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ORIGINAL INVESTIGATION

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Evidence for early opioid modulation of licking responses to sucrose and Intralipid: a microstructural analysis in the rat

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Abstract The behavioural mechanisms underlying the effects of the opioid antagonist naloxone (0.3–3 mg/kg IP), and the opioid agonists morphine (0.3–3 mg/kg SC), and U-50, 488H (0.3–3 mg/kg SC) on ingestive behaviour were investigated using a microstructural analysis of licking patterns for sucrose solutions and Intralipid (fat emulsions) in a brief contact test. Naloxone dose-dependently decreased the total number of licks and the number of bouts for sucrose and Intralipid, but did not affect mean bout duration. Morphine dose-dependently increased the total number of licks and the number of bouts for both test fluids. For Intralipid but not for sucrose drinking, morphine actually decreased mean bout duration. U-50, 488H significantly affected total licks, although the dose-effect relationship showed an inverted U-shaped function. There was a dose-dependent increase in mean bout duration following administration of U-50, 488H and an increase in bout number, although only the lowest dose differed significantly from the control condition. The results show that microstructural analysis can distinguish between the effects of naloxone, morphine and U-50, 488H on licking behaviour and indicate that selective opioid receptor subtypes may be differentially involved in ingestive processes.

Key words Mu-opioid · Kappa-opioid · Licking behaviour · Bout structure · Incentive salience · Palatability · Rat

Introduction

There is considerable agreement that drug actions at central opioid receptors influence ingestive behaviour (Cooper et al. 1988). Thus, opioid receptor agonists have been shown to increase food consumption under a variety of experimental conditions (Sanger and McCarthy 1981; Morley et al. 1982; Gosnell et al. 1983; Levine and Billington 1989), while opioid receptor antagonists reduce food consumption (Holtzman 1974, 1975; Cooper 1980; Apfelbaum and Mandenoff 1981; Levine et al. 1982). In recent pharmacological work, emphasis has been placed on evaluating the relative contributions of specific receptor subtypes in mediating opioid effects on ingestive responses (Bodnar 1996; Gosnell and Levine 1996; Kelley et al. 1996). Great effort has also been expended in attempts to identify the central sites of action of opioids on feeding behaviour (Bakshi and Kelley 1993a,b; Gosnell and Levine 1996; Kelley et al. 1996; Kotz et al. 1997; Zhang and Kelley 1997). However, despite these considerable advances, details of the behavioural changes that underlie opioid-induced changes in food consumption remain undefined.

One prominent hypothesis is that opioids modify the palatability or hedonic evaluation of food-related stimuli (e.g. Cooper and Kirkham 1993). In support of this, opioid antagonists have been found to reduce or abolish both sweet (Le Magnen et al. 1980; Cooper 1983; Lynch and Libby 1983), and salt taste preferences (Cooper and Gilbert 1984), and to reduce food preference (Cooper and Turkish 1989). Conversely, opioid agonists have been reported to enhance taste acceptance and preference (Calcagnetti and Reid 1983; Bertino and Abelson 1988; Gosnell and Majchrzak 1989). Furthermore, opioid antagonists reduce sucrose sham feeding in satiated rats (Rockwood and Reid 1982; Kirkham and Cooper 1988a,b; Kirkham 1990), indicating a possible effect on palatability factors. Some taste reactivity studies also reinforce the view that there is opioid modulation of palatability. For example, morphine has been shown to enhance the positive hedonic responses elicited by intraoral infusions of sucrose solutions (Doyle et al. 1993; Pecina and Berridge 1995;

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Rideout and Parker 1996), and naltrexone to diminish them (Parker et al. 1992). These data imply that opioids can modify the positive evaluation of sweet taste stimuli.

An alternate view is that opioids affect food ingestion through a change in motivation. Recently, Cleary and colleagues (1996) showed that in rats trained under a progressive reinforcement schedule, naloxone dose-dependently reduced the motivation to respond for various concentrations of sucrose. Work from the same laboratory has also ruled out the possibility that the anorectic effect of naloxone is due to a change in the discrimination of sweet taste (O'Hare et al. 1997).

There is good evidence that insight into the behavioural processes underlying ingestive responding can be gained by studying the microstructural characteristics of licking in the rat (Davis 1973; Davis and Smith 1992; Davis and Perez 1993). This approach has been fruitfully employed to investigate the effects on ingestion of the dopamine D_2 antagonist raclopride (Schneider et al. 1990), serotonergic (fenfluramine and fluoxetine), and catecholaminergic drugs (amphetamine and phenylpropanolamine) (Asin et al. 1992), the neuropeptides bombesin and cholecystokinin (Hsiao and Spencer 1983; Moran et al. 1996), and the benzodiazepine agonist midazolam (Higgs and Cooper 1997,1998). In the present studies, our aim was to investigate for the first time the microstructural features of the effects of opioid receptor ligands on licking in non-deprived rats. Testing was limited to 60 s per fluid concentration to reduce postingestional factors, and to focus attention on the early stages of ingestion where palatability factors are postulated to be maximally effective. The responses to several concentrations of test fluid were measured within the same session, and presentation of the stimuli was separated by short intertrial intervals. This method has advantages over other tests using longer intertrial intervals because it allows licking patterns to a range of fluid concentrations to be determined rapidly within a short period.

Because previous work on licking responses in other laboratories has almost exclusively examined drug effects on carbohydrate consumption, we included comparisons between licking patterns for a sweet tasting carbohydrate solution (sucrose) and a fat emulsion (Intralipid). In addition, because there is evidence that selective opioid receptor subtype agonists and antagonists may differentially affect ingestive responding (see Bodnar 1996 for reviews; Gosnell and Levine 1996), we also examined the effect of three different opioid receptor ligands on licking. Experiment 1 investigated the effect of the relatively nonselective opioid antagonist naloxone on licking for sucrose and Intralipid. In experiment 2, the effect of the μ opioid agonist morphine on licking for sucrose and Intralipid was examined. Morphine was chosen as an example of an opioid ligand that stimulates ingestion, as opposed to having an anorectic effect, to examine bidirectional control of ingestion via action at opioid receptors. Finally, the effect of the selective κ agonist U-50, 488H on licking for sucrose was also investigated. The reason for this was that there is some evidence to suggest that different mechanisms may be responsible for the effects of μ and κ ligands on feeding (e.g. Noel and Wise 1993; Zhang and Kelley 1997). In addition, the number of sucrose concentrations was extended in experiment 3 to test for the possibility that any drug effects were concentration-dependent.

Materials and methods

Subjects

Fifty-two non-deprived adult male hooded Lister rats (Charles River, UK) weighing 300–400 g at the beginning of training were used. They were housed in pairs in plastic cages in a room with a constant temperature of 21 ± 2°C, and were maintained under a 12-h light/dark cycle (lights on at 0600 hours). Rats were allowed ad lib access to food pellets [Special Diet Services, RM1(E), Cambridge, UK] and water, except during testing. All testing was carried out in the light phase. The experiments were carried out in accordance with the terms of the Animals (Experimental Procedures) Act, 1986, under licence from the UK Home Office.

Drugs

Naloxone hydrochloride, at doses of 0.3, 1, and 3 mg/kg (Sterling Winthrop, UK) was dissolved in distilled water and injected intraperitoneally (IP) 20 min before testing. This dose range was used because previous studies have demonstrated a decrease in food intake in non-deprived rats using similar doses (Cooper 1980). Morphine sulphate (Macfarland Smith, Edinburgh, UK) was dissolved in distilled water and injected subcutaneously (SC), 60 min before experimentation. The doses were 0.3, 1, and 3 mg/kg morphine or its vehicle, shown previously to stimulate intake in non-deprived rats (Sanger and McCarthy 1980). An injection-test period of 60 min was used because morphine produces an initial decrease in food intake up to 60 min following injection, probably due to side-effects of the drug (Leshem 1984). Increases in intake are then observed once the initial sedative effect has worn off. The κ opioid agonist U-50, 488H (Sigma, Poole, UK) (trans-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate) was dissolved in distilled water and injected SC, 30 min before testing. The doses were 0.3, 1, and 3 mg/kg, and have been shown to enhance intake in non-deprived rats (Jackson and Cooper 1986). All drugs were injected in a volume of 1 ml/kg.

