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Centrally administered orexin A increases motivation for sweet pellets in rats

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Abstract *Rationale:* Centrally administered orexin A induces both feeding and locomotion in rats. Thus, the feeding response following orexin A administration may be secondary to general increases in activity rather than a specific motivation to eat. *Objective:* The aim of the study is to determine whether orexin A increases the motivation to eat. *Methods:* The effect of orexin A (0, 31.25, 62.5, 125, 250, and 500 pmol) on breakpoint was determined in male Sprague–Dawley rats with rostral-lateral hypothalamic cannulae under a progressive ratio of five schedule (PR5). The effect of orexin A (0, 31.25, 125, and 500 pmol) on pressing rate under a fixed ratio (20) schedule was obtained to analyze the time course of orexin-A-induced pressing. The effect of 24-h food deprivation on breakpoint under PR5 and the effect of orexin A (125 pmol) on free feeding (sweet pellets) and on open-field locomotor

activity (0, 100, 500, and 1,000 pmol) were also tested. *Results:* Orexin A significantly augmented free feeding of sweet pellets, open-field locomotor activity, rate of pressing (FR20 schedule), and breakpoint (PR5 schedule), although compared to 24-h deprivation, the effect of orexin A on breakpoint was mild. However, there was a differential dose response relationship and time course of stimulation between orexin A's effects on locomotion and lever pressing. *Conclusion:* These data indicate that infusion of orexin A enhances free feeding by enhancing and possibly prolonging motivation to eat.

Keywords Hypocretin · Progressive ratio · Fixed ratio · Neuropeptide · Lateral hypothalamus · Deprivation · Feeding

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Introduction

The manifestation of appetite and feeding behavior is the result of the coordinated communication between brainstem, hypothalamic, and other central nervous system nuclei as well as peripheral circulating signals. Understanding the neurochemical encoding of appetite within the central nervous system is a valuable component of developing treatment strategies for obesity. The lateral hypothalamic area (LHA) has long been implicated as an important part of the network that regulates feeding behavior (Bernardis and Bellinger 1996). Thus, upon discovery of the novel peptides orexin A and orexin B (33 and 28 amino acid residue peptides cleaved from a common precursor), produced in neurons located in the LHA, the logical supposition was that the orexins may play a role in the neurochemical encoding of appetite and feeding (de Lecea et al. 1998; Sakurai et al. 1998).

It has become clear that orexins play an important role in maintaining and regulating states of arousal (Hagan et al. 1999; Mignot 2001; Sutcliffe and De Lecea 2002). For instance, the canine condition of narcolepsy is caused by a mutation in the *canarc-1* gene, which encodes the orexin receptor, OX2R (Lin et al. 1999). Indeed, loss of orexin neurons

may be responsible for the human condition of narcolepsy (Nishino et al. 2000; Peyron et al. 2000; Thannickal et al. 2000). In rodent studies, increases in orexin signaling and neural activity appear to be coincident with changes in the light phase, with orexin signaling highest during the dark/active phase (Fujiki et al. 2001; Yoshida et al. 2001). In line with these data, central infusion of orexins decreases REM and increases arousal (Hagan et al. 1999). Interestingly, sensitivity to orexin-A-increased locomotion is not light phase sensitive as is orexin-A-induced feeding (Kotz et al. 2002).

Ample evidence supports the hypothesis that orexins also play an important role in modulating appetite. Orexin-containing neurons show increased *c-fos* immunoreactivity following a 48-h fast, orexin receptors are expressed in hypothalamic nuclei, and orexin and neuropeptide Y (NPY) neurons appear to have reciprocal connectivity (Backberg et al. 2002; Broberger et al. 1998; Cai et al. 1999; Cluderay et al. 2002; Muroya et al. 2004). Antagonism of the orexin-1 receptor inhibits feeding (Haynes et al. 2000). However, reports of orexin-induced feeding have been inconsistent, with feeding behavior sometimes seen following orexin A administration and not orexin B, or not at all (Haynes et al. 1999; Ida et al. 1999; Marsh et al. 1999; Sunter et al. 2001). Furthermore, orexin-induced feeding behavior has typically not been as robust as that for other orexigenic neuropeptides such as NPY (Edwards et al. 1999; Haynes et al. 1999; Sahu 2002).

It was our aim to examine the extent to which orexin-A-induced feeding was the result of a motivation to eat. To this end, a progressive ratio (PR) schedule of reinforcement was used to examine the amount of work rats were willing to exert, or their motivation, for a reinforcer following centrally administered orexin A. PR schedules are well suited to evaluate the relative reinforcing efficacy of stimulants (Arnold and Roberts 1997; Richardson and Roberts 1996) and have been used to evaluate the relative reinforcing efficacy of appetitive stimulants such as food deprivation, opioids, and neuropeptide Y (Hodos 1961; Hodos and Kalman 1963; Jewett et al. 1995; Zhang et al. 2003). In the present experiment, food deprivation was used as a positive control to which any changes in orexin-induced motivation to eat could be compared. Additionally, a fixed ratio schedule of reinforcement (FR20) was used to analyze the time course of orexin-A-augmented lever pressing, and the effect of orexin A on spontaneous open-field locomotor activity was assessed to analyze the time course of orexin-A-augmented activity.

