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Opioids in the perifornical lateral hypothalamus suppress ethanol drinking

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Abstract

The opioid system is known to enhance motivated behaviors, including ethanol drinking and food ingestion, by acting in various reward-related brain regions, such as the nucleus accumbens, ventral tegmental area and medial hypothalamus. There is indirect evidence, however, suggesting that opioid peptides may act differently in the perifornical lateral hypothalamus (PF/LH), causing a suppression of consummatory behavior. Using brain-cannulated Sprague-Dawley rats trained to voluntarily drink 7% ethanol, the present study tested the hypothesis that opioids in the PF/LH can reduce the consumption of ethanol, with animals receiving PF/LH injections of the δ -opioid receptor agonist D-Ala2-met-enkephalinamide (DALA), the μ -receptor agonist [D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO), the κ -receptor agonist ($\pm$)-trans-U-50,488 methanesulfonate (U-50,488H), or the general opioid antagonist methylated naloxone (m-naloxone). The consumption of ethanol, lab chow, and water was monitored for 4 hours after injection. The results showed that the three opioid receptor agonists injected into the PF/LH specifically and significantly reduced ethanol intake, while causing little change in chow or water intake, and the opposite effect, enhanced ethanol intake, was observed with the opioid antagonist. Of the three opioid agonists, the δ -agonist appears to produce the most consistent and long-lasting suppression of consumption. This effect was not observed with injections 2 mm dorsal to this area, focusing attention on the PF/LH as the main site of action. These results suggest that the opioid peptides have a specific role in the PF/LH of reducing ethanol drinking, which is distinct from their more commonly observed appetitive actions in other brain areas. The additional finding, that m-naloxone in the PF/LH stimulates ethanol intake in contrast to its generally suppressive effect in other regions, focuses attention on this hypothalamic area and its distinctive role in contributing to the variable effects sometimes observed with opioid antagonist therapy for alcoholism.

Keywords

perifornical lateral hypothalamus; opioids; ethanol; δ -receptor; μ -receptor; κ -receptor

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INTRODUCTION

Ethanol, which is a beverage containing calories as well as a drug of abuse, is known both to affect and be modulated by some of the same neurochemical mechanisms that control feeding behavior (Barson et al., 2011). These include the opioid peptide systems, which have important functions related to reward processing and consummatory behavior (Bodnar, 2011; Oswald and Wand, 2004). These systems include the peptides, enkephalin (ENK) and dynorphin (DYN) (Kakidani et al., 1982; Noda et al., 1982), with ENK binding most strongly to δ - and μ -receptors and DYN binding to κ -receptors (Singh et al., 1997) which are widely distributed throughout the brain (Mansour et al., 1988; Oswald and Wand, 2004). There is extensive evidence showing that these peptides, in mesolimbic brain areas such as the nucleus accumbens (NAc), ventral tegmental area (VTA), and the hypothalamic paraventricular nucleus (PVN), can have a strong stimulatory effect on the consumption of ethanol (Barson et al., 2010; Margolis et al., 2008; Zhang and Kelley, 2002). However, recent studies have identified brain sites through which the opioid peptides may act to produce a very different effect, a suppression of consummatory behavior. For example, in the ventral pallidum, infusion of a μ -agonist suppresses self-administration of ethanol, general feeding, and hedonic responses to sucrose (Kempainen et al., 2012; Smith and Berridge, 2005). Also, activation of the δ -receptor in the VTA can suppress ethanol consumption (Margolis et al., 2008). These new findings show that the opioids, instead of consistently promoting appetitive behaviors, may have different roles that vary as a function of the specific brain area and specific opioid receptor subtype.