Test meal

In experiments 1 and 2, rats had access to three concentrations of sucrose (1, 3, and 10%) (granulated cane sugar, Tate and Lyle, London, UK), or three concentrations of Intralipid (1, 3, and 10%) (Pharmacia Ltd, Milton Keynes, UK) which were made up freshly each day. In experiment 3, rats had access to four concentrations of sucrose (1, 3, 10, and 30%) which were also made up freshly each day. Intralipid consists of fractionated soya bean oil, fractionated egg phospholipids and glycerol. The Intralipid emulsions were made by diluting a 20% commercial preparation with tap water, and the sucrose solutions were made up weight/volume each day using tap water. The number of kcal available was 100 kcal per 100 ml for a 10% emulsion of Intralipid, and 39 kcal per 100 ml

for a 10% sucrose solution. The concentrations were chosen to stimulate a range of sampling, from low (approximately 50 licks) to just below asymptotic levels (approximately 300 licks) and were based on pilot data.

Apparatus

Testing was carried out using an MS80 multistation lick analysis system (Dilog Instruments, Tallahassee, Florida, USA) described in detail previously (Higgs and Cooper 1997). Briefly, rats were placed in a Perspex chamber that had an opening in the centre of the front wall allowing access to a drinking spout. Bottles containing the fluids were mounted in a line on a metal platform that could be moved backward and forward by a reversible motor. This enabled several concentrations to be presented within the same test session. The lickometer was connected to an amplifier that passed less than 60 nA through the rat every time tongue contact was made with the tube. This current was fed to a computer (Opus Technology, Surrey, UK) that stored the time of each tongue contact to the nearest ms.

Procedure

Training

Three groups of ten rats and two groups of 11 rats underwent training. The groups were well familiarized with the test apparatus and procedure. This involved placing each rat in the test chamber where they had access to a range of sucrose or Intralipid concentrations presented in a random order to avoid the influence of contrast effects. Each concentration was presented once only in a session for 60 s and there was a 10-s intertrial interval. Therefore, in each daily session, rats were exposed to three or four 60-s presentations, each of which was separated by 10-s. A trial did not start until the rat made its first lick. This procedure continued until steady baseline levels of licking (measured as total number of licks) were observed across days and the latency to initiate licking did not exceed 30 s (approximately 10 days). Two days before testing each rat received a sham injection of distilled water to familiarize it with the injection procedure.

Testing

Following the familiarization period, rats received injections of naloxone (0.3, 1, and 3 mg/kg), morphine (0.3, 1, and 3 mg/kg), U-50, 488H (0.3, 1, and 3 mg/kg) or the appropriate vehicle. After injection, the rats were placed in the lickometer chamber where they had access to all concentrations of the fluid with which they had been trained. The testing procedure was identical to that used during training. Half of the naloxone-treated rats had access to sucrose ($n = 10$), and the other half had access to Intralipid ($n = 11$). Similarly, half of the morphine-treated rats had access to sucrose ($n = 10$) and the other half had access to Intralipid ($n = 11$). The U-50, 488H-treated rats ($n = 10$) only had access to sucrose.

A repeated-measures design was used in which each rat was tested at every dose. The order of injections was randomised and 48 h elapsed between treatments to avoid carry-over effects. Therefore, each animal was exposed to a total of four test sessions, where a session comprised 60-s access to three or four fluid concentrations (with which they had been trained), presented in a random order and separated by short intervals.

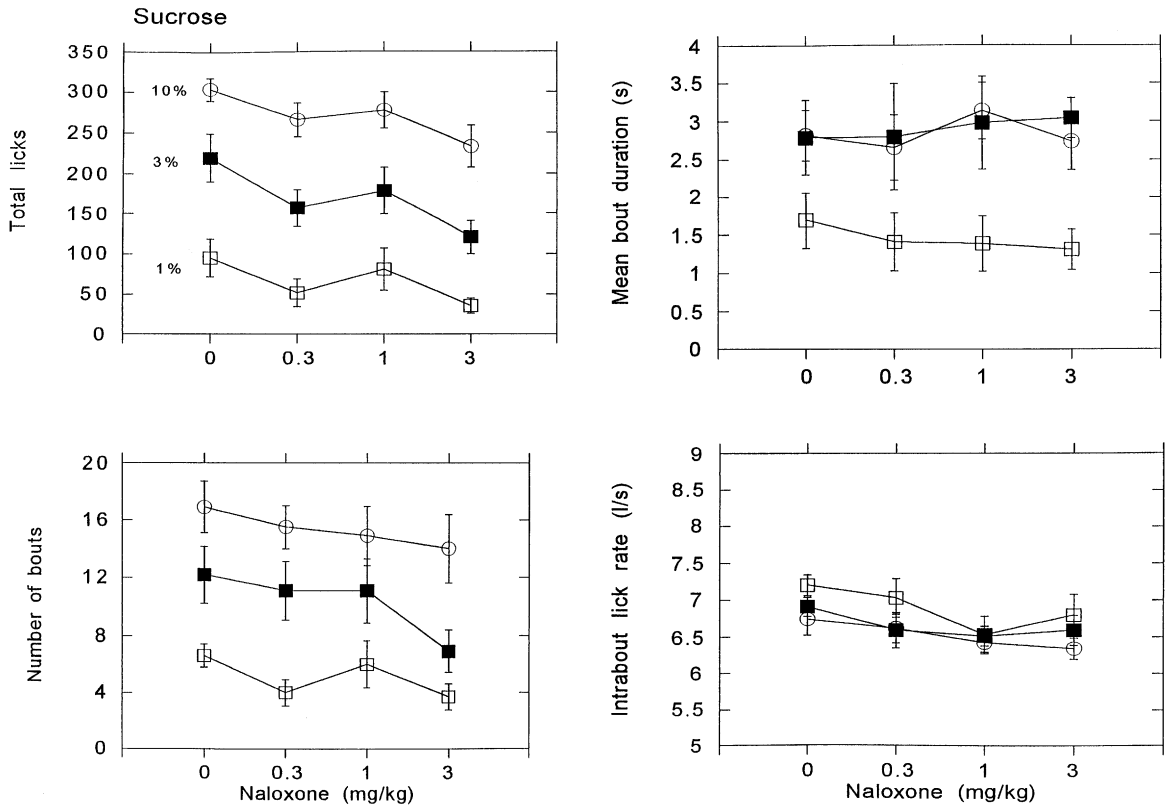

Fig. 1 The effects of naloxone (0.3–3 mg/kg) on total licks, mean bout duration, number of bouts and intrabout lick rate for three concentrations of sucrose in a brief contact test (60-s access to each concentration). *Open squares* (❑) indicate 1%, *filled squares* (■) indicate 3%, *open circles* (○) indicate 10% concentrations. $n = 10$. Data shown are mean ± SEM

Data analysis

The lick-time data were analyzed using Dilog software (Henderson 1994), followed by further processing using a Microsoft Excel spreadsheet. The lick data were grouped into bouts by specifying an upper interlick interval (ILI) of 400 ms. This definition was used because it had been established in previous studies that an interval of 400 ms was just longer than the break point in a log survivor plot of ILIs (Morris 1993). Various microstructural variables were examined: the total number of licks, mean bout duration, number of bouts, and the intrabout lick rate (licks per second within bouts). Where the intrabout lick rate was significantly affected, a more detailed analysis of the interlick interval (ILI) distribution was also carried out. A frequency distribution of ILIs was compiled by calculating the proportion of intervals falling within consecutive 10-ms time bins for each rat, and then averaging across animals.

