experiment 1 there was a trend towards lower overall consumption in groups trained with fructose as the reinforcer. A contrast analysis of the effect of drug dose revealed that consumption was higher at all doses of the midazolam than it was after vehicle treatment [lowest $F(1,27)=10.41$, $p=0.003$]. Unlike in Experiment 1 there was no CS×reinforcer interaction [$F(1,27)=1.07$, $p=0.310$] indicating that the preference for the CS+ was not affected by reinforcer type. There was a CS×drug dose interaction [$F(3,81)=4.83$, $p=0.004$] a contrast analysis revealed that the magnitude of the difference in CS+ and CS− consumption was higher at after the 0.3 mg/kg and 3 mg/kg doses of midazolam than it was after saline control [lowest $F(1,27)=5.75$, $p=0.024$] although the difference was not significant after the 1 mg/kg dose [$F(1,27)=2.33$, $p=0.138$]. There was a significant CS×reinforcer×drug-train interaction [$F(1,27)=4.72$, $p=0.039$]: When fructose was the reinforcer the magnitude of the difference between CS+ and CS− consumption was lower in drug-trained rats than it was in control rats [$F(1,27)=5.13$, $p=0.032$] but this was not true when maltodextrin was the reinforcer [$F<1$]. There were no other significant interactions [highest $F(3,81)=1.6$, $p=0.196$ for the dose×reinforcer interaction].

The percentage preference for the CS+ during test is also shown in Fig. 2. Unlike in Experiment 1 there was no significant effect of reinforcer type [$F<1$] which confirms the analysis of raw consumption in indicating that in this experiment both reinforcers produced the same CS+ preference. As in Experiment 1 there was a significant interaction between reinforcer type and drug training [$F(1,27)=6.84$, $p=0.014$]. Simple effect analyses revealed that there was a significant effect of drug training when fructose was the reinforcer [$F(1,27)=7.65$, $p=0.010$] but not when the reinforcer was maltodextrin [$F<1$]. This confirms that that the size of the CS+ preference was reduced by training under the influence of midazolam when the reinforcer was fructose but not when it was maltodextrin. There was no effect of dose [$F(3,81)=1.65$, $p=0.384$] indicating that midazolam did not affect the relative preference for the CS+ over the CS−. As in Experiment 1 the fact that the analysis of raw consumption indicated that some doses of midazolam resulted in a greater difference in consumption between the CS+ and CS− simply reflects the fact that the greater consumption overall in the midazolam-treated animals on test would be most obvious in the solution for which they had an existing preference. There were no other significant main effects or interactions [highest $F(1,28)=1.86$, $p=0.184$ for main effect of drug-train].

In summary, as in Experiment 1 there was a preference for the CS+ over the CS− in all groups although unlike in Experiment 1 the size of this effect was not influenced by the nature of the reinforcer. Again, midazolam had general facilitatory effect on test consumption. This general increase in consumption produced by midazolam acted to increase the consumption of both the CS+ and the CS− (albeit that CS+ consumption increased more). However, this does not appear to have produced a greater preference for the CS+ as there was no effect of midazolam on the percentage preference for the CS+. As in Experiment 1 the effects of midazolam during training depended on the nature of the reinforcer: when fructose was the reinforcer giving midazolam during training produced an increase in CS− consumption during test but no such effect was seen with maltodextrin as the reinforcer. This extra CS− consumption reduced the CS+ preference in the fructose trained animals, while when maltodextrin was the reinforcer giving midazolam during training had little effect on the preference for the CS+ on test. Therefore capping the consumption during training to 10 ml/session reduced the overall differences between reinforcer types but did not remove the interaction between drug training and reinforcer type: the CS+ preference was reduced by training under midazolam only when the reinforcer was fructose.

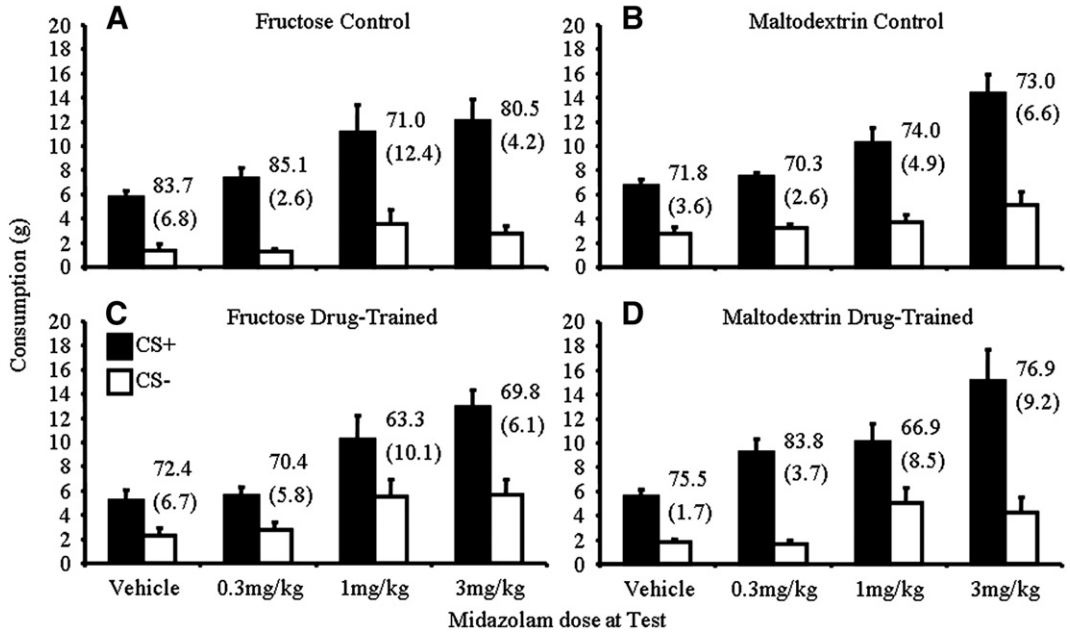

Fig. 2. Reports the mean consumption (with SEM) from Experiment 2 as a function of both cue type (black bars=CS+, white bars=CS−) and midazolam dose at test: Panels A and B show the data from animals given vehicle injections during training while Panels C and D show the data from animals given 1 mg/kg midazolam during training. Panels A and C show the data from animals trained with fructose as the US while Panels B and D show the data from animals trained with maltodextrin as the US. Numbers beside each pair of bars report the mean percentage preference for the CS+ (with SEM).

7. General discussion

The present findings confirm prior reports that rats learn to prefer flavours paired with either orally consumed fructose or maltodextrin. The preference in the fructose trained animals can be attributed to the formation of an association between the CS+ flavour and the sweet taste of fructose because previous studies have shown that fructose has very weak (if any) post-ingestive reinforcing effects (Sclafani and Ackroff, 1994; Sclafani et al., 1993, 1999). The preference in the maltodextrin-trained animals can be attributed to the formation of an association between the CS+ flavour and the post-ingestive effects of the nutrients contained in maltodextrin. Previous studies have shown that maltodextrin has very weak (if any) reinforcing effect on the basis of its taste alone when nutrient effects are minimised (Elizalde and Sclafani, 1988; Bonacchi et al., in press). The new findings are that treatment with midazolam had no effect on the acquisition of preferences based on maltodextrin and actually attenuated (but did not prevent) preferences based on fructose. In addition, midazolam treatment had minimal detectable effects on the expression of learned flavour preferences based on either fructose or maltodextrin in terms of the relative amount of the CS+ and CS− consumed during 2-bottle tests. The absolute size of the difference in consumption of CS+ and CS− flavours was affected by treatment with midazolam but this appears to reflect the fact that midazolam produced a general increase in consumption during test. In addition, midazolam treatment during training increased consumption of the CS− flavour, but not consumption of the CS+ flavour. These results reflect those obtained with manipulations of the opioid system which also produced little or no evidence for impact on the acquisition or expression of preferences despite large effects on unconditioned consumption (e.g. Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999). The similarity between the results obtained with opioid and benzodiazepine manipulations is not particularly surprising in light of the fact that some of the effects of the benzodiazepines on palatability are opioid dependant (e.g. Higgs and Cooper, 1997; Richardson et al., 2005).

Although the current experiments have so far been discussed entirely in terms of flavour preferences the designs used allow the possibility that other effects might have contributed to the observed results. It is well documented that when rats are initially exposed to a high concentration of a palatable substance before being switched to a low concentration they show a marked decrease in consumption and will consume less in the first few post-switch trials than control animals that have only ever received the low concentration (this is referred to as successive negative contrast, for reviews see Flaherty, 1982; 1996). The fact that the CS+ flavour was presented in simultaneous compound with the reinforcer during training before being presented alone during test is similar to the negative contrast situation and although there is no evidence that consumption of the CS+ is lower than that of the CS− (which would be a direct demonstration of negative contrast) it is possible that contrast effects might have reduced the preference for the CS+. If so, it would be expected that the magnitude of the CS+ preference should actually increase over non-reinforced test trials as the impact of the switch from high to low concentrations dissipates. The current study does not possess enough power to examine this possibility independently of drug dose during test but previous analyses of the extinction of flavour preferences do not typically show an increase in preference over time (e.g. Harris et al., 2004; Sclafani and Ackroff, 1994). In addition, when the test data from the current experiments are collapsed over drug dose to allow for an analysis of the effect of test session there is no suggestion that the CS+ preference is lower on the first test than on subsequent tests (details of this analysis are available from the author on request). It has also been shown that anxiolytic compounds, including benzodiazepines, can reduce the degree of negative contrast when given after the switch to the low concentration (for a review see Flaherty, 1990). Although there is no direct evidence for contrast effects in the current study it is at least possible that such effects were present and they may have been attenuated by the administration of midazolam during test with the effect of increasing the apparent CS+ preference. However, midazolam given during test did not in fact influence the relative preference for the CS+ over the CS− here although it should be noted that these experiments were not designed with sufficient power to detect an interaction between test session and drug dose on test.

Another possible factor in the current experiments is that the drug-trained animals would have received pairings of the CS flavours with the effects of midazolam thus allowing for direct CS to drug associations to form. The current experiments were designed to minimise such conditioning by presenting the drug before each day's training session began. In addition, midazolam was presented with both the CS+ and the CS− which should prevent any conditioning to the effects of the drug from influencing the relative preference for the CS+ over the CS−. Moreover, previous examination of the effects of benzodiazepines as reinforcers in flavour conditioning designs have actually shown that they condition aversions (e.g. Parker et al., 1998) but there was no evidence that training under midazolam produced a general decrease in consumption during test as might be expected if a taste aversion had been created. Finally, the contrast between the effects of midazolam during training and test is confounded in the current studies by the fact that only the drug-trained animals had experienced midazolam prior to test allowing for the possibility that tolerance to the effects of the drug might have affected their test responding. This would be particularly serious had there been an interaction between the effects of giving midazolam during training and during test, but no such interactions were observed here.

In an apparent contrast to the present findings several studies have reported that benzodiazepine agonists selectively enhance the preference for palatable over non-palatable foods and fluids as opposed to having very general effects on consumption (for reviews see Berridge and Pecina, 1995; Cooper, 2005). This contrast might be taken to suggest that different neurochemical systems mediate the expression of learned and unlearned flavour preferences but there is an important methodological difference between these studies and those reported here that questions such a conclusion. For example, Cooper and Yerbury (1988; see also Parker, 1991) report that benzodiazepine agonists enhance the preference for saccharin over water and attribute this to the selective increase in the palatability of the already preferred saccharin without a change in the palatability of water. However, those experiments only gave a choice between one sweet and one neutral solution while in the current experiments the choice was between two solutions that were both sweetened with saccharin. It is thus possible that in the current experiments the palatability of both the CS+ and the CS− was enhanced by treatment with midazolam during test which would explain why there was no selective effect of the drug on the preference for the CS+ seen here. If true, this would indicate that while benzodiazepine agonists increase the preference for a preferred solution over a neutral solution they would not have such a selective effect if the choice were between two already palatable solutions (for example 8% and 16% fructose). In line with this prediction, Higgs and Cooper (1998) used brief-access tests to show that midazolam increased the palatability of several different concentrations of either sucrose or maltodextrin and that the increase in palatability was similar at all concentrations.

The fact that treatment with midazolam only increased consumption of the CS− solution and had no effect on the consumption of the CS+ and reinforcer compound during training in Experiment 1 appears to contradict the fact that previous studies have shown that benzodiazepines selectively enhance the consumption by animals of palatable foods and fluids much more than that of ordinary chow or water (for reviews see Berridge and Pecina, 1995; Cooper, 2005). Again, it should be noted that these studies have typically compared palatable flavours with neutral flavours rather than comparing the

consumption of differently palatable flavours. Here the CS− is actually a flavoured saccharin solution and is thus a palatable flavour itself, so the fact that midazolam increased the consumption of the CS− during one-bottle training is not surprising. With respect to the fact that treatment with midazolam had little effect on the consumption of the CS+ solution perhaps the most obvious possibility is that the animals' consumption had already reached maximal levels in the control group and so there was no room for any increase to be seen. This ceiling effect might be a physical limit but more probably reflects other mechanisms. When the consumption of carbohydrate solutions is examined as a factor of concentration it is typically observed that while increasing concentration initially results in an increase in consumption maximal levels of consumption are actually seen at intermediate concentrations (e.g. Davis, 1996; Dwyer, 2008; Nissenbaum and Sclafani, 1987; Spector et al., 1998). This inverted-U shaped relationship between consumption and concentration does not appear to be a factor of higher concentrations being less palatable than intermediate concentrations because studies of licking microstructure indicate that the palatability of more concentrated solutions is actually higher than that of less concentrated solutions despite the lower levels of absolute consumption (e.g. Davis, 1996; Davis and Smith, 1992; Spector et al., 1998). So while midazolam may have enhanced the palatability of the CS+ this would not be reflected in additional consumption because the concentration of the reinforcer (either fructose or maltodextrin) was such that it already supported maximal levels of consumption.

Perhaps the most surprising outcome from the current studies was that treatment with midazolam during training attenuated preference for the CS+ during subsequent tests when the reinforcer was fructose, but not when it was maltodextrin. Given the evidence that benzodiazepines actually increase palatability, and the fact that fructose but not maltodextrin supports flavour preferences on the basis of palatability, this seems a rather counterintuitive result. One possible explanation relies on the fact that midazolam was given before all training sessions and thus it should have acted to increase the palatability of the flavoured saccharin solution that served as the CS−. So in the drug-trained animals the CS− flavour was actually paired with saccharin which had its palatability enhanced by midazolam. Of course the same would happen on CS+ trials. It should also be noted that saccharin alone can support flavour preference conditioning when paired with a neutral CS flavour (e.g. Holman, 1975). Because both the CS+ and CS− flavours contained saccharin the preference test actually reflects a choice between a flavour previously paired with particularly palatable fructose (plus saccharin) and a flavour paired with palatable saccharin. So the degree of preference for the CS+ during test should reflect the relative palatability of the tastes with which the CS+ and CS− were paired (as well as the association between the CS+ and the reinforcer). If it is assumed that enhancing the palatability of saccharin by treatment with midazolam reduced the difference in relative palatability of saccharin and fructose then this could explain the fact that drug-trained animals showed a smaller preference during test. But why should the same effect not be present in the maltodextrin-trained animals? It should be remembered that it is the nutrient content of maltodextrin rather than its palatability that is effective as a reinforcer in flavour preference conditioning. As discussed above, benzodiazepines specifically enhance the positive evaluation of tastes without modulating motivational state variables directly (for reviews see Berridge and Pecina, 1995; Cooper, 2005) and so should not have any influence on the post-ingestive reinforcing effects of nutrients. In maltodextrin-trained animals the choice on test is between the CS+ which was previously paired with nutrients and the CS− which was not. If midazolam does not influence the rewarding qualities of nutrients there is no reason to expect that drug-trained animals would differ from controls. Of course the palatability of the saccharin paired with both the CS+ and CS− would have been enhanced by training with midazolam but this would not affect the CS+ preference because it should be similarly enhanced for both solutions.

The preceding analysis should not be taken as arguing that the modulation of palatability by benzodiazepine-dependant mechanisms is unrelated to the acquisition of flavour preferences. Indeed, if the reduction in the size of palatability-based preferences in drug-trained animals is the result of midazolam increasing the preference for the CS− flavour then the current results are actually consistent with the idea that enhancing the palatability of a flavour with benzodiazepine treatment could create an enduring preference for that flavour, although this should be confirmed empirically. Similarly, the fact that testing under midazolam did not affect the preference for the CS+ but did increase overall consumption should not be taken as evidence that modulation of the benzodiazepine system does not affect the hedonic evaluation of flavours that have undergone conditioning in the same way that it affects unconditioned palatability reactions. Instead, the current results are consistent with the idea that midazolam increased the palatability of both the CS+ and the CS− thus leaving their relative values unchanged.

In summary, the present experiments demonstrated that the benzodiazepine agonist midazolam had minimal effects on the expression of flavour preferences conditioned by either fructose or maltodextrin. Treatment with midazolam during training did suppress the acquisition of flavour preferences conditioned fructose but not maltodextrin. Despite not having a selective effect on the CS+ flavour midazolam did increase the total intake of the saccharin-sweetened CS solutions during test, which is consistent with prior findings obtained with unflavoured saccharin solutions. These findings indicate that increasing activity in the benzodiazepine system does not influence the expression of learnt flavour preferences although they are consistent with previous work demonstrating that benzodiazepine agonists increase consumption by enhancing palatability.

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