



## Short communication

## Feeding association between the nucleus of the solitary tract and the ventral tegmental area

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## ABSTRACT

Male Sprague–Dawley rats were fitted with two cannulae in the VTA and one cannula in the NTS for co-administration of the  $\mu$ -opioid receptor agonist DAMGO in one site and the opioid antagonist naltrexone in the other. Injection of DAMGO into the VTA or the NTS stimulated feeding. The increase in food intake after DAMGO injection into the VTA was decreased following injection of naltrexone into the NTS. Furthermore, the increase in food intake after DAMGO injection into the NTS was decreased following injection of naltrexone into the VTA. These results suggest an opioid-mediated feeding association between the VTA and NTS.

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Feeding reflects the interaction of external environmental influences and internal neurochemical and neurobiological processes (Barbano & Cador, 2005). Indicators of energy status, including levels of glucose, insulin, leptin, and catecholamines are involved in the regulation of feeding (Saper, Chou, & Elmquist, 2002; Schwartz, Woods, Porte, Seeley, & Baskin, 2000) via function-specific, interacting brain nuclei (Kim, Quinn, Levine, & O'Hare, 2004; Orsini, 2003). Two such nuclei, the nucleus of the solitary tract (NTS) and the ventral tegmental area (VTA) are important nuclei involved in the control of feeding (Kotz, Billington, & Levine, 1997). The NTS receives first order neuronal projections conveying gustatory and somathetic sensations, and shares neural connections with many brain regions involved in nutrient and energy homeostasis (Li, Davis, & Smith, 2003). The VTA is one of the major nodes in the mesolimbic dopamine pathway, which mediates reward for various behaviors, including feeding (Wightman & Robinson, 2002).

Several studies have demonstrated that both the NTS and the VTA are involved in opioid-mediated feeding. Administration of opioid receptor agonists into the NTS (Giraudo, Kotz, Billington, & Levine, 1988; Kotz et al., 1997) or VTA (Echo, Lamonte, Ackerman, & Bodnar, 2002; Lamonte, Echo, Ackerman, Garrison, & Bodnar, 2002; Noel & Wise, 1993, 1995) result in increased food intake;

conversely administration of opioid antagonists into these nuclei results in decreased food intake (Echo et al., 2002; Giraudo et al., 1988; Kotz, Glass, Levine, & Billington, 2000; Segall & Margules, 1989). Previous investigations have indicated that the  $\mu$ -opioid receptor agonist [D-Ala<sup>2</sup>, NMe-Phe<sup>4</sup>, Gly-ol<sup>5</sup>]-enkaphalin (DAMGO), and non-selective opioid antagonists such as naltrexone (NTX) and naloxone are potent regulators of feeding in rats when injected into the NTS (Giraudo et al., 1988; Kotz et al., 1997) or the VTA (Lamonte et al., 2002; MacDonald, Jewett, Billington, & Levine, 2003; Quinn, O'Hare, Levine, & Kim, 2003). Administration of DAMGO into the rostral or medial NTS increases feeding (Giraudo et al., 1988; Kotz et al., 1997), and administration of NTX into the NTS blocks DAMGO-induced feeding in the central nucleus of the amygdala (CeA) (Giraudo et al., 1988), and neuro peptide-Y-induced feeding in the paraventricular nucleus (PVN) (Kotz et al., 1997). Bilateral injection of DAMGO into the VTA increases food intake (MacDonald et al., 2003; Quinn et al., 2003), and injection of naloxone decreases consumption of sweet solutions in free-fed rats and food-deprived rats (Segall & Margules, 1989). In addition, DAMGO-induced feeding in the VTA is reduced by co-administration of NTX into the VTA (Lamonte et al., 2002), into the nucleus of the accumbens (NAc) (MacDonald et al., 2003), into the PVN (Quinn et al., 2003), and by subcutaneous injection (Ragnauth, Ruegg, & Bodnar, 1997).