The microstructural data were analyzed using a two-way repeated-measures analysis of variance (ANOVA), with drug treatment and fluid concentration as variables. Post hoc analysis of the main effect of drug was made using a Dunnett's *t*-test. Statistical tests were performed using Super Anova and Statview SE+graphics (Abacus Concepts Inc., Berkeley, Calif., USA). A result was considered statistically significant if $P < 0.05$.

Results

Experiment 1: The effect of naloxone on licking responses for sucrose and Intralipid

Sucrose

Total licks. Figure 1 (top left) shows the effect of naloxone on the number of licks for sucrose. There was a main effect of both drug treatment [$F(3,27) = 4.39$, $P < 0.05$] and concentration [$F(2,18) = 113.74$, $P < 0.001$], but no significant interaction term [$F(6,54) = 0.2$, NS]. Post hoc analysis showed that the 3 mg/kg dose of naloxone significantly decreased the total number of licks ($P < 0.01$]. Figure 1 (top left) also shows that an increase in sucrose concentration led to an increase in the total number of licks.

Mean bout duration. Naloxone did not significantly affect mean bout duration for sucrose drinking [$F(3,27) = 0.17$, NS], but a two-way repeated-measures ANOVA did reveal a main effect of concentration on this parameter [$F(2,18) = 9.8$, $P < 0.01$]. There was no significant drug × concentration interaction [$F(6,54) = 0.31$, NS]. Figure 1 (top right) shows that mean bout duration increased with increasing concentration of sucrose.

Number of bouts. For sucrose drinking a two-way, repeated-measures ANOVA revealed a significant effect of both drug treatment [$F(3,27) = 4.4$, $P < 0.05$] and concentration [$F(2,18) = 30.0$, $P < 0.001$] on bout number but no significant interaction between these two variables [$F(6,54) = 0.52$, NS]. A post hoc test revealed that naloxone significantly reduced bout number at the 3 mg/kg dose ($P < 0.01$) (Fig. 1, lower left). Bout number increased with increasing concentration of sucrose.

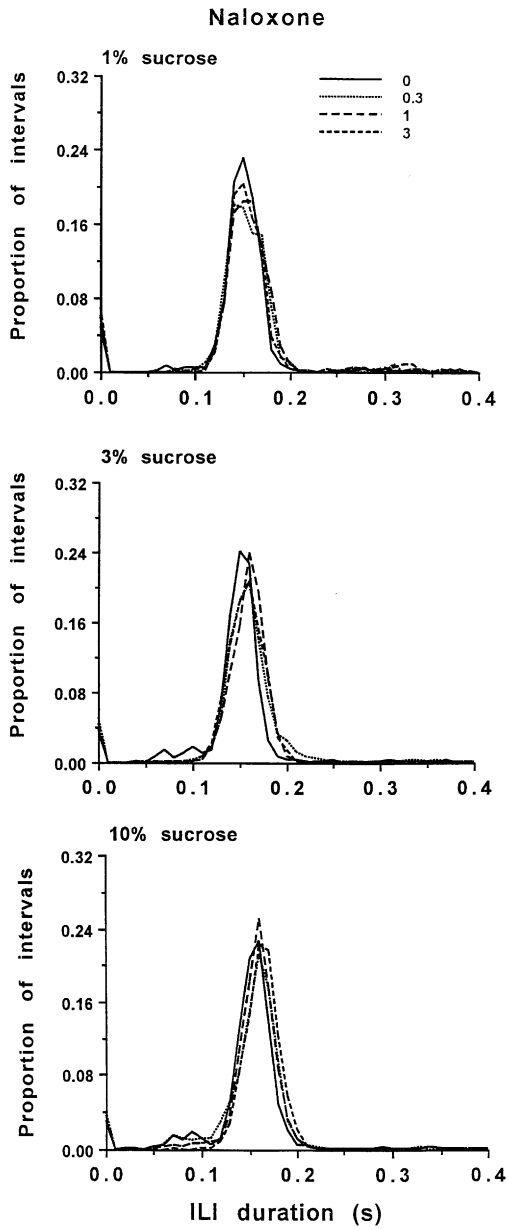

Fig. 2 Relative frequency distributions of interlick intervals (*ILIs*) obtained following treatment with naloxone (0.3–3 mg/kg) at each level sucrose concentration (1,3 and 10%). Data are averaged across ten rats and shown as proportions of total

Intrabout lick rate. A two-way repeated-measures ANOVA revealed a marginally significant effect of naloxone on the intrabout lick rate [$F(3,27) = 2.84$, $P = 0.056$] (Fig. 1, lower right). A post hoc test showed that naloxone significantly reduced the intrabout lick rate at the 1 mg/kg and 3 mg/kg doses ($P < 0.05$). There was also a significant effect of concentration [$F(2,18) = 5.8$, $P < 0.05$]. An increase in sucrose concentration led to a decrease in intrabout lick rate,

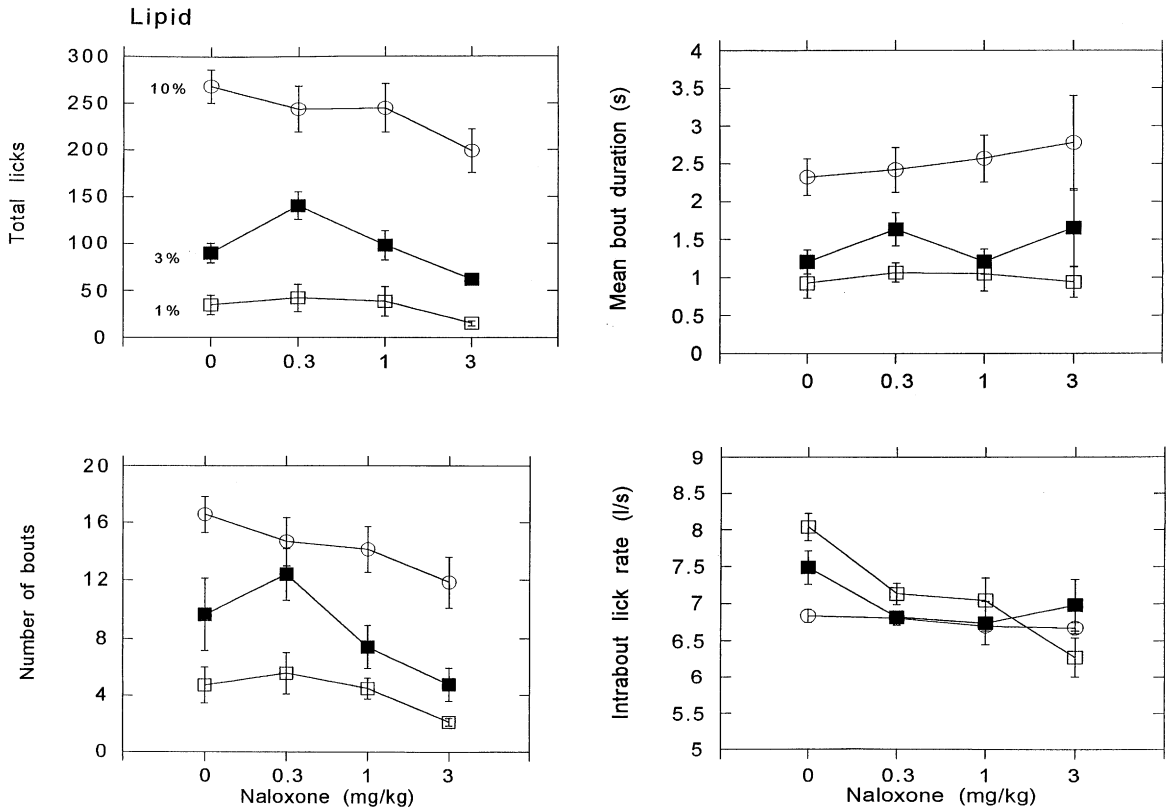

Fig. 3 The effects of naloxone (0.0–3 mg/kg) on total licks, mean bout duration, number of bouts and intrabout lick rate for three concentrations of Intralipid in a brief contact test (60-s access to each concentration). *Open squares* (❑) indicate 1%, *filled squares* (■) indicate 3%, *open circles* (❍) indicate 10% concentrations. $n = 11$. Data shown are mean ± SEM

although there was no significant interaction with drug treatment [$F(6,54) = 0.6$, NS].