There is some evidence that the perifornical lateral hypothalamus (PF/LH), which contains functional δ -, μ -, and κ -receptors (Li and van den Pol, 2006, 2008; Olds and Williams, 1980) and integrates motivational signals with ingestive behavior (Berthoud and Munzberg, 2011), may be another site where the opioids are behaviorally inhibitory. In this area, the excitatory amino acid glutamate has recently been shown to stimulate the consumption of ethanol (Chen et al., 2012), similar to its effect on food intake (Stanley et al., 2011), suggesting that the opioid peptides ENK and DYN which are neuronally inhibitory (Li and van den Pol, 2006; Nicoll et al., 1977; Singh et al., 1997) may induce the opposite effect, a suppression of ethanol drinking behavior. These opioids could act through the orexigenic peptide, orexin/hypocretin (OX), which is expressed in neurons of the PF and nearby LH area (de Lecea et al., 1998; Sakurai et al., 1998) and colocalizes with the opioid receptors (Georgescu et al., 2003; Li and van den Pol, 2006, 2008). This peptide has a stimulatory effect on ethanol intake, while an OX antagonist suppresses ethanol self-administration (Schneider et al., 2007; Shoblock et al., 2011). Together with evidence that opioids inhibit transcription of OX (Georgescu et al., 2003; Zhou et al., 2006), these findings raise the possibility that opioid peptides may act in the PF/LH to suppress consummatory behavior such as ethanol drinking.

The goal of this study was to test, in ethanol drinking rats with implanted brain cannulae, the hypothesis that the opioid peptides in the PF/LH have a specific role in reducing the consumption of ethanol. In Sprague-Dawley rats trained to voluntarily drink 7% ethanol, tests were conducted with PF/LH injections of three opioid receptor agonists, the δ -receptor agonist D-Ala2-met-enkephalinamide (DALA), the μ -receptor agonist [D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO), and the κ -receptor agonist ($\pm$)-trans-U-50,488 methanesulfonate (U-50,488H), and also the general opioid antagonist, methylated naloxone (m-naloxone). Their effects on ethanol, food, and water consumption were examined over the next 4 hours. The results provide clear evidence that opioids in the PF/LH have a specific effect of suppressing voluntary ethanol drinking.

MATERIAL AND METHODS

Subjects

Adult male Sprague-Dawley rats (225 to 250 g at the start of experiments) were obtained from Taconic Farms (Germantown, NY). Rats were individually housed in hanging wire cages and maintained on a reversed 12:12-hour light–dark cycle (lights off from 6:00 am). Subjects had *ad libitum* access to LabDiet Rodent Chow 5001 (St Louis, MO) prior to ethanol training, and *ad libitum* access to water throughout the experiment. All animals were allowed 1 week to acclimate to the facility before experiments began. In the present study, 44 rats were included in the analysis. Adequate measures were taken to minimize pain or discomfort of the subject. All procedures were approved by the Princeton University Institutional Animal Care and Use Committee, and conformed to the National Institutes of Health guidelines on the ethical use of animals.

Ethanol Training

Subjects were acclimated to unsweetened ethanol by using a variant of the 2-bottle choice procedure (Martinetti et al., 2000). To encourage animals to drink and adapt to the unsweetened ethanol, the concentration of ethanol was gradually increased every 4 days, from 1, 2, 4, to 7% (v/v). Animals had access to ethanol solutions for 12 h per day, starting 3 h into the dark period. Using this procedure, animals can consume 2.50 ± 0.34 g/kg over a 12 h period, which leads to blood ethanol concentrations of 29–38 mg/dl (Morganstern et al., 2010). Chow was also provided for 12 h per day, along with the ethanol solution. Animals received cannulation surgery after a minimum of 4 days of access to 7% ethanol.

Surgery

Subjects were anesthetized using ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), supplemented with ketamine when necessary. Stainless steel 21-gauge guide shafts (10 mm in length) were implanted bilaterally, aimed at the PF/LH (A -2.9 , L ± 1.6 , V 3.9 mm, with reference to bregma, the midsagittal sinus, and the level skull surface). Subjects had 1 week to recover before testing. Stainless steel stylets were left in the guide shafts between injections to prevent occlusion.