Materials and methods

Subjects

Male Sprague–Dawley rats (Harlan, Madison, WI), weighing 250–275 g at the beginning of the experiments, were individually housed in hanging wire bottom cages (17.8×17.8×24.8 cm) on a 12-h light/dark cycle, with lights on at

0700 hours and lights off at 1900 hours, in a climate-controlled room (21.7°F). Experiment 1 initially began with 20 rats, experiment 2 with 22 rats, and experiment 3 with ten rats. Rat chow (Harlan Teklad 8604, average content 24.5% protein, 4.4% fat, 71.1% carbohydrate, 3.93 kcal/g) and water were available ad libitum unless otherwise specified. Current studies received local institutional animal care and use committee approval.

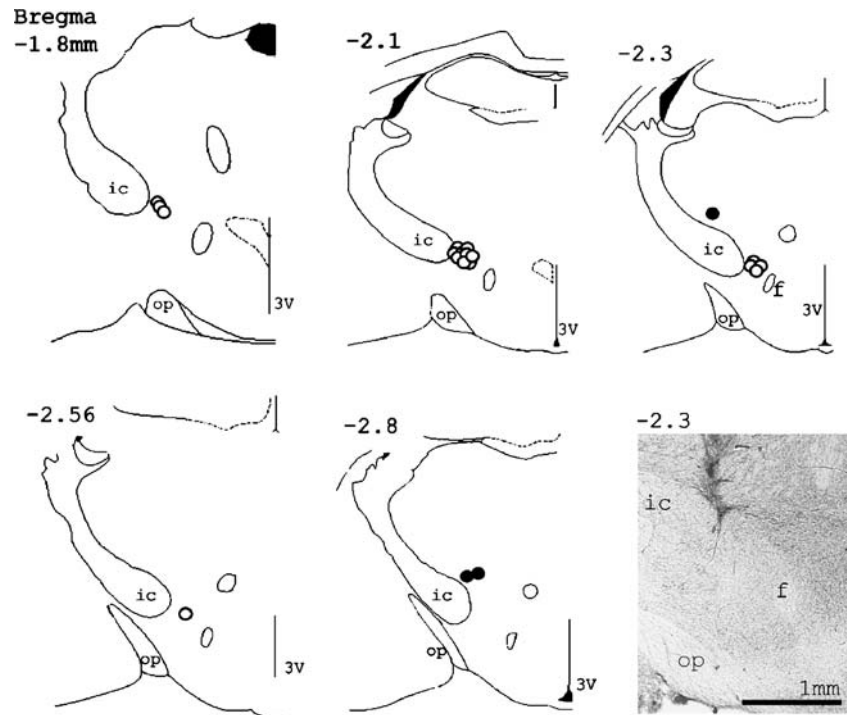
Surgery and drug infusions

Rats were anesthetized with a combination of ketamine (90 mg/kg) and xylazine (15 mg/kg) and implanted with unilateral stainless-steel cannulae (Plastics One, Roanoke, VI). Cannulae were held in place with dental cement (Dentsply, York, PA) and three anchor screws (Plastics One) that were embedded into the skull. Flunixin (2.5 mg/kg Burn's Veterinary Supply, Westbury, NY) was administered as a postoperative analgesic. Cannulae were directed 0.5 mm above the rostral lateral hypothalamic area (−2.2 mm posterior, ±1.9 mm lateral, 7.2 mm ventral; relative to bregma). We previously demonstrated that orexin A injected into this site robustly stimulates feeding when compared to orexin A injected into other sites along the rostral–caudal axis of the lateral hypothalamus (Thorpe et al. 2003), suggesting that the behavioral effects seen following orexin A injection into this aspect of the central nervous system are acting at, or within, a 1-mm radius of the present coordinates. The incisor bar was set at −3.3 mm for all surgeries. Dummy cannulae, designed such that they extended to the tip of the guide cannulae, were inserted into the guide cannulae and left in place until injection. Infusions were made manually with a microsyringe (Hamilton Company, Reno, NV) attached to polyurethane tubing (PE20) back-filled with sterile water. A 2- μ l air bubble separated the drug from the sterile water. Injectors (33 g; Plastics One) extended 1.0 mm beyond the tip of the guide cannulae (26 g). Infusions, 0.5 μ l per injection, took place over 30 s with the injector left in position for an additional 15 s to allow for diffusion.

Cannula verification

Correct cannulae placement was determined with postmortem histological verification (see Fig. 1 for experiment 1 placement). Data from four rats were excluded from the final analysis of experiments 1a and 1b, two due to misplaced cannulae (shown in Fig. 1) and two due to loss of the implant during the course of the study (one of the animals that lost a cannula also had a misplaced cannula; see Fig. 1). Data from two additional rats were excluded from experiment 1c due to loss of implant. Data from one rat were excluded from experiment 3 due to a misplaced cannula.

Fig. 1 a Schematic representation of microinjection locations (tips of injectors) for experiment 1. Closed circles represent incorrect placement; open circles represent correct placement. *ic* Internal capsule, *3V* third cerebroventricle, *op* optic tract. Schematic drawings derived from *The Rat Brain in Stereotaxic Coordinates* (Paxinos 1998). b Photomicrograph of a representative microinjection site (tip of injector)



Apparatus

Operant conditioning experiments took place in 18 two-lever operant conditioning chambers designed for rats (l=30.5 cm; h=24 cm; w=21.0 cm; Coulbourn Instruments, Lehigh Valley, PA) housed in sound-attenuating chambers. MED-PC software (MED Associates, East Fairfield, VT) and was employed to control operant conditioning contingencies and to perform data collection. Locomotor activity was assessed in 43.2×43.2 cm open-field activity chambers equipped with three 16 beam infrared arrays (MED Associates). Data collection and processing was performed using “Open Field Activity Software” (MED Associates).