It has been shown that  $\mu$ -opioid systems in the NTS and in the VTA are interconnected with other specific brain nuclei (Giraudo

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et al., 1988; MacDonald et al., 2003; Quinn et al., 2003) known to be involved in opioid-mediated feeding. These neuronal pathways include bi-directional opioid–opioid connections of the NTS with the CeA (Giraud et al., 1988), and bi-directional opioid–opioid connections of the VTA with the PVN (Quinn et al., 2003) and the NAc (MacDonald et al., 2003). There is no direct evidence suggesting a neuronal connection between the NTS and the VTA. In fact, conventional wisdom would suggest that the rostral NTS is involved in sensory reward of gustatory signals while the VTA is more involved in incentive motivation. However, Olszewski, Cedernaes, Olsson, Levine, and Schloth (2008), employing an *in situ* hybridization database, have recently reported that molecules involved in feeding were co-expressed in multiple feeding-related sites. This supports a growing body of work by Berthoud (2003, 2004, 2006), Shin, Zheng, and Berthoud (2009), Zheng and Berthoud (2008), and Zheng, Patterson, and Berthoud (2007) which suggests that there might be a greater level of communication between brain nuclei traditionally thought of as being responsible for reward of gustatory signals and those thought responsible for incentive motivation. Consequently, the current study investigated whether reciprocal opioid agonist and antagonist administration in the NTS and the VTA affected feeding in the rat. To assess this interaction, the present study examined the effect of intra-VTA injections of the opioid antagonist NTX on DAMGO-induced feeding in the NTS, and the effect of intra-NTS injections of NTX on DAMGO-induced feeding in the VTA.

## Method

### Subjects

Twelve male Sprague–Dawley rats (Harlan, UK), weighing 225–250 g, were individually housed in conventional wire bottomed cages with the temperature maintained at 23 °C under a 12-h light/12-h dark cycle (lights on at 0800). Subjects were given *ad libitum* access to water and a standard laboratory chow diet (Harlan, UK) until each experimental trial.

### Cannulae implantation

Subjects were anaesthetised with sodium pentobarbital (60 mg/kg) and fitted with 23 gauge stainless steel cannulae (Plastics One, Austin, TX); one placed into the NTS and two placed (bilaterally) into the VTA. It has previously been shown that a unilateral injection of NTX into the VTA does not antagonize food intake following DAMGO injection into the NAc, whereas a bilateral injection of NTX into the VTA antagonises food intake following DAMGO injection into the NAc (MacDonald et al., 2003). Consequently, the bilateral procedure for NTX injection into the VTA was adopted. Stereotaxic co-ordinates (Paxinos & Watson, 1988) with the incisor bar set at 3.3 mm below the intraural line were, NTS; 12.7 mm posterior, –1.4 mm lateral to the bregma and 6.9 mm below the surface of the skull, and VTA; 5.7 mm posterior, ±2.4 mm lateral (angled 10° toward the sagittal suture) to the bregma and 8 mm below the surface of the skull. The injector extended 1 mm beyond the tip of the guide cannula. All animals were allowed one week for recovery from surgery before experimental testing. In order to test the sensitivity of the injection sites, an additional five animals were fitted with a single cannula in the NTS (co-ordinates as above) and were bilaterally cannulated peripherally to the VTA (co-ordinates; 5.7 mm posterior, ±3.2 mm lateral (angled 10° toward the sagittal suture) to the bregma and 7.4 mm below the surface of the skull), and five animals were fitted with bilateral cannulae in the VTA (co-ordinates as above) and a single cannula peripheral to the NTS (co-ordinates; 12.7 mm posterior, –0.5 mm lateral to the bregma and 8 mm below the surface of the skull).

### Experimental procedure

During experimental trials, immediately before drug injection, the laboratory chow diet was removed from the cages. Following injection, pre-weighed chow diet was placed into the cages. Unilateral NTS and bilateral VTA injections were administered in a 0.5 µl volume over 30-s, the antagonist being injected first and the agonist injected after a 30-s interval. At least one day elapsed between injections and each subject received all treatments in a counter-balanced fashion. Food intake was measured at 1-, 2-, and 4-h post-injection and corrected for spillage. Injections were performed between the 2nd and 3rd hour of the light phase of the light/dark cycle.

In the first part of the study, subjects were injected with NTX into the NTS followed by DAMGO injections into each side of the VTA. Subjects were randomly assigned to treatment groups and received either, (a) saline (0.5 µl) into the NTS and saline (0.5 µl) into each side of the VTA, (b) NTX (26.5 nmol, 5 µg/0.5 µl) into the NTS and saline into the each side of the VTA, (c) saline into the NTS and DAMGO (2.4 nmol, 2.5 µg/µl) into each side of the VTA, (d) NTX (6.6 nmol, 1.25 µg/0.5 µl) into the NTS and DAMGO (2.4 nmol, 2.5 µg/µl) into each side of the VTA, (e) NTX (13.2 nmol, 2.5 µg/0.5 µl) into the NTS and DAMGO (2.4 nmol, 2.5 µg/µl) into each side of the VTA, (f) NTX (26.5 nmol, 5 µg/0.5 µl) into the NTS and DAMGO (2.4 nmol, 2.5 µg/µl) into each side of the VTA.