Microinjection Procedures

Drugs were delivered through 26-gauge stainless steel microinjectors with fused-silica tubing inside (74 μ m ID, 154 μ m OD, Polymicro Technologies, Phoenix AZ) that protruded beyond the stainless steel to reach the region of interest (V 8.4 mm for PF/LH, V 6.4 mm for the anatomical control). Doses were chosen based on previous central injection studies that found opioid ligands to affect ethanol drinking (Barson et al., 2009; Barson et al., 2010) and on pilot tests. Two doses were tested for each opioid receptor agonist, and one dose was used for the antagonist m-naloxone, which has a better site specificity than naloxone (Schroeder et al., 1991). The specific drugs and doses used were as follows: (i) the δ -receptor agonist, DALA (3.6 nmol, 7.1 nmol per side); (ii) the μ -receptor agonist, DAMGO (1.5 nmol, 2.9 nmol per side); (iii) the κ -receptor agonist, U-50,488H (2.7 nmol, 5.4 nmol per side); and (iv) the opioid receptor antagonist, m-naloxone (2.1 nmol per side). The compounds DAMGO and m-naloxone were purchased from Sigma-Aldrich Co. (St Louis, MO), while DALA was purchased from American Peptide Co. Inc. (Sunnyvale, CA) and U-50,488H from Tocris Bioscience Co. (Ellisville, MO). All drugs were dissolved in preservative-free 0.9% NaCl solution (Hospira Inc., Lake Forest, IL) immediately prior to microinjection. Injections were counterbalanced, so each animal received vehicle or drug in counterbalanced order on 2 consecutive days ($n = 5$ –11/group). To minimize stress, animals were handled on an almost daily basis during the 1–2 weeks prior to injections. Injections

were given 3 hours into the dark cycle, immediately prior to daily ethanol and food access. A syringe pump delivered 0.5 μ l during 47 seconds and microinjectors were left in place for an additional 47 seconds to allow for diffusion. The intake of ethanol, food, and water was measured at 1 h, 2 h, and 4 h after injection. Within each group, at least a week elapsed between sets of injections to allow for receptor recovery. Tests of each dose included a counterbalanced control injection of saline vehicle. For the anatomical control injections, the highest behaviorally effective dose of each drug was tested.

Histological Analysis

To verify the sites of microinjections, rats were sacrificed by rapid decapitation. Their brains were sliced on a freezing microtome as 40 μ m coronal sections and examined microscopically. Animals (1–3 rats/group) with probes 0.5 mm or farther from the PF/LH were discarded from the analysis.

Statistical Data Analysis

Ethanol, food, and water intake was analyzed for each dose of drug using a two-way repeated measures analysis of variance (ANOVA), with treatment and time as within-subject factors. Since the behavioral effects of opioids have been shown to vary over time (Barson et al., 2010; Craft, 2008), an *a priori* decision was made to analyze differences at specific time points (1 h, 2 h, and 4 h) using pairwise 2-tailed *t*-tests, with the *p* value set at 0.017 after Bonferroni correction for each of the 3 pairwise comparisons. Data from animals (1–4 rats/group) consuming little or no ethanol (< 0.2 g/kg in 4 hours) after vehicle injection in all experiments were excluded. Data are expressed as mean $\pm$ standard error of the mean. The vehicle intake presented in the figures is the average from the 2 sets of injections, as intake after vehicle did not significantly differ within each group.

RESULTS

Experiment 1: Delta-agonist DALA in the PF/LH suppresses ethanol intake

Histological examination of the injection sites revealed that the PF/LH injections were made between bregma -2.76 mm and -3.00 mm, along the lateral edges of the PF/LH (Fig. 1A). The anatomical control injections, which were made at the same rostrocaudal coordinates, were located in the ventrolateral thalamic nucleus (Fig. 1B).

As shown in Fig. 2, injection of the δ -receptor agonist DALA in the PF/LH selectively decreased the intake of 7% ethanol when compared to vehicle injection. Two-way repeated measures ANOVAs showed that DALA significantly decreased ethanol intake at both doses tested (7.1 nmol: $F(1,10) = 16.35$, $p < 0.05$; 3.6 nmol: $F(1,9) = 6.56$, $p < 0.05$). This suppression effect was long-lasting at the higher dose of 7.1 nmol/side, which decreased ethanol intake across the 4 h test period (-45%) ($t = 3.8$, $df = 10$, $p < 0.017$). Also, it was specific to ethanol, as food intake was unchanged by DALA at both doses (not significant, *n.s.*), and water consumption was unaffected by the lower dose while increased by the higher dose ($F(1,10) = 13.96$, $p < 0.05$), an effect observed at 2 h ($+94\%$) ($t = 4.1$, $df = 10$, $p < 0.017$) and 4 h ($+44\%$) ($t = 3.1$, $df = 10$, $p < 0.017$) post-injection. In the anatomical control site 2 mm dorsal to the PF/LH, DALA had no significant effects on the intake of ethanol, food or water (*n.s.*) (Table 1). Taken together, these results demonstrated that the δ -receptor agonist DALA in the PF/LH produces a dose-dependent suppression specifically of ethanol intake and that this effect can last for at least 4 h.