ILI distributions

Figure 2 shows the effect of drug administration on the distribution of interlick intervals (ILIs) at each level of sucrose concentration. The distribution of ILIs cluster characteristically around 0.15 s (which is equivalent to a rate of licking of six to seven licks per second). Naloxone caused a slight shift to the right of the ILI distribution that was most pronounced for the 3% sucrose condition.

Intralipid

Total licks. As shown in Fig. 3 (top left), naloxone decreased the total number of licks for Intralipid [$F(3,30) = 5.2$, $P < 0.01$]. Post hoc analysis showed that the 3 mg/kg dose of naloxone differed significantly from the vehicle control condition ($P < 0.01$). Conversely, an increase in Intralipid concentration significantly increased the total number of licks [$F(2,20) = 83.7$, $P < 0.001$]. There was no significant drug × concentration interaction [$F(6,60) = 0.8$, NS].

Mean bout duration. Figure 3 (top right) shows the effect of naloxone on mean bout duration for Intralipid drinking. There was no significant effect of naloxone on this parameter [$F(3,30) = 0.48$, NS]. However, an increase in the concentration of Intralipid did lead to a monotonic increase in mean bout duration [$F(2,20) = 42.2$, $P < 0.001$]. No interaction between the effects of drug treatment and Intralipid concentration was observed [$F(6,60) = 0.6$, NS].

Number of bouts. Naloxone significantly decreased the number of bouts for Intralipid [$F(3,30) = 7.9$, $P < 0.001$] (Fig. 3, lower left) and this was significant at the 3 mg/kg dose ($P < 0.01$). There was also a main effect of Intralipid concentration on bout number [$F(2,20) = 44.3$, $P < 0.001$]. An increase in concentration led to an increase in bout number. There was no significant drug × concentration interaction [$F(6,60) = 1.1$, NS].

Intrabout lick rate. Figure 3 (lower right) shows the effect of naloxone on the intrabout lick rate for Intralipid. There was a main effect of both drug treatment [$F(3,30) = 8.3$, $P < 0.001$] and Intralipid concentration [$F(2,20) = 8.6$, $P < 0.01$] but no drug concentration interaction [$F(6,60) = 1.4$, NS]. At all doses,

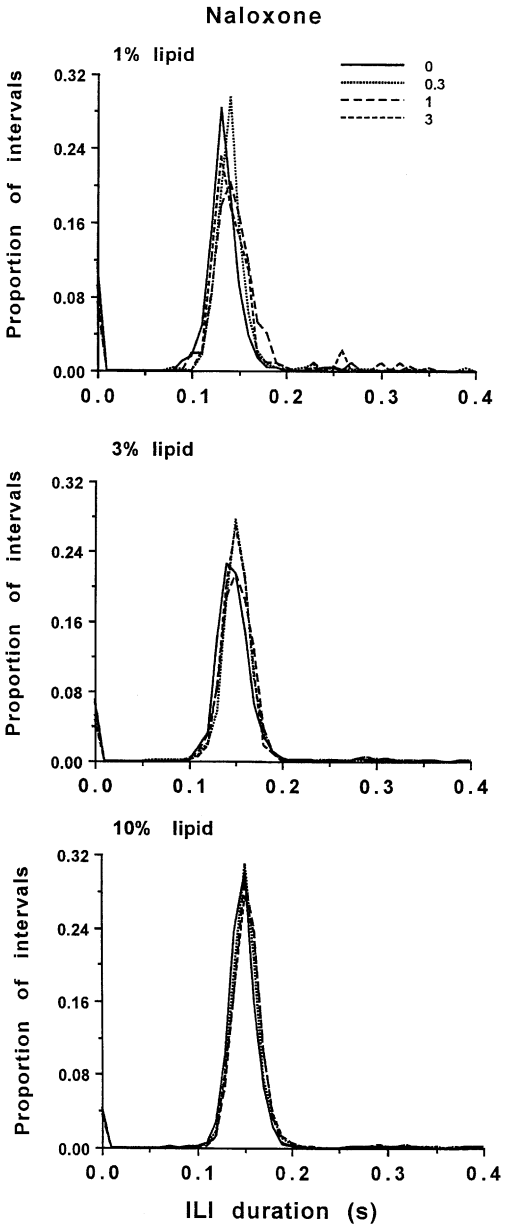

Fig. 4 Relative frequency distributions of interlick intervals (*ILIs*) obtained following treatment with naloxone (0.3–3 mg/kg) at each level of Intralipid concentration (1,3 and 10%). Data are averaged across 11 rats and shown as proportions of total

naloxone significantly decreased the rate of licking within bouts ($P < 0.01$). Increasing concentration also led to a decrease in the intrabout lick rate.

ILI distributions

The effect of naloxone on the distribution of ILIs at each level of Intralipid concentration is shown in Fig. 4. The distribution of ILIs for Intralipid drinking is similar to that observed for sucrose drinking with most of ILIs clustering around 0.15 s. There was a shift of the ILI distribution to the right following naloxone adminstration and this was more pronounced at the 1% and 3% concentrations.

Summary

The results show that the total number of licks emitted in the 60-s test period increased significantly as a function of both sucrose and Intralipid concentration. This in turn was associated with concentration-dependent increases in both mean bout duration and bout number. In both cases, there was evidence of a reduction in the rate of licking within bouts with higher concentrations. Naloxone reduced the total number of licks in 60 s and reduced bout number without affecting mean bout duration. There was evidence that naloxone reduced the rate of licking within bouts.

Experiment 2: The effect of morphine on licking responses for sucrose and Intralipid

Sucrose

Total licks. There was a significant effect of both drug treatment [$F(3,27) = 3.42$, $P < 0.05$] and concentration [$F(2,18) = 100.4$, $P < 0.001$] on the total number of licks. A post hoc test showed that morphine significantly increased the number of licks for sucrose at the 3 mg/kg dose ($P < 0.05$) (Fig. 5, top left). Total licks also increased monotonically as a function of increasing concentration of sucrose. There was no significant interaction between drug treatment and concentration [$F(6,54) = 0.93$, NS], although as shown in Fig. 5 (top left), the effect of morphine on the total number of licks was less pronounced at the 10% concentration.

Mean bout duration. Morphine did not significantly affect the mean bout duration for sucrose drinking [$F(3,27) = 0.78$, NS]. However, a two-way repeated-measures ANOVA did reveal a main effect of concentration on this parameter [$F(2,18) = 16.97$, $P < 0.001$], although there was no significant drug × concentration interaction [$F(6,54) = 0.46$, NS]. Figure 5 (top right) shows that mean bout duration increased with increasing concentration of sucrose.