In the second part of the study, subjects were injected with NTX into each side of the VTA followed by DAMGO injection into the NTS. Subjects were randomly assigned to treatment groups and received either, (a) saline (0.5 µl) into each side of the VTA and saline (0.5 µl) into the NTS, (b) NTX (26.5 nmol, 5 µg/µl) into the each side of the VTA and saline into the NTS, (c) saline into the each side of the VTA and DAMGO (2.4 nmol, 2.5 µg/0.5 µl) into the NTS, (d) NTX (6.6 nmol, 1.25 µg/µl) into each side of the VTA and DAMGO (2.4 nmol, 2.5 µg/0.5 µl) into the NTS, (e) NTX (13.2 nmol, 2.5 µg/µl) into each side of the VTA and DAMGO (2.4 nmol, 2.5 µg/0.5 µl) into the NTS, (f) NTX (26.5 nmol, 5 µg/µl) into each side of the VTA and DAMGO (2.4 nmol, 2.5 µg/0.5 µl) into the NTS.

### Histology

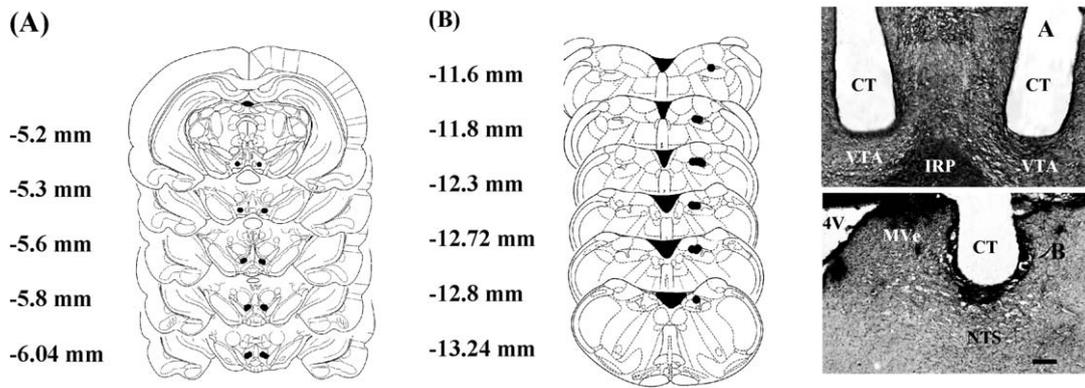
On the completion of these studies, all subjects received an overdose of sodium pentobarbital (60 mg/kg) and were perfused through the ascending aorta with 300 ml of 0.1 M PBS (pH 7.4) followed by 4% paraformaldehyde (w/v) in 0.1 M PBS. The brain was removed and stored in a 10% sucrose (w/v) solution in 0.1 M PBS. For cannula placement verification, coronal sections (50 µm) were cut on a cryostat and mounted on gelatine-coated glass microscope slides. The sections were stained with a 0.1% thionin solution (Sigma, UK), dried and cover-slipped.

### Statistics

Food intake data at each time-point and cumulative food intake in each part of the study were analysed by one-way repeated measures analysis of variance. Post hoc analysis was performed using Fisher's protected least significant difference tests. To verify differences in injection sites a two-way repeated measures analysis of variance was conducted.

## Results

Fig. 1 indicates representative cannulae placements in the NTS and the VTA of the animals included in the core study. The NTS cannulae terminated in a region 11.6–13.24 mm posterior to bregma, and the VTA cannulae terminated in a region 5.2–6.04 mm posterior to bregma (Paxinos & Watson, 1988). In the first part of



**Fig. 1.** Location of cannulae terminations in the VTA (A) and the NTS (B) and representative photomicrographs of cannula placements in the VTA and the NTS. CT: cannula tract; 4V: fourth ventricle; IPR: interpeduncular nucleus; MVe: medial vestibular nucleus. Scale bar = 100  $\mu$ m.

the study, there was no significant effect of treatment on food intake at 1-h, however there were significant treatment effects on cumulative food intake at 2- and 4-h post-injection (Fig. 2, 2-h,  $F_{5,55} = 4.312$ ,  $p = 0.012$ , and 4-h,  $F_{5,55} = 10.302$ ,  $p = 0.0001$ ). Bilateral administration of DAMGO (2.4 nmol) into the VTA significantly increased cumulative food intake at 2- and 4-h post-injection as compared to VTA saline injections. Administration of NTX (26.5 nmol) into the NTS significantly reduced DAMGO-induced food intake at 2- and 4-h post-injection, and 13.2 nmol NTX in the NTS significantly reduced food intake at 4-h post-injection.