Experiment 2: Mu-agonist DAMGO in the PF/LH suppresses ethanol intake

With injection of the μ -agonist, DAMGO, the results revealed a suppressive effect on consumption of 7% ethanol (Fig. 3), which was smaller in magnitude and shorter in duration compared to that observed with DALA. While two-way repeated measures ANOVAs failed to show main effects of DAMGO (2.9 nmol: $F(1,10) = 1.46$, $p = 0.26$; 1.5 nmol: $F(1,5) = 2.86$, $p = 0.15$), analyses at specific time points showed that DAMGO at both doses significantly reduced ethanol drinking during the first hour after injection. At the higher dose (2.9 nmol/side), DAMGO caused a small but significant decrease in ethanol intake during 1 h (–19%) ($t = 2.9$, $df = 10$, $p < 0.017$), and this first-hour suppressive effect was also observed at the lower dose (1.5 nmol/side), which significantly reduced ethanol consumption by 76% ($t = 3.7$, $df = 5$, $p < 0.017$). This effect was short-lived, not evident during hours 2–4 post-injection. It was also specific to ethanol, as DAMGO produced no change in food or water intake (*n.s.*). In the anatomical control site 2 mm dorsal to the PF/LH, DAMGO had no significant effects on the consumption of ethanol, food, or water at any time (*n.s.*) (Table 1). Thus, the μ -receptor agonist DAMGO in the PF/LH produced a suppressive effect specifically on ethanol intake which, while initially strong, was shorter-lasting than that observed with DALA.

Experiment 3: Kappa-agonist U-50,488H in the PF/LH suppresses ethanol intake

Injection of the κ -agonist U-50,488H in the PF/LH also selectively reduced the intake of 7% ethanol when compared to vehicle injection, with the effect again being smaller and shorter in duration compared to that observed with DALA (Fig. 4). A two-way repeated measures ANOVA showed that U-50,488H, at the higher dose of 5.4 nmol/side, significantly decreased ethanol intake ($F(1,6) = 9.43$, $p < 0.05$). This was evident only at 1 h (–61%) ($t = 4.0$, $df = 6$, $p < 0.017$) and not at hours 2–4 post-injection. It had no effect on food or water intake at any time period (*n.s.*). The lower dose of U-50,488H (2.7 nmol/side) also marginally reduced ethanol intake ($F(1,4) = 7.04$, $p = 0.06$), showing a tendency to suppress drinking at 1 h ($t = 3.2$, $df = 4$, $p = 0.033$). In the anatomical control site 2 mm dorsal to the PF/LH, U-50,488H had no significant effect on the intake of ethanol, food, or water (*n.s.*) (Table 1). Thus, the κ -receptor agonist U-50,488H in the PF/LH caused a specific suppression of ethanol intake that was evident only during the first hour post-injection.

Experiment 4: Opioid antagonist m-naloxone in the PF/LH stimulates ethanol intake

In contrast to the effect of opioid agonists, m-naloxone in the PF/LH selectively increased the intake of 7% ethanol when compared to vehicle injection (Fig. 5). While there was no significant main effect ($F(1,8) = 1.79$, $p = 0.22$), at 1 h post-injection, m-naloxone (2.1 nmol/side) increased specifically ethanol intake by 100% ($t = 2.9$, $df = 8$, $p = 0.021$), without affecting food or water consumption (*n.s.*). M-naloxone had little effect on ethanol intake during hours 2–4 (*n.s.*). In the anatomical control site 2 mm dorsal to the PF/LH, mnaloxone had no significant effects on the intake of ethanol, food, or water (*n.s.*) (Table 1). Together, these results demonstrate that the opioid antagonist m-naloxone leads to an effect opposite to that produced by the opioid agonists, presumably by blocking endogenously-active opioid receptors.

DISCUSSION

The present study assessed the role of opioids in the PF/LH in affecting consummatory behavior in ethanol-drinking rats. Our results demonstrate that opioid agonists injected into the PF/LH can significantly and specifically suppress ethanol drinking and that blockade of these receptors with m-naloxone can produce the opposite effect. The evidence suggests that this suppressive effect, while occurring with all three δ -, μ -, and κ -agonists, appears to be strongest and most prolonged when induced by the δ -agonist DALA, in that it occurs at both

doses and lasts for at least 4 h. A more extensive dose-response analysis should help to substantiate this conclusion regarding a more consistent effect with δ -receptor activation in the PF/LH.