Number of bouts. There were significant effects of both morphine [$F(3,27) = 3.12$, $P < 0.05$] and sucrose concentration [$F(2,18) = 20.75$, $P < 0.001$] on bout number. Figure 5 (lower left) shows that bout number increased as a function of both increasing dose of morphine and sucrose concentration. The effect of morphine on bout number was significant at the 3 mg/kg dose [$P < 0.05$]. There was no interaction between drug

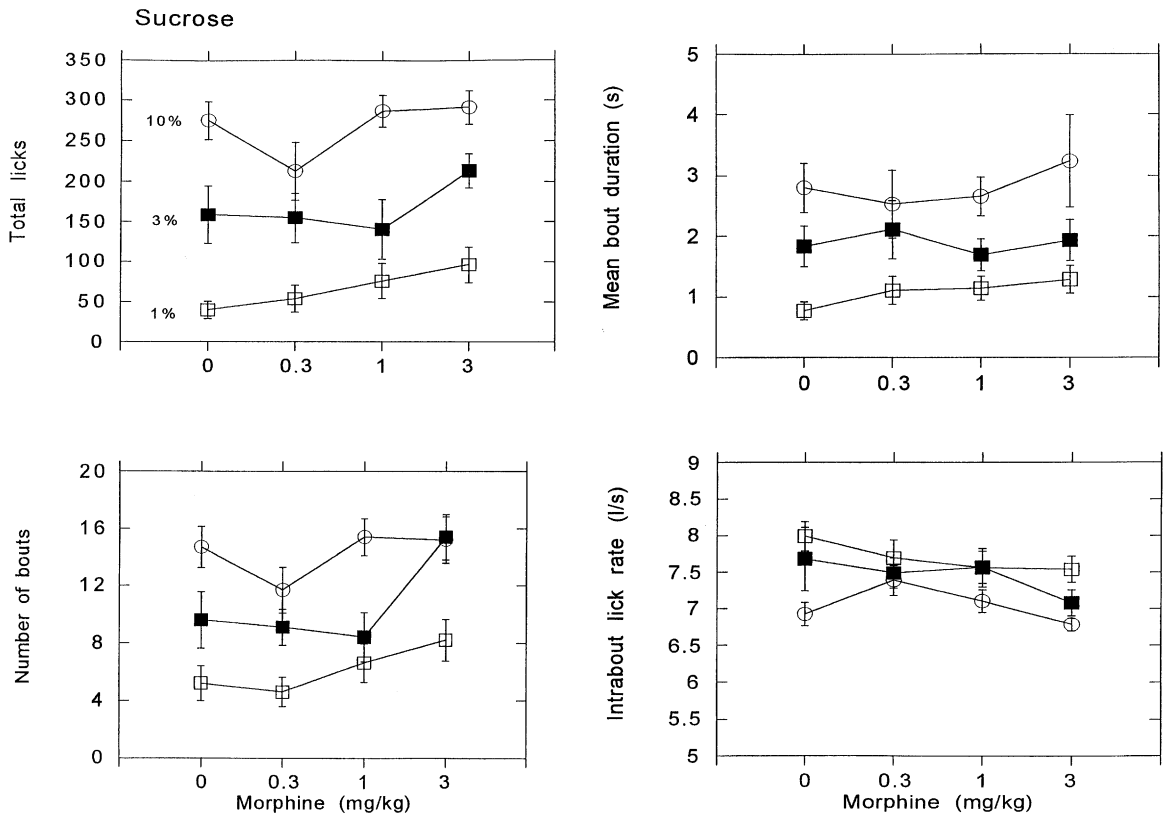

Fig. 5 The effects of morphine (0.3–3 mg/kg) on total licks, mean bout duration, number of bouts and intrabout lick rate for three concentrations of sucrose in a brief contact test (60-s access to each concentration). *Open squares* (❑) indicate 1%, *filled squares* (■) indicate 3%, *open circles* (○) indicate 10% concentrations. $n = 10$. Data shown are mean ± SEM

treatment and sucrose concentration for bout number [$F(6,54) = 2.0$, NS].

Intrabout lick rate. Morphine did not significantly affect the intrabout lick rate for sucrose drinking [$F(3,27) = 1.23$, NS]. There was a main effect of concentration [$F(2,18) = 18.64$, $P < 0.001$] but no drug × concentration interaction [$F(6,54) = 1.4$, NS]. A decrease in the intrabout lick rate was observed with increasing sucrose concentration (Fig. 5, lower right).

Intralipid

Total licks. There was a main effect of morphine treatment on the total number of licks [$F(3,30) = 3.2$, $P < 0.05$]. Figure 6 (top left) shows a dose-related increase in the number of licks that was significant at the 3 mg/kg dose ($P < 0.05$). There was also a main effect of concentration [$F(2,20) = 56.2$, $P < 0.001$], with total licks increasing as a function of increasing concentration. There was no significant drug × concentration interaction [$F(6,60) = 1.8$, NS], although Fig. 3 (top left) shows that the effect of morphine was reduced at the 10% concentration of Intralipid.

Mean bout duration. There was a main effect of morphine on mean bout duration for Intralipid drinking [$F(3,30) = 4.7$, $P < 0.01$] (Fig. 6, top right). However, the effect of morphine on this parameter was opposite to that on total number of licks. Increasing the dose of morphine led to a decrease in the mean bout duration and this was significant at the 0.3 mg/kg, 1 mg/kg ($P < 0.05$), and 3 mg/kg doses ($P < 0.01$). An increase in Intralipid concentration led to an increase in the duration of bouts [$F(2,20) = 9.7$, $P < 0.001$]. There was no significant interaction between drug treatment and concentration [$F(6,60) = 1.0$, NS].

Number of bouts. Morphine dose-dependently increased bout number for Intralipid drinking [$F(3,30) = 26.9$, $P < 0.001$] (Fig. 6, lower left). A post hoc test revealed that the 1 mg/kg and 3 mg/kg doses of morphine produced a significant increase in bout number [$P < 0.01$]. Increasing the concentration of Intralipid also significantly increased the number of bouts [$F(2,20) = 37.9$, $P < 0.001$]. No interactions were observed between drug dose and concentration on bout number [$F(6,60) = 1.5$, NS].

Intrabout lick rate. As shown in Fig. 6 (lower, right), there was a main effect of drug treatment on the rate of licking within bouts [$F(3,30) = 22.3$, $P < 0.001$].