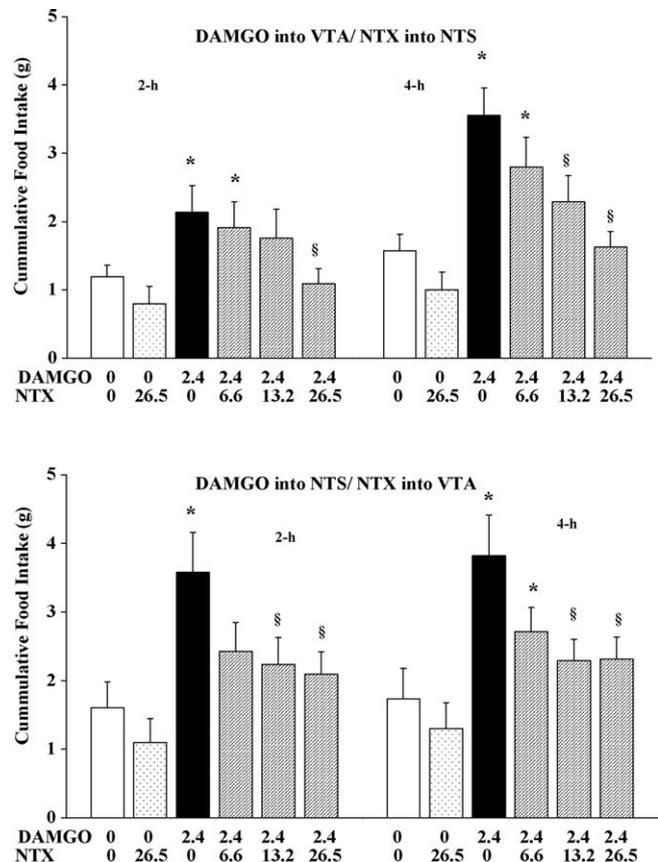
In the second part of the study, there was no significant treatment effect on food intake at 1-h, however there were significant treatment effects on cumulative food intake at 2- and 4-h post-injection (Fig. 2, 2-h,  $F_{5,55} = 4.834$ ,  $p = 0.0026$ , and 4-h,  $F_{5,55} = 4.408$ ,  $p = 0.0083$ ). Administration of DAMGO (2.4 nmol) into the NTS significantly increased cumulative food intake at 2- and 4-h post-injection. Bilateral administration of NTX into the VTA at 13.2 and 26.5 nmol significantly decreased NTS DAMGO-induced food intake at 2- and 4-h post-injection. In addition, comparisons between the saline/saline control group and the DAMGO and 13.2 and 26.5 nmol NTX combination groups showed no significant differences in food intake (2-h: 13.2 nmol VTA NTX,  $p = 0.367$ , 26.5 nmol VTA NTX,  $p = 0.485$ ; 4-h: 13.2 nmol VTA NTX,  $p = 0.449$ , 26.5 nmol VTA NTX,  $p = 0.428$ ). A two-way repeated analysis of variance test indicated that there was a significant site difference ( $F_{1,22} = 8.901$ ,  $p = 0.0083$ ) in 2-h post-injection food intake only, but that there was no interaction between site and food intake at this time-point ( $F_{5,110} = 1.284$ ,  $p = 0.2813$ ).

In the animals that were cannulated peripherally to the VTA and the NTS, NTX administration (26.5 nmol) in either site did not significantly reduce DAMGO-induced (2.4 nmol) feeding in the alternative site (NTS-peripheral VTA, 0–4-h food intake: DAMGO/saline;  $2.63 \pm 0.69$  g, DAMGO/NTX;  $2.45 \pm 0.64$  g; VTA-peripheral NTS, 0–4-h food intake: DAMGO/saline;  $2.84 \pm 0.61$  g, DAMGO/NTX;  $2.78 \pm 0.72$  g;  $p$ 's > 0.05).