Training

Following a 1-week acclimation period, rats were exposed daily to the reinforcer, sweet pellets, used in the operant studies (45-mg Dustless Precision Pellets, 4.00×3.3 mm, content=18.5% protein, 61.5% carbohydrate, 4.9% fat, 3.62 kcal/g; Bio-Serv, Frenchtown, NJ). Rats were food deprived overnight for the initial training trial. Training began on a fixed ratio (FR1) schedule where reception of a sweet pellet was contingent upon a single press of either the left or right lever. The session ended after 60 min or reception of 20 pellets, whichever came first. Upon successful completion of the FR1 schedule for three consecutive sessions (i.e., reception of 20 pellets prior to session expiration), the ratio was stepwise increased to an FR5, where reception of each pellet is contingent upon five lever presses. Upon successful completion of FR5, rats were subsequently trained on a progressive ratio schedule beginning

with a PR1, then a PR3, and finally a PR5. Only the left lever was presented during PR sessions, and there was a 2-h cutoff time for the PR studies. Rats did not reach the 2-h cutoff under the present experimental conditions.

Under a PR schedule of reinforcement, the number of lever presses required to receive a reinforcer (sweet pellet) is sequentially increased after reception of each additional reinforcer. The first pellet is delivered after five presses; thereafter, each successive pellet requires five more presses than the previous pellet. The session ended when the animal did not receive a pellet for 30 min. The number of presses required for the last pellet received is termed breakpoint and is thought to correlate to a change in motivation to eat. Mock injections were carried out daily where dummy cannulae were removed and reinserted. Following the mock injections, animals were returned to their home cage for 25 min, with chow removed prior to placement in operant chambers. Thus, animals were habituated to the conditions under which injections would take place. Experimental treatments began when breakpoints were stable for seven consecutive days. All sessions took place between 1230 and 1430 hours.

Experiments

Experiment 1

- (a) Rats implanted with rostral LHa (rLHa) cannulae received orexin A (0, 125, 250, and 500 pmol, American Peptides, Sunnyvale, CA; dissolved in artificial cerebrospinal fluid) in a Latin square design such that each treatment was given during each session and

every animal received each treatment ($n=16$). With this design, all possible treatment orders are represented so that treatment order does not confound results. Following injection, animals were returned to their home cage for 25 min to avoid any sedative effects resulting from high doses of orexin A, as were previously reported to occur immediately following infusions (Rodgers et al. 2000), where food had been removed. Animals were then placed in the operant conditioning chambers where breakpoint was determined as described above. Breakpoint was assessed for at least 2 days between treatments to ensure a return to their baseline breakpoint. One week following the above-described experiment, a lower dose was included due to the efficacy of orexin A at the previous lowest doses. Thus, orexin A (0, 62.5, and 125 pmol) was administered as described above, and means were calculated for the 0- and 125-pmol doses in the two studies for use in statistical analyses. Final analysis included the following doses: 0, 31.25, 62.5, 125, 250, and 500 pmol orexin A.

- (b) Thirteen days following experiment 1a, as a positive control, the effect of 24-h food deprivation on breakpoint was evaluated. Animals were deprived in a crossover design. Thus, on the two experiment days, half of the animals were food deprived and half were maintained on an ad libitum diet. At least 72 h elapsed between sessions, with breakpoint determined between sessions to ensure a return to baseline and during the 13 days prior to the beginning of the experiment.
- (c) After experiment 1b, rats were placed on a fixed ratio schedule of reinforcement where reception of a single sweet pellet was contingent upon 20 lever presses (FR20). Sessions ran for 1 h. Stable baseline pressing was defined as five consecutive days with approximately 5% variation in response. After animals had achieved a stable baseline response (20 days), orexin A (0, 31.25, 125, and 500 pmol) was injected intra-rLHA in a counterbalanced design as described above. Rate of pressing was recorded in 5-min bins and later processed as 20-min bins for statistical analysis.

Experiment 2

In a separate group of rats ($n=22$), free access to sweet pellets was granted 2 h daily at 1300 hours for 3 days, prior to treatment sessions. Thus, rats were acclimated to the sweet pellets prior to experimental sessions. Animals received orexin A (0 or 125 pmol dissolved in artificial cerebrospinal fluid, 0.5 μ l per injection) in a randomly assigned crossover design such that each treatment was given during each treatment session and every animal received each treatment. Similar to the design used in experiment 1, both possible treatment orders were represented so that treatment order did not confound results. Following injection, animals were returned to their home cage where food access was removed. Twenty-five minutes after injection, animals were allowed ad libitum access to sweet

pellets and water. Sweet pellet intake was measured 1 h postintroduction of sweet pellets. At least 48 h elapsed between treatments.

Experiment 3

In a separate group of rats ($n=9$), locomotor activity was assessed. Rats were acclimated to the open-field chambers and injection procedures with three mock test sessions (one session per day). During these sessions, animals were handled in the same manner as during injection sessions and placed in the open-field chamber for 2 h. During treatment sessions, animals were injected intra-rLHA with orexin A (0, 100, 500, and 1,000 pmol in 0.5 μ l aCSF) in a within-subjects randomized design as described above. Animals were removed from their home cage, injected, and placed in the open-field activity monitor. After a 20-min acclimation period, locomotor activity was recorded for 1 h. Activity data were placed in 20-min bins for statistical analysis. At least 48 h elapsed between test sessions.