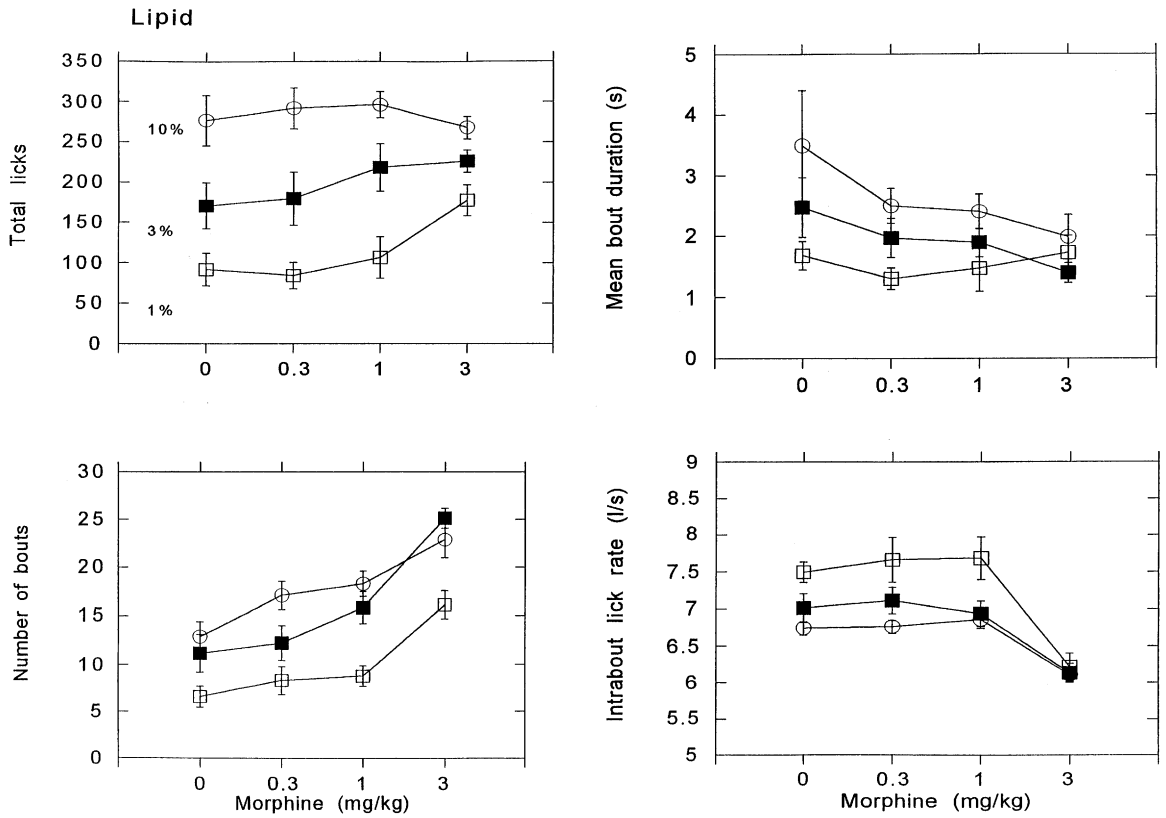

Fig. 6 The effects of morphine (0.3–3 mg/kg) on total licks, mean bout duration, number of bouts and intrabout lick rate for three concentrations of Intralipid in a brief contact test (60-s access to each concentration). *Open squares* (❑) indicate 1%, *filled squares* (■) indicate 3%, *open circles* (○) indicate 10% concentrations. $n = 11$. Data shown are mean ± SEM

A Dunnett's *t*-test showed that only the 3 mg/kg dose significantly decreased the intrabout lick rate ($P < 0.01$). Increasing concentration also significantly decreased the intrabout lick rate [$F(2,20) = 32.8$, $P < 0.001$]. The lack of a significant interaction term suggests that the effect of morphine was constant at all levels of concentration [$F(6,60) = 1.7$, NS].

ILI distributions

The effect of drug administration on the distribution of ILIs at each level of Intralipid concentration is shown in Fig. 7. Morphine (3 mg/kg) induced a shift of the ILI distribution to the right, which is consistent with the reduction in intrabout lick rate observed at this dose.

Summary

The effects of fluid concentration were consistent between the sucrose and Intralipid conditions and confirmed the findings of experiment 1. Morphine increased the number of licks and the number of bouts of licking although the effect of morphine was less evident at the highest concentrations of sucrose and Intralipid. This was probably due to ceiling effects because the animals were licking close to the maximum number of licks that can be emitted in 60 s at a rate of six or seven licks per second (360–420 licks). There was no effect of morphine on mean bout duration in the case of sucrose drinking and, unexpectedly, morphine produced a decrease in the case of Intralipid drinking. At 3 mg/kg, morphine reduced the intrabout lick rate for Intralipid drinking but not sucrose drinking, and this was reflected in a rightward shift of the ILI distribution.

Experiment 3: The effect of U-50, 488H on licking responses for sucrose

Sucrose

Total licks. A two-way repeated-measures ANOVA revealed a main effect of both drug treatment [$F(3,27) = 4.4$, $P < 0.05$] and concentration [$F(3,27) = 276.3$, $P < 0.001$] on the total number of licks. The relationship between dose of U-50, 488H and total licks showed an inverted U-shaped function. As shown in Fig. 8 (top left) there was an increase in total licks following administration of 0.3 mg/kg ($P < 0.05$) and 1 mg/kg

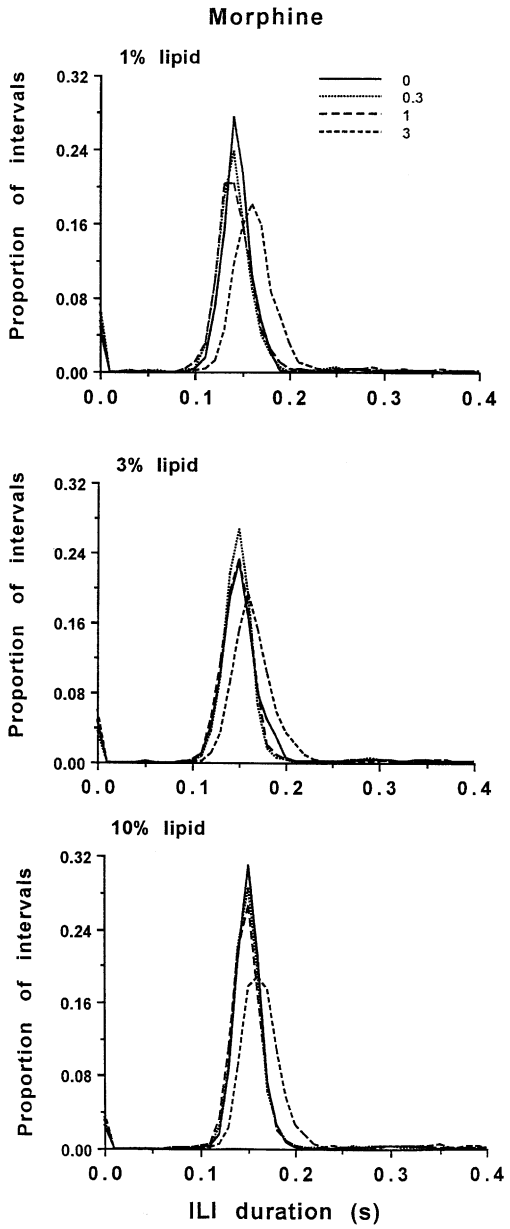

Fig. 7 Relative frequency distributions of interlick intervals (*ILIs*) obtained following treatment with morphine (0.3–3 mg/kg) at each level of Intralipid concentration (1,3 and 10%). Data are averaged across 11 rats and shown as proportions of total

($P < 0.01$) doses of U-50, 488H, but the 3 mg/kg condition did not differ significantly from the vehicle control condition. There was no interaction between the effects of drug treatment and sucrose concentration [$F(9,81) = 0.4$, NS].

Mean bout duration. U-50, 488H dose-dependently increased mean bout duration [$F(3,27) = 5.7$, $P < 0.01$] (Fig. 8, upper right). A post hoc test revealed that both the 1 mg/kg and 3 mg/kg doses of U-50, 488H produced a significant increase in mean bout duration ($P < 0.01$). Increasing the concentration of sucrose also led to a significant increase in mean bout duration [$F(3,27) = 49.6$, $P < 0.001$]. No significant interactions were observed between drug dose and concentration [$F(9,81) = 0.8$, NS].

Number of bouts. Administration of U-50, 488H significantly affected the number of bouts [$F(3,27) = 4.0$, $P < 0.05$]. However, a post hoc test revealed that only the 0.3 mg/kg dose increased the number of bouts ($P < 0.01$). Figure 8 (lower left) shows that bout number increased with increasing concentration of sucrose [$F(2,18) = 9.8$, $P < 0.01$). There was no significant drug × concentration interaction [$F(9,81) = 0.4$, NS].

Intrabout lick rate. Figure 8 (lower, right) shows that there was a main effect of drug treatment on the intrabout lick rate [$F(3,27) = 19.1$, $P < 0.001$]. A Dunnett's *t*-test revealed that the 1 mg/kg ($P < 0.01$) and 3 mg/kg ($P < 0.01$) doses of U-50, 488H significantly decreased the intrabout lick rate. Increasing concentration also decreased the rate of licking within bouts, although this was only marginally significant [$F(2,27) = 2.9$, $P = 0.052$]. There was also a significant interaction term [$F(9,81) = 2$, $P < 0.05$], suggesting that the effect of U-50, 488H on the intrabout lick rate was not the same at all concentrations.

ILI distributions

Figure 9 shows that U-50, 488H had a marked effect on the distribution of ILIs with the 1 mg/kg and 3 mg/kg doses inducing a shift to the right in the distribution at each concentration of sucrose. The effect of U-50, 488H on ILI distribution was most pronounced at the 1% concentration of sucrose.