Role of opioid peptides in the PF/LH in controlling ethanol intake

As reviewed in the Introduction, opioid peptides are well-known for their stimulatory effect on consummatory behavior through different brain areas related to reward processing, such as the NAc, VTA, and PVN. There is additional evidence, however, indicating that the opioids may have the opposite effect, a suppression of consummatory behavior, when acting through different brain areas and particularly when acting through the δ -receptor. This has been observed in the VTA, where administration of a δ -agonist suppresses the drinking of ethanol (Margolis et al., 2008), and also in the NAc, where infusion of a δ -agonist suppresses DAMGO-induced hyperphagia and a δ -receptor antagonist increases sucrose drinking (Katsuura and Taha, 2010; Kelley et al., 1996). In the PF/LH area, the published studies have revealed mixed effects, with some describing a stimulatory effect of the opioids on chow intake and others showing no change in food intake (Gulati, 1995; Li et al., 2006). Our findings, with injections in rats trained to drink ethanol, provide strong evidence for an inhibitory effect specifically on the consumption of ethanol. This suppression is not due to a general inhibition of ingestive behavior, as the injections had little impact on the consumption of water or food. The fact that this phenomenon occurs through stimulation specifically of the PF/LH, rather than areas reached by drug reflux up the implanted cannula, is supported by our additional finding that opioid injections dorsal to the PF/LH have little impact on consumption and by published evidence showing that microinjections of 0.5 μ l solutions remain relatively confined to the injection site (Melzacka et al., 1985; Pecina and Berridge, 2005; Schroeder et al., 1991). Together, these results support the possibility that opioid peptides in the PF/LH of rats have a specific function in reducing ethanol drinking in animals trained to drink it.

Possible differential roles of δ -, μ -, and κ -receptors in controlling ethanol intake

The results presented in this study indicate that the inhibitory effect on ethanol drinking can be achieved through activation in the PF/LH of any of the three opioid receptors, δ , μ and κ , with drinking selectively suppressed during the first hour after injection of DALA, DAMGO or U-50,488H. Further examination of the results focused attention on the δ -receptor, with the current doses of DALA apparently producing the strongest effect and most prolonged response. This specific effect of the δ -receptor in the PF/LH is consistent with other studies showing that δ -receptor activation in specific brain regions can produce a strong suppression of motivational states and feeding responses (Katsuura and Taha, 2010; Kelley et al., 1996; Margolis et al., 2008). Moreover, mice lacking the δ -receptor show increased preference for ethanol (Roberts et al., 2001), and peripheral administration of a δ -receptor agonist reduces ethanol consumption and preference (van Rijn and Whistler, 2009). The shorter-lasting suppressive effect of the μ -agonist may be related to the well-known competing action of the μ -receptor in enhancing positive motivation (Kelley et al., 2002), which may curtail the response, and the inhibitory effect of the κ -agonist may reflect the role of the κ -receptor in producing aversion, which can reduce the consumption of drugs such as ethanol (Barson et al., 2010; Wee and Koob, 2010). Overall, our results with opioid injections in the PF/LH reveal a consistent suppressive effect on ethanol drinking, which occurs with all three receptor subtypes.

Site-specific mechanisms determining the opioid-induced effects

There is evidence suggesting that the differential effects, a stimulation and suppression, of opioids on consummatory behavior may depend on a variety of factors in addition to the specific receptor subtype, including the specific brain area and local neurochemical systems