Results

Experiment 1a

Orexin A (125 and 250 pmol) significantly augmented breakpoint and the number of pellets received (Fig. 2). A one-way repeated-measures analysis of variance (ANOVA) was computed using treatment (0, 31.25, 62.5, 125, 250, and 500 pmol orexin A) as the within-subjects factor and breakpoint or number of pellets received as the dependent variables. A main effect of treatment ($F_{5,75}=2.8$, $P=0.023$) was seen. A Fisher's PLSD post hoc test revealed significant differences between 125 pmol orexin A and vehicle ($P=0.019$), as well as 250 pmol orexin A and vehicle ($P=0.015$).

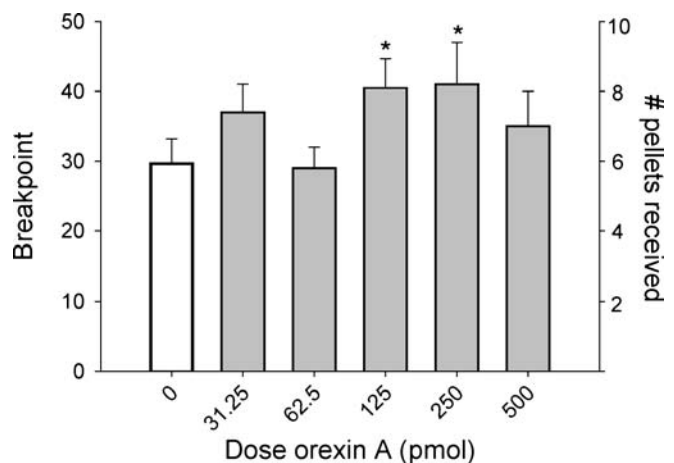
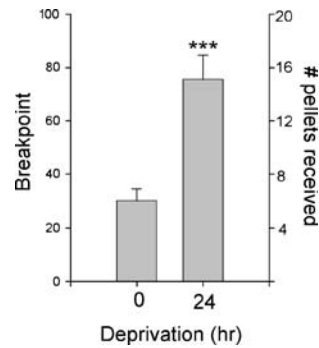


Fig. 2 Under a PR5, breakpoint was augmented by intra-rLHA orexin A. Data represent means \pm standard error. * $P < 0.005$ compared to control

Fig. 3 Twenty-four-hour food deprivation increases breakpoint. Data represent means \pm standard error. *** $P < 0.0005$ compared to control



Analysis of the data from animals with misplaced cannulae indicated that orexin A did not affect breakpoint when injected outside of the target site. Although there were too few animals with misplaced cannulae for sufficient power to detect statistical differences, orexin A injection showed no effect on breakpoint as compared to vehicle when injected into an incorrect site [“incorrect placement” 250 pmol orexin A mean = 27.5 ± 2.5 ; vehicle = 21.3 ± 6.3 ($n=2$); “correct placement” 250 pmol orexin A mean = 40.9 ± 5 , vehicle = 29.7 ± 3.5 ($n=16$)].

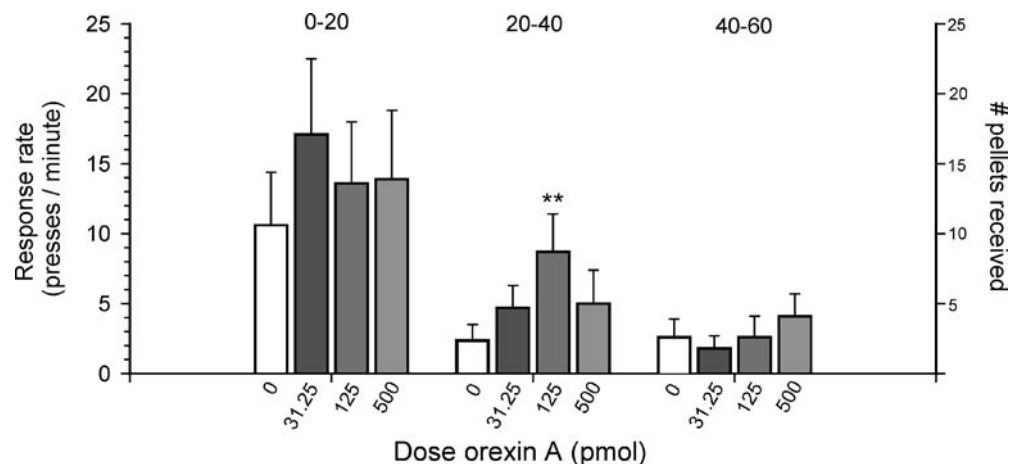
Experiment 1b

Twenty-four hour food deprivation significantly augmented breakpoint (Fig. 3). A one-way repeated-measures analysis of variance (ANOVA) was computed using treatment (ad libitum fed, 24-h food deprivation) as the within-subjects factor and breakpoint as the dependent variable. A main effect of treatment ($F_{1,15} = 38.13$, $P \leq 0.0001$) was seen.

Experiment 1c

Under an FR20 schedule, orexin A (125 pmol) significantly increased the rate of lever pressing during the second third of the test session (Fig. 4). A separate one-way repeated-measures analysis of variance was computed using treatment (0, 31.25, 125, and 500 pmol orexin A) as

Fig. 4 Under an FR20, orexin A augmented rate during the second third of the 1-h session. Data represent means \pm standard error. * $P < 0.05$ compared to control



the within-subjects factor and breakpoint as the dependent variable for each third of the test session (20-min bins). A main effect of treatment was seen during the second third of the test session ($F_{3,42} = 104.1$, $P = 0.032$). Fisher PLSD post hoc test demonstrated that 125 pmol orexin A significantly ($P = 0.0037$) increased rate of pressing compared to vehicle during this time interval. Analysis of 60-min total rates did not yield significant results.