Summary

U-50, 488H had a dose-related effect on total number of licks that was characterised by an inverted U-shaped function. Mean bout duration was dose-dependently increased following administration of U-50, 488H. The effect on mean bout duration was evident at all concentrations of sucrose. No increase in total number of licks was observed for 30% sucrose, although this was probably due to ceiling effects. U-50, 488H also increased the number of bouts although only the lowest dose of 0.3 mg/kg differed significantly from the vehicle control condition. At 1 mg/kg and 3 mg/kg, U-50, 488H reduced the intrabout lick rate and this was reflected in a rightward shift of the ILI distribution. This effect was most pronounced at the 1% concentration, which probably accounted for the significant interaction between drug dose and concentration observed for this parameter.

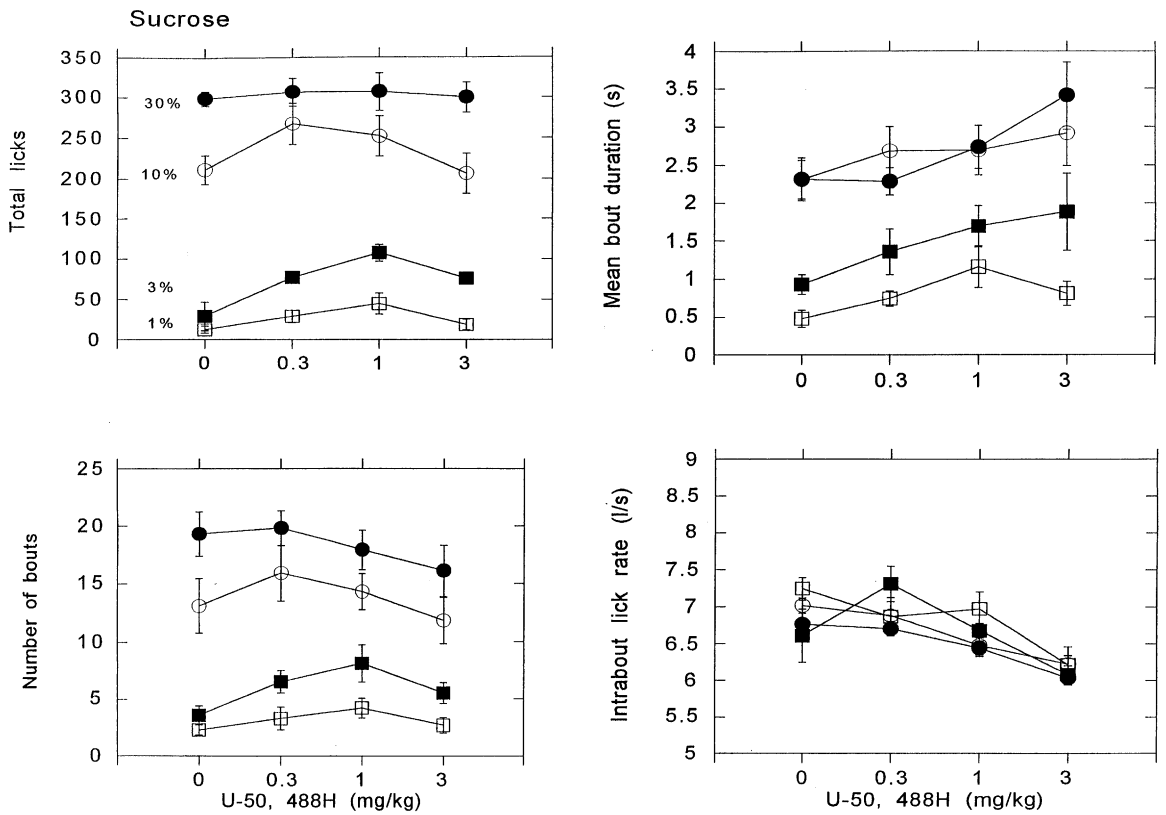

Fig. 8 The effects of U-50, 488H (0.3–3 mg/kg) on total licks, mean bout duration, number of bouts and intrabout lick rate for four concentrations of sucrose in a brief contact test (60-s access to each concentration). *Open squares* (❑) indicate 1%, *filled squares* (■) indicate 3%, *open circles* (○) indicate 10%, *filled circles* (●) indicate 30% concentration $n = 10$. Data shown are mean ± SEM

Discussion

In the present experiments, we investigated for the first time the effects of the opioid antagonist naloxone, and the opioid agonists morphine and U-50, 488H on the microstructure of licking in the non-deprived rat. Generally speaking, previous work on the effects of opioids on ingestive behaviour has used longer tests (e.g. Jackson and Cooper 1986), but our present results demonstrate that the effects of these drugs can be detected within a 60-s access period. These early drug effects focus attention on factors that determine the initiation and maintenance of ingestive behaviour, as distinct from those which contribute to feeding satiation. In addition, our results indicate that the effects of opioid receptor ligands are not dependent on sweet taste because the responses elicited to sucrose and Intralipid were very similar.

We found that naloxone (3 mg/kg) reduced the total number of licks for both sucrose and Intralipid, whereas morphine (3 mg/kg) and U-50, 488H (0.3–1 mg/kg) increased the total number of licks. These data are consistent with earlier reports of the anorectic effects of opioid antagonists (Cooper 1980; Apfelbaum and Mandenoff 1981; Levine et al. 1982), versus the hyperphagic effects of opioid agonists (Sanger and McCarthy 1981; Gosnell et al. 1983; Levine and Billington 1989). It has been shown previously that initial rates of licking in short access tests are concentration-dependent and so may reflect changes in palatability (Davis 1973). This conclusion is supported by the results of the present studies because the total number of licks emitted for both sucrose and Intralipid varied in a concentration-dependent manner. There appear to be grounds, therefore, for arguing that naloxone diminishes palatability, while morphine and U-50, 488H enhance it. Nevertheless, we would like to argue that there are problems with interpretations couched in terms of palatability without further qualification.

Because there is evidence that microstructural analysis of licking behaviour may provide information concerning the mechanisms underlying the effect of drug treatments on ingestion, we also analyzed the effect of opioid administration on bout structure. The results showed that differences were evident in the effects of these drugs on the number and duration of bouts. Specifically, naloxone reduced the number of bouts for sucrose and Intralipid but did not alter the mean bout duration. The effect of morphine was similar but in the opposite direction; there was an increase in the number of bouts for both sucrose and Intralipid following