through which the opioids may act. This has been suggested by studies in the ventral pallidum, where the inhibitory effect of an opioid agonist on consummatory behavior is thought to be mediated by direct inhibition of postsynaptic sites (Kemppainen et al., 2012) and the stimulatory effect on behavior may be mediated by its presynaptic disinhibition of GABA release (Tang et al., 2005). In the PF/LH, it is possible that the suppressive effect of the opioids on ethanol intake may occur, in part, through their interaction with local populations of neurons that express orexigenic peptides, such as OX, that are known to promote ethanol drinking. There is extensive evidence supporting a role for OX in the stimulation of appetitive behavior, including the drinking of alcohol (Aston-Jones et al., 2010; Sakurai et al., 1998), with hypothalamic injection of OX found to enhance ethanol intake (Schneider et al., 2007) and local expression and levels of OX stimulated by ethanol exposure or an ethanol-paired cue (Dayas et al., 2008; Morganstern et al., 2010). An inhibitory effect of the opioids on the OX neurons in the PF/LH has been demonstrated by studies showing withdrawal from the general opioid agonist, morphine, to increase OX expression (Georgescu et al., 2003; Zhou et al., 2006), and administration of ENK and DYN as well as morphine to hyperpolarize OX neurons (Li and van den Pol, 2006, 2008). There is additional evidence suggesting that the opioids in the PF/LH may suppress ethanol intake through inhibition of the local glutamatergic system. Behavioral studies demonstrate that glutamate agonists in the PF/LH can stimulate ethanol drinking (Chen et al., 2012), and electrophysiological studies show that opioid agonists decrease the firing of OX neurons, in part, through inhibition of synaptic glutamate release (Li and van den Pol, 2006, 2008). This evidence supports the idea that the actions of opioids in the PF/LH may differ from their actions in other areas due to the specific local neurochemical circuitry that is affected and that their suppression of ethanol intake may be mediated by inhibitory effects on the OX and glutamate systems in the PF/LH. With this evidence suggesting that site-specific mechanisms may underlie the actions of opioids, one may ask whether the intake of substances other than ethanol, such as sucrose or saccharin, is also affected by the PF/LH opioids. Since OX injection in the ventricles increases sucrose and saccharin intake (Baird et al., 2009; Furudono et al., 2006) and OX knockout mice consume less sucrose solution than wild-type (Matsuo et al., 2011), it is possible that opioids in the PF/LH act similarly through the OX system to reduce the consumption of both sucrose and saccharin. Further studies are needed to establish the generalizability of these inhibitory actions of the opioids.

Functional significance of PF/LH opioids

The suppressive effect of PF/LH opioids on periods of ethanol drinking may have an important function in controlling the overall amount of ethanol consumed. Whereas opioids in most brain regions enhance drinking and may function within a positive feedback circuit to cause a prolonged increase in ethanol intake (Barson et al., 2010; Chang et al., 2007), the PF/LH opioids may serve as a negative feedback signal for preventing the overconsumption of this substance. This protective role of PF/LH opioids mirrors the actions of the δ -receptor in certain other brain regions, which when activated can reduce excess ethanol intake (Margolis et al., 2008) and suppress emotional responses such as anxiety that can further promote ethanol drinking (Perrine et al., 2006; Saitoh et al., 2004; Saitoh et al., 2005). With the PF/LH known to have a primary function in integrating diverse signals mediating consummatory behavior (Berthoud and Munzberg, 2011), the present finding suggests that the opioid peptides, after acting in different brain areas to stimulate consumption, can provide an inhibitory signal to the PF/LH to prevent over-activation and restore balance to this system.

Conclusions

Our results in rats trained to drink ethanol show that opioids in the PF/LH can specifically suppress ethanol drinking. We propose that the functional role of the opioid receptors and their endogenous ligands is mediated, in part, by the inhibition of local orexigenic systems in the PF/LH, where these receptors may provide an important inhibitory signal for controlling opioid actions in other brain regions. This specific inhibitory action in the PF/LH, which is clearly distinct from the opioids' appetitive actions in other brain areas, may help to explain the inconsistent effects sometimes observed with opioid antagonist therapy for alcoholism.

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(A)

(B)

Figure 1.

Injection sites of animals included in the analysis. (A) Sites for PF/LH injections, as indicated by black dots. (B) Sites for anatomical control injections. Adapted from *The Rat Brain*, compact 6th edition, G. Paxinos and C. Watson, Copyright 2007.

Figure 2.

DALA injected in the PF/LH significantly decreased ethanol intake for up to 4 h after injection, but it had no effect on food intake and increased water intake at 2 h and 4 h after injection of the higher dose (7.1 nmol: $n = 11/\text{group}$; 3.6 nmol: $n = 10/\text{group}$). Values are mean $\pm$ S.E.M.. The vehicle intake presented is the average from the 2 sets of injections. $^*p < 0.017$, $^{\#}p < 0.050$ vs. individual paired vehicle injection at specific time points.

Figure 3.