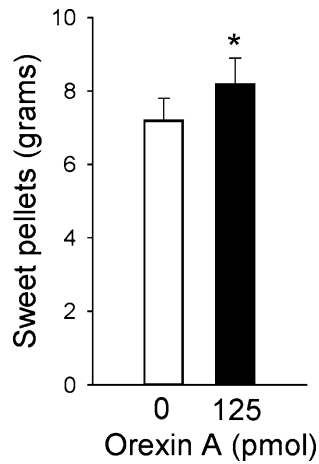
Experiment 2

Orexin A significantly augmented ad libitum feeding of sweet pellets (Fig. 5). A repeated-measures ANOVA was computed using treatment (0 or 125 pmol orexin A) as the within-subjects factor and sweet pellet intake at 1 h after introduction of sweet pellets as the dependent variable. There was a main effect of treatment ($F_{1,20} = 5.03$, $P = 0.036$).

Experiment 3

Orexin A significantly increased spontaneous locomotor activity throughout the 1-h test session (Fig. 6). Separate repeated-measures ANOVAs (per time period) revealed a main effect of treatment (0, 100, 500, and 1,000 pmol orexin A) during all three 20-min bins (0–20 min, $F_{3,24} = 16.9$, $P < 0.00001$; 20–40 min, $F_{3,24} = 4.8$, $P = 0.0096$; 40–60 min, $F_{3,24} = 5.2$, $P = 0.0064$). During the first 20 min of the session, Fisher’s PLSD post hoc analysis demonstrated that locomotor activity after vehicle injection was significantly lower than that after 100 ($P = 0.0003$), 500 ($P < 0.0001$), and 1,000 pmol orexin A ($P < 0.0001$). During the second 20-min bin, Fisher’s PLSD post hoc analysis demonstrated that locomotor activity after vehicle injection was significantly lower than that after 500 pmol orexin A ($P = 0.0013$). During the third 20-min bin, Fisher’s PLSD post hoc analysis demonstrated that locomotor activity after vehicle injection was significantly lower than that after 500 ($P = 0.02$) and 1,000 pmol orexin A ($P = 0.0029$).

Fig. 5 Intra-rLHa orexin A (125 pmol in 0.5 μ l aCSF) significantly increased 1 h free feeding of sweet pellets in the home cage. Data represent means \pm standard error. * P <0.05 compared to control



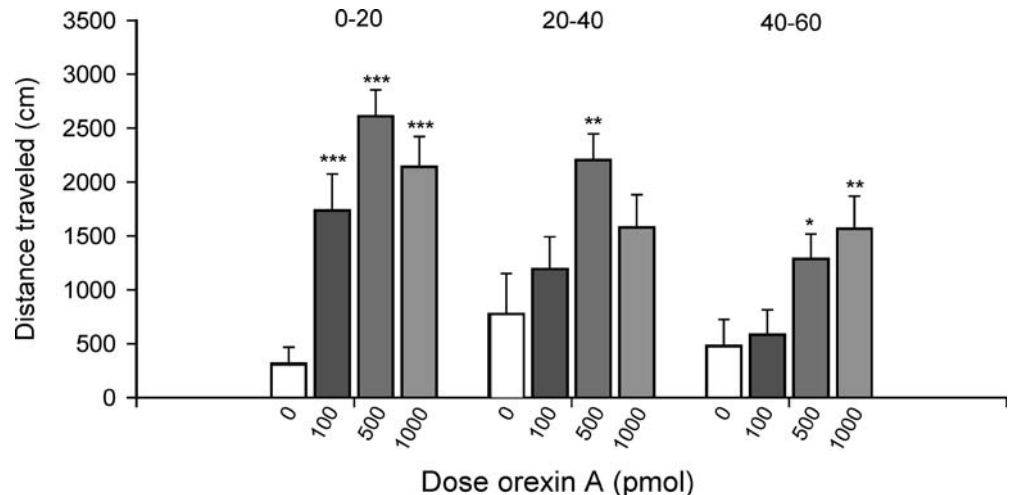
Discussion

Orexin modulation of arousal and activity does not preclude it from having different, yet significant and behaviorally relevant, modulatory functions within other systems, such as the feeding network. Here we demonstrate that orexin A administration to the dorsal portion of the rLHa not only augmented feeding (Fig. 5), but also did so in a feeding-specific, motivated fashion (Fig. 2). Motivation was measured using a progressive ratio of reinforcement (PR5), with sweet pellets as the reinforcer. Relative to vehicle (0 pmol), orexin A (125 and 250 pmol) treatment significantly increased breakpoint. However, the effect on motivated feeding behavior, as measured by a PR5, is mild relative to the effects of 24-h food deprivation (Fig. 3). All doses of orexin A significantly augmented spontaneous locomotor activity during the first 20 min of locomotor assessment (Fig. 6). Five hundred picomoles of orexin A augmented locomotion throughout the entire test session, although this dose did not augment lever pressing under an FR schedule or at any point during a PR schedule. When rats were placed on a fixed ratio schedule of reinforcement (FR20), rate of pressing was augmented in the second third of the test session, indicating that orexin A-stimulated mo-

tivation for sweet pellets occurred approximately 40 min following injection (Fig. 4). However, a similar dose failed to significantly affect locomotor activity at this same time point, indicating that a time course distinction may exist between orexin A's effects on locomotion and feeding. Additionally, the fact that orexin A augmented the rate of pressing between 20 and 40 min under the FR20 schedule, and did not affect pressing during the first 20 min, suggests a prolongation of a motivation to eat. This has otherwise been stated as a "decrease in the onset of satiety" and has been previously observed (Rodgers et al. 2000).