**Discussion**

The current study investigated the possibility of an opioid-mediated association between the NTS and the VTA in the control of feeding. Bilateral injections of the  $\mu$ -opioid receptor agonist DAMGO into the VTA, or unilateral injection of DAMGO into the NTS stimulated feeding, observations in agreement with previous studies (Giraud et al., 1988; Kotz et al., 1997; MacDonald et al., 2003; Quinn et al., 2003). The current study also investigated whether increased food intake stimulated by DAMGO injected into either the NTS or VTA was decreased by injection of varying

concentrations of the opioid antagonist NTX into the non-DAMGO injected site. Bilateral injections of NTX (13.2 and 26.5 nmol) into the VTA decreased DAMGO-induced feeding in the NTS, and injection of NTX into the NTS decreased DAMGO-induced feeding in the VTA. These findings do not suggest a direct opioid pathway between the VTA and the NTS, as it is feasible that the effects of the opioid manipulations observed were due to indirect communication via other sites (Garzon & Pickel, 2004), or other neurotransmitters (Khairnova et al., 2004; Laviolette & van der Kooy, 2004; Laviolette, Gallegos, Henriksen, & van der Kooy, 2004). However,



**Fig. 2.** Effects of NTX (0, 6.6, 13.2 and 26.5 nmol) injections into the NTS on food intake induced by bilateral DAMGO injection (2.4 nmol) into the VTA, and effects of bilateral NTX (0, 6.6, 13.2 and 26.5 nmol) injections into the VTA on food intake induced by DAMGO (2.4 nmol) injection into the NTS. Values are means  $\pm$  S.E.M. \* $p < 0.05$  as compared to saline injection in both areas and § $p < 0.05$  as compared with DAMGO injection into the VTA and saline injection into the NTS ( $n = 12$  per group).

they strongly suggest a reciprocal opioid-mediated association between the NTS and the VTA, which is of particular interest given the known involvement of the NTS in gustatory reward of sensory signals and the role of the VTA in incentive motivation. This is not surprising, as there is a hypothalamic loop to the parabrachial nucleus, NTS, VTA and back to the hypothalamus, which is influenced by opioids at each step of the way.

Opioid peptides have consistently been shown to be involved in feeding and their receptors can be found in the mesolimbic and mesostriatal systems, in the hypothalamus, and the NTS (Aicher, Goldberg, Sharma, & Pickel, 2000; Davis & Kream, 1993; Ding, Kaneko, Nomura, & Mizuno, 1996; Mansour, Akil, & Watson, 1995; Nomura, Ding, Kaneko, Li, & Misuno, 1996). Research has shown that with respect to feeding, the  $\mu$ -opioid system in the VTA (e.g., MacDonald et al., 2003; Quinn et al., 2003) and in the NTS (e.g., Giraudo et al., 1988) shares a functional relationship with  $\mu$ -opioid systems in the NAc, the PVN, and the CeA. The opioid systems in these areas also appear to interact with the dopamine and GABA systems with regard to feeding regulation (Echo et al., 2002; Huang, Wang, & Pickel, 2000; Laviolette et al., 2004; MacDonald, Billington, & Levine, 2004). Traditionally, opioid-mediated feeding in the NTS has been considered to relate to sensory reward of gustatory signals, while the activity of the VTA contributes to incentive motivation. However, the concept of discrete brain nuclei being independently involved or associated simply with reward of gustatory signals, or incentive motivation appears increasingly unlikely. Thus data presented here, along with previous findings, have begun to challenge and contradict the concept of function-specific discrete brain nuclei mediating feeding behavior. For example, opioid receptor antagonists reduce hunger-driven feeding induced by food deprivation (Hayward & Low, 2001; Koch, Glass, Cooper, & Bodnar, 1995), and the  $\mu$ -opioid receptor plays a greater role in motivation to seek food than in the reward aspect of ingestion (Papaleo, Kieffer, Tabarin, & Contarino, 2007). A recent report by Olszewski et al. (2008) and considerable work by Berthoud (2003, 2004, 2006), Shin et al. (2009), Zheng and Berthoud (2008) and Zheng et al. (2007) also questions the concept of specific brain sites as independent centers. The findings of the current study similarly suggest that feeding, mediated by both sensory reward and incentive motivation drivers, and by exchanges of information between sites such as the VTA and NTS, might be regulated in a more interdependent manner than previously thought.

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