DAMGO injected in the PF/LH significantly suppressed ethanol intake for 1 h post-injection, while having no effect on food or water intake (2.9 nmol: $n = 11/\text{group}$; 1.5 nmol: $n = 6/\text{group}$). Values are mean $\pm$ S.E.M.. The vehicle intake presented is the average from the 2 sets of injections. * $p < 0.017$, vs. individual paired vehicle injection at specific time points.

Figure 4.

U-50,488H injected in the PF/LH dose-dependently and significantly decreased ethanol intake for 1 h post-injection, without affecting food or water intake (5.4 nmol: $n = 7/\text{group}$; 2.7 nmol: $n = 5/\text{group}$). Values are mean $\pm$ S.E.M.. The vehicle intake presented is the average from the 2 sets of injections. $*p < 0.017$, $\#p < 0.050$ vs. individual paired vehicle injection at specific time points.

Figure 5.

Injection of the general opioid receptor antagonist, m-naloxone, in the PF/LH significantly increased ethanol intake for 1 h post-injection, but left food and water intake unaffected ($n = 9/\text{group}$). Values are mean $\pm$ S.E.M.. $\#p < 0.050$, vs. vehicle injection at specific time points.

TABLE 1

DALA, DAMGO, U-50,488H, and m-naloxone injected 2.0 mm dorsal to the target area produced no change in ethanol, food, or water intake, showing that effects were region-specific.

Nutrient	Hour 1	Hour 2	Hour 4
Ethanol (g/kg)			
Vehicle	0.17 ± 0.03	0.36 ± 0.07	0.54 ± 0.09
DALA 7.1 nmol	0.17 ± 0.03	0.35 ± 0.08	0.53 ± 0.10
Food (g)			
Vehicle	9.40 ± 0.94	10.68 ± 0.78	17.73 ± 1.28
DALA 7.1 nmol	10.13 ± 0.75	10.99 ± 0.62	18.19 ± 0.83
Water (ml)			
Vehicle	9.41 ± 0.62	12.85 ± 1.01	22.31 ± 1.15
DALA 7.1 nmol	10.26 ± 0.48	12.79 ± 0.57	23.40 ± 0.66
Ethanol (g/kg)			
Vehicle	0.43 ± 0.10	0.85 ± 0.21	1.30 ± 0.32
DAMGO 2.9 nmol	0.30 ± 0.09	0.56 ± 0.15	1.06 ± 0.34
Food (g)			
Vehicle	9.91 ± 1.78	11.54 ± 2.17	17.46 ± 2.39
DAMGO 2.9 nmol	8.66 ± 0.72	10.24 ± 1.12	15.23 ± 1.13
Water (ml)			
Vehicle	3.83 ± 1.52	6.04 ± 2.01	11.43 ± 4.10
DAMGO 2.9 nmol	5.64 ± 1.75	8.06 ± 2.20	13.03 ± 3.74
Ethanol (g/kg)			
Vehicle	0.21 ± 0.04	0.41 ± 0.10	0.63 ± 0.22
U-50,488H 5.4 nmol	0.14 ± 0.04	0.28 ± 0.06	0.43 ± 0.09
Food (g)			
Vehicle	9.94 ± 1.14	10.88 ± 0.83	15.12 ± 1.58
U-50,488H 5.4 nmol	9.86 ± 0.96	10.60 ± 0.61	15.46 ± 0.87
Water (ml)			
Vehicle	6.80 ± 1.21	10.80 ± 1.19	17.60 ± 2.67
U-50,488H 5.4 nmol	7.22 ± 0.46	12.10 ± 1.04	20.74 ± 0.90
Ethanol (g/kg)			
Vehicle	0.23 ± 0.03	0.38 ± 0.03	0.60 ± 0.05
m-Naloxone 2.1 nmol	0.21 ± 0.05	0.40 ± 0.08	0.55 ± 0.08
Food (g)			
Vehicle	9.47 ± 0.48	11.45 ± 0.72	15.78 ± 0.39
m-Naloxone 2.1 nmol	8.70 ± 0.84	11.52 ± 0.16	16.25 ± 0.66
Water (ml)			
Vehicle	7.52 ± 1.16	12.65 ± 1.22	20.22 ± 1.57
m-Naloxone 2.1 nmol	7.22 ± 0.89	12.50 ± 0.60	19.63 ± 0.94

Values are mean ± S.E.M.. DALA: n = 8/group; DAMGO: n = 7/group; U-50,488H: n = 5/group; m-Naloxone: n = 6/group.