Progressive ratio schedules (PR) were designed as a means to obtain a rate-independent measure of the reinforcing efficacy of differing sweetened solutions (Hodos 1961). PRs have since been modified to study a range of behaviors including intracranial self-stimulation and drug self-administration (Richardson and Roberts 1996). PRs have also been used to examine the effects of drugs on food-motivated behavior (Brown et al. 1998; Jewett et al. 1995; Zhang et al. 2003). Here we designed a PR with arithmetically increasing response requirement (increments of five). Under this schedule, ad libitum fed rats reached a baseline breakpoint of 29, which equates to approximately six sweet pellets (or 0.3 g). Following orexin A (125 pmol), the average breakpoint was 40 or eight sweet pellets (or 0.4 g). Thus, the PR design here examined the relative motivational properties of orexin A independent of interoceptive satiety cues associated with gastric fill. When allowed free access to sweet pellets in their home cage, orexin A (125 pmol) stimulated consumption of 1.1 g above baseline of sweet pellets (Fig. 5). Thus, our data show that orexin A augmented sweet pellet intake by approximately 20% in both a free-feeding situation (no work) and in FR- and PR-pressing paradigms (work contingent). It is possible that different (e.g., "easier") PR schedules may have yielded more robust responses. However, under both "no work" and "work-contingent" conditions, there was a 20% augmentation of food intake after orexin A. Thus, changing the workload did not change the "relative" amount orexin-A-augmented feeding, suggesting that the PR schedule

Fig. 6 Locomotor activity recorded in open-field chambers was significantly increased by orexin A. Data represent means \pm standard error. * P <0.05, ** P <0.005, *** P <0.0005, compared to control



used in the current studies was an appropriate surrogate measure of orexin-A-induced motivation to feed.

In the present study, to ensure that breakpoint was measured during orexin A's window of efficacy, two important controls were implemented. First, in all experiments, following central infusions, the animals were placed back in their home cages for 25 min without access to chow in both the feeding and operant studies or placed in an open-field chamber for 20 min in the locomotor activity assessment sessions. Thus, any sedative effects resultant from high doses of orexin A, as previously reported to occur immediately following infusions (Rodgers et al. 2000), had passed. However, altering the amount of time between the infusion of orexin A and the beginning of the operant conditioning experiment may have had an effect on the time course of orexin A's effects on lever pressing. Second, in a separate experiment, the effect of orexin A infusion on "free" sweet pellet feeding was evaluated by allowing rats free access to sweet pellets in their home cages. This control verified that a positive feeding effect (of sweet pellets) was evident following orexin A infusion (Fig. 5). Importantly, the augmented free feeding seen with infusions of orexin A reached statistical significance within the period of time that breakpoint was assessed. That is, orexin A induced "free" feeding within the mean amount of time it took an animal to reach breakpoint (approximately 1 h). In previous studies, feeding responses following orexin A infusion, either intracerebroventricular or intra-rLHa, occurred within the first hour following infusion and sometimes extended into the fourth hour following infusion (España et al. 2002; Haynes et al. 1999; Ida et al. 1999; Sakurai et al. 1998; Sweet et al. 1999). With these data in mind, the mechanism by which orexin A elicits a feeding response was "in effect" during breakpoint analysis.

Due to orexin A's importance to arousal, some doubt has been raised as to whether or not there is an endogenous role for orexin A in modulating feeding behavior. Exogenous administration of orexin A consistently increases arousal at all circadian phases, whereas orexin A has an inconsistent record for the induction of feeding during the dark phase, the time when rats normally consume the greatest amount of their daily food intake (España et al. 2002; Haynes et al. 1999; Thorpe et al. 2003; Yamanaka et al. 1999). Since the most consistent reports of orexin-A-induced feeding appear to be during the light phase, one argument has been that augmented feeding following orexin A administration was secondary to its effects on arousal. The ability of orexin A to augment feeding during the light phase may be explained by the fact that orexin A is increasing wakefulness during a period otherwise predominated by sleep, and that baseline feeding is already low at this time. However, orexin A has also been shown to augment feeding when baseline feeding is high, such as following 24-h food deprivation, restricted feeding, or presentation of a highly palatable diet (Clegg et al. 2002; Szekely et al. 2002; Thorpe et al. 2003). Furthermore, orexin-A-induced loco-

motor activity is not always coincident with an increase in feeding, as orexin A in the rLHa increases running wheel activity during the light and dark phases, yet only increases feeding during the light phase (Kotz et al. 2002). Reported inconsistencies in the ability of orexin A to augment feeding may be due to the sensitivity of the complex behavior of feeding to changes in the environment or the modality of drug infusion (cerebroventricular vs site specific).

Neuroanatomical and behavioral evidence indicates that orexin A's feeding effects are at least partially contingent upon NPY signaling. Neuroanatomical evidence demonstrates reciprocal connectivity between orexin neurons and neurons in the arcuate nucleus, including NPY and proopiomelanocortin (POMC)-producing neurons, where it appears that orexin A stimulates NPY neurons and inhibits POMC neurons (Broberger 1999; Guan et al. 2001; Horvath et al. 1999; Muroya et al. 2004). An increase in *c-fos* immunoreactivity is seen in the arcuate nucleus, following injection of orexin A either in the rostral lateral hypothalamic area or intracerebroventricularly (Date et al. 1999; Horvath et al. 1999; Mullett et al. 2000). Furthermore, administration of NPY receptor antagonists, prior to administration of orexin A, significantly decreases the feeding response seen with administration of orexin A alone (Ida et al. 2000; Jain et al. 2000; Yamanaka et al. 2000). Thus, it appears that the feeding response elicited by orexin A shares common properties and possibly pathways with NPY.

Orexin signaling may be a common pathway whereby feeding and arousal mechanisms are coordinated in order to maintain energy homeostasis (Mignot 2001; Sakurai 2003). In fact, orexins may likely have a role that facilitates the activities that are important for gathering food while maintaining vigilance (Rodgers et al. 2002). The present data support this hypothesis by suggesting that orexin A enhances motivation to eat, an obviously important component of foraging behavior.

The complexity of feeding behavior is highlighted by the many disparate stimuli that can influence both human and rodent feeding behavior. To date, few attempts have been made to characterize the properties of orexin-induced feeding. At question is whether the feeding response following orexin A infusion is due to its effects on arousal or a specific motivation to eat. Here we have demonstrated that central infusion of orexin A increases ad libitum intake of sweet pellets and the amount of work animals are willing to exert for sweet pellets over the same period of time, suggesting that the internal mechanism by which orexin A induces feeding, at least following central infusion in this area, is a feeding-related, motivated behavior. The fact that under an FR20 schedule orexin A did not enhance initial pressing rates but enhanced pressing rates after the first 20 min suggests a possible impairment of satiety. Thus, we submit evidence that orexin-induced feeding is due to a prolongation in motivation, as defined by a stimulant's ability to increase breakpoint, to eat.

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References

- Arnold JM, Roberts DC (1997) A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. *Pharmacol Biochem Behav* 57:441–447
- Backberg M, Hervieu G, Wilson S, Meister B (2002) Orexin receptor-1 (OX-R1) immunoreactivity in chemically identified neurons of the hypothalamus: focus on orexin targets involved in control of food and water intake. *Eur J Neurosci* 15:315–328
- Bernardis LL, Bellinger LL (1996) The lateral hypothalamic area revisited: ingestive behavior. *Neurosci Biobehav Rev* 20:189–287
- Broberger C (1999) Hypothalamic cocaine- and amphetamine-regulated transcript (CART) neurons: histochemical relationship to thyrotropin-releasing hormone, melanin-concentrating hormone, orexin/hypocretin and neuropeptide Y. *Brain Res* 848:101–113
- Broberger C, De Lecea L, Sutcliffe JG, Hokfelt T (1998) Hypocretin/orexin- and melanin-concentrating hormone-expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to the neuropeptide Y and agouti gene-related protein systems. *J Comp Neurol* 402:460–474
- Brown CM, Fletcher PJ, Coscina DV (1998) Neuropeptide Y-induced operant responding for sucrose is not mediated by dopamine. *Peptides* 19:1667–1673
- Cai XJ, Widdowson PS, Harrold J, Wilson S, Buckingham RE, Arch JR, Tadayyon M, Clapham JC, Wilding J, Williams G (1999) Hypothalamic orexin expression: modulation by blood glucose and feeding. *Diabetes* 48:2132–2137
- Clegg DJ, Air EL, Woods SC, Seeley RJ (2002) Eating elicited by orexin-a, but not melanin-concentrating hormone, is opioid mediated. *Endocrinology* 143:2995–3000
- Cluderay JE, Harrison DC, Hervieu GJ (2002) Protein distribution of the orexin-2 receptor in the rat central nervous system. *Regul Pept* 104:131–144
- Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, Sakurai T, Yanagisawa M, Nakazato M (1999) Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. *Proc Natl Acad Sci U S A* 96:748–753
- de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS II, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A* 95:322–327
- Edwards CM, Abusnana S, Sunter D, Murphy KG, Ghatei MA, Bloom SR (1999) The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. *J Endocrinol* 160:R7–R12
- Espana RA, Plahn S, Berridge CW (2002) Circadian-dependent and circadian-independent behavioral actions of hypocretin/orexin. *Brain Res* 943:224–236
- Fujiki N, Yoshida Y, Ripley B, Honda K, Mignot E, Nishino S (2001) Changes in CSF hypocretin-1 (orexin A) levels in rats across 24 hours and in response to food deprivation. *NeuroReport* 12:993–997
- Guan JL, Saotome T, Wang QP, Funahashi H, Hori T, Tanaka S, Shioda S (2001) Orexinergic innervation of POMC-containing neurons in the rat arcuate nucleus. *NeuroReport* 12:547–551
- Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, Benham CD, Taylor SG, Routledge C, Hemmati P, Munton RP, Ashmeade TE, Shah AS, Hatcher JP, Hatcher PD, Jones DN, Smith MI, Piper DC, Hunter AJ, Porter RA, Upton N (1999) Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc Natl Acad Sci U S A* 96:10911–10916
- Haynes AC, Jackson B, Overend P, Buckingham RE, Wilson S, Tadayyon M, Arch JR (1999) Effects of single and chronic intracerebroventricular administration of the orexins on feeding in the rat. *Peptides* 20:1099–1105
- Haynes AC, Jackson B, Chapman H, Tadayyon M, Johns A, Porter RA, Arch JR (2000) A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regul Pept* 96:45–51
- Hodos W (1961) Progressive ratio as a measure of reward strength. *Science* 134:943–944
- Hodos W, Kalman G (1963) Effects of increment size and reinforcer volume on progressive ratio performance. *J Exp Anal Behav* 6:387–392
- Horvath TL, Diano S, van den Pol AN (1999) Synaptic interaction between hypocretin (orexin) and neuropeptide Y cells in the rodent and primate hypothalamus: a novel circuit implicated in metabolic and endocrine regulations. *J Neurosci* 19:1072–1087
- Ida T, Nakahara K, Katayama T, Murakami N, Nakazato M (1999) Effect of lateral cerebroventricular injection of the appetite-stimulating neuropeptide, orexin and neuropeptide Y, on the various behavioral activities of rats. *Brain Res* 821:526–529
- Ida T, Nakahara K, Kuroiwa T, Fukui K, Nakazato M, Murakami N (2000) Both corticotropin releasing factor and neuropeptide Y are involved in the effect of orexin (hypocretin) on the food intake in rats. *Neurosci Lett* 293:119–122
- Jain MR, Horvath TL, Kalra PS, Kalra SP (2000) Evidence that NPY Y1 receptors are involved in stimulation of feeding by orexins (hypocretins) in sated rats. *Regul Pept* 87:19–24
- Jewett DC, Cleary J, Levine AS, Schaal DW, Thompson T (1995) Effects of neuropeptide Y, insulin, 2-deoxyglucose, and food deprivation on food-motivated behavior. *Psychopharmacology (Berl)* 120:267–271
- Kotz CM, Teske JA, Levine JA, Wang C (2002) Feeding and activity induced by orexin A in the lateral hypothalamus in rats. *Regul Pept* 104:27–32
- Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* 98:365–376
- Marsh DJ, Hollopeter G, Huszar D, Laufer R, Yagaloff KA, Fisher SL, Burn P, Palmiter RD (1999) Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides. *Nat Genet* 21:119–122
- Mignot E (2001) A commentary on the neurobiology of the hypocretin/orexin system. *Neuropsychopharmacology* 25:S5–S13
- Mullett MA, Billington CJ, Levine AS, Kotz CM (2000) Hypocretin I in the lateral hypothalamus activates key feeding-regulatory brain sites. *NeuroReport* 11:103–108
- Muroya S, Funahashi H, Yamanaka A, Kohno D, Uramura K, Nambu T, Shibahara M, Kuramochi M, Takigawa M, Yanagisawa M, Sakurai T, Shioda S, Yada T (2004) Orexins (hypocretins) directly interact with neuropeptide Y, POMC and glucose-responsive neurons to regulate Ca²⁺ signaling in a reciprocal manner to leptin: orexigenic neuronal pathways in the mediobasal hypothalamus. *Eur J Neurosci* 19:1524–1534
- Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E (2000) Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* 355:39–40
- Paxinos GW (1998) *The Rat Brain In Stereotaxic Coordinates*, Fourth Edition edn. Academic Press, Academic Press

- Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R, Lammers GJ, Bouras C, Kucherlapati R, Nishino S, Mignot E (2000) A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med* 6:991–997
- Richardson NR, Roberts DC (1996) Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *J Neurosci Methods* 66:1–11
- Rodgers RJ, Halford JC, Nunes de Souza RL, Canto de Souza AL, Piper DC, Arch JR, Blundell JE (2000) Dose-response effects of orexin-A on food intake and the behavioural satiety sequence in rats. *Regul Pept* 96:71–84
- Rodgers RJ, Ishii Y, Halford JC, Blundell JE (2002) Orexins and appetite regulation. *Neuropeptides* 36:303–325
- Sahu A (2002) Interactions of neuropeptide Y, hypocretin-I (orexin A) and melanin-concentrating hormone on feeding in rats. *Brain Res* 944:232–238
- Sakurai T (2003) Orexin: a link between energy homeostasis and adaptive behaviour. *Curr Opin Clin Nutr Metab Care* 6:353–360
- Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92:1 page following 696
- Sunter D, Morgan I, Edwards CM, Dakin CL, Murphy KG, Gardiner J, Taheri S, Rayes E, Bloom SR (2001) Orexins: effects on behavior and localisation of orexin receptor 2 messenger ribonucleic acid in the rat brainstem. *Brain Res* 907:27–34
- Sutcliffe JG, De Lecea L (2002) The hypocretins: setting the arousal threshold. *Nat Rev Neurosci* 3:339–349
- Sweet DC, Levine AS, Billington CJ, Kotz CM (1999) Feeding response to central orexins. *Brain Res* 821:535–538
- Szekely M, Petervari E, Balasko M, Hernadi I, Uzsoki B (2002) Effects of orexins on energy balance and thermoregulation. *Regul Pept* 104:47–53
- Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM (2000) Reduced number of hypocretin neurons in human narcolepsy. *Neuron* 27:469–474
- Thorpe A.J. MMA, Wang C, Kotz M (2003) Regional, metabolic and circadian specificity of LH orexin A. *Am J Physiol Regul Integr Comp Physiol*, 284:R1409–R1417
- Yamanaka A, Sakurai T, Katsumoto T, Yanagisawa M, Goto K (1999) Chronic intracerebroventricular administration of orexin-A to rats increases food intake in daytime, but has no effect on body weight. *Brain Res* 849:248–252
- Yamanaka A, Kunii K, Nambu T, Tsujino