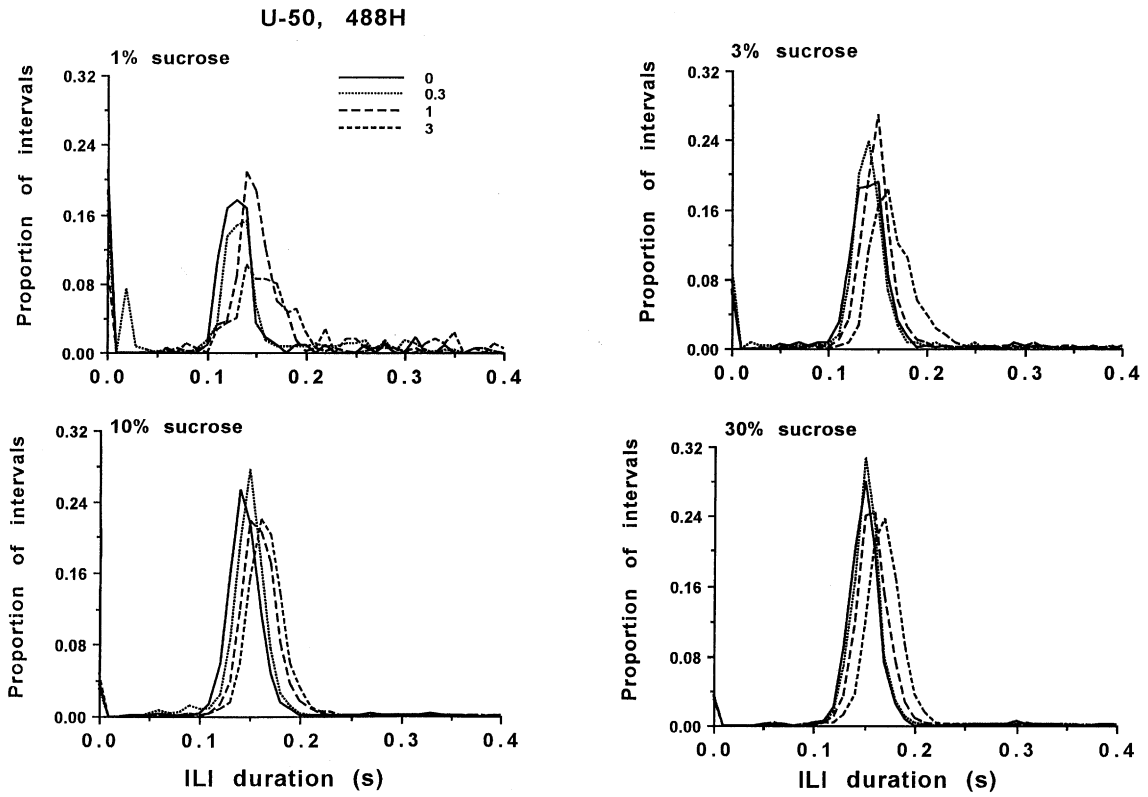

Fig. 9 Relative frequency distributions of interlick intervals (*ILIs*) obtained following treatement with U-50, 488H (0.3–3 mg/kg) at each level of sucrose concentration (1,3,10 and 30%). Data are averaged across ten rats and shown as proportions of total

morphine administration, but no effect on the mean bout duration for sucrose drinking. Morphine did significantly affect the mean bout duration for Intralipid ingestion, although this effect was the converse of that on total number of licks because morphine actually decreased mean bout duration for Intralipid drinking. Interestingly, the primary microstructural effect of U-50, 488H was to increase mean bout duration. This drug also increased the number of bouts, but this effect was only significant at the lowest dose (0.3 mg/kg).

It has been shown that increasing the concentration of sugar solutions in longer term tests results in longer bouts of licking without affecting the frequency of these behaviours (Davis and Smith 1992). Consequently, it has been suggested that mean bout duration may provide a sensitive measure of palatability. This result resembles that of the benzodiazepine agonist midazolam which selectively increased mean bout duration without affecting bout number in a 60-s test (Higgs and Cooper 1997). Benzodiazepines probably provide the best documented case of drug-induced enhancement of palatability (Berridge and Pecina 1995; Cooper and Higgs 1996), and so this result is entirely consistent with the proposal that mean bout duration is a sensitive index of palatability.

With respect to the present data, this suggests that the increase in mean bout duration caused by U-50, 488H may have been due to an enhancement of palatability, whereas the lack of consistent effect of naloxone and morphine on mean bout duration suggests that the primary effect of these drugs is not on palatability. However, in the current studies, we observed concentration-dependent effects for bout number and mean bout duration. Therefore, we wish to suggest that the "palatability" determining the characteristics of ingestive behaviour in a brief contact test can be split into two components. We would draw a distinction between a component that helps to maintain contact with the licking tube, and thus contributes to the determination of bout duration, and one that returns the animal to the licking tube more often, thus contributing to bout number.

We should like tentatively to ascribe the increase in bout duration to an increase in hedonic evaluation and the increase in bout number to an incentive effect. This is similar to Berridge's (1996) distinction between "liking" (hedonic evaluation) and "wanting" (incentive salience) and suggests that whereas naloxone and morphine may act to alter incentive salience, U-50, 488H may affect hedonic responding. However, this proposal requires further thorough investigation.

The present results can also be interpreted in terms of differential effects of μ and κ ligands on ingestion. Morphine and naloxone had similar but opposing effects on licking, and this may have been due to action

at μ receptors. Morphine has a high affinity for μ receptors and although naloxone is a relatively nonselective antagonist, it does have a higher affinity for μ receptors, relative to δ and κ receptors (Goldstein and Naidu 1989). U-50, 488H on the other hand, which affected licking in a manner distinguishable from that of naloxone and morphine, has a selective action at κ receptors. Therefore, the data suggest that different mechanisms may underlie the effects of μ and κ receptors on feeding. It is noteworthy that differential effects of μ and κ opioids on ingestion and associated behaviours have been observed in several previous studies. For example, Badiani and colleagues (1995) reported that injection of the μ opioid agonist DAMGO into the ventral tegmental area (VTA), but not U-50, 488H, increased the amount of time spent eating food pellets. This effect was due to an increase in the number of feeding bouts rather than their duration. Similarly, it has been reported that although intra-VTA injection of both μ and κ agonists can lower the threshold required for lateral hypothalamic stimulation-induced feeding (Jenck et al. 1987b), only morphine (and not U-50, 488H) reduces self-stimulation thresholds (Jenck et al. 1987a). It has been suggested that changes in ingestive behaviour induced by action at μ receptors may be due to activation of the mesolimbic dopamine system, whereas the effects of κ receptor ligands may be dopamine independent (Noel and Wise 1993; Zhang and Kelley 1997). Given the proposed role of dopamine in the attribution of incentive salience (Robinson and Berridge 1993), this distinction fits in with the hypothesis, based on the current data that μ receptors may be primarily involved in salience attribution, whereas κ receptors may be preferentially involved in palatability.

The conclusion has to be tempered by the fact that morphine did affect the mean bout duration for Intralipid drinking. However, this result was not responsible for the change in total number of licks, because the total number of licks was increased following morphine treatment whereas mean bout duration was decreased. The reason for this decrease is not clear, but given the evidence that morphine may have some aversive as well as rewarding effects on behaviour (Bechara and van der Kooy 1985), it is possible that the increase in bout number reflects modulation of reward while the decrease in bout duration reflects modulation of aversive responding.

It is also necessary to consider that the effect of naloxone, morphine and U-50, 488H on licking patterns may have been due to motoric effects. This point is especially important, given that the rate at which the sedative effect of morphine wears off is probably dose-related. Therefore, the influence of sedative effects in the present experiments was examined by measuring the intrabout lick rate and distribution of interlick intervals (ILIs). Morphine reduced the intrabout lick rate for Intralipid drinking, but did not have any effect in the case of sucrose drinking. Therefore, there was no general effect of morphine on licking rate which could explain the changes in bout structure. Naloxone, on the other hand, reduced the intrabout lick rate for both Intralipid and sucrose drinking. However, this effect would not be expected to contribute to any change in bout number, because although the frequency distribution of ILIs was shifted to the right following naloxone administration, the average ILI still did not exceed the 400-ms cut-off point used to define a bout. U-50, 488H had a significant effect on the intrabout lick rate and this was very pronounced at the 3 mg/kg dose, suggesting a sedative effect. Such an effect of U-50, 488H was probably responsible for the failure of the highest dose to increase the total number of licks, leading to the inverted U-shape dose-effect function. However, it is interesting to note that the mean bout duration did not show an inverted U-shaped function, suggesting that this measure may not be as susceptible to motoric side-effects as the number of total licks parameter. The implication of this dissociation of mean bout duration and total number of licks is that the former may provide a more reliable measure of palatability than the latter.

In summary, our data indicate that naloxone, morphine and U-50, 488H can exert significant effects on licking responses in non-deprived rats within a 60-s access period. Microstructural analysis distinguished between the effects of these drugs, suggesting that different mechanisms may underlie the effects of μ and κ receptor ligands on ingestion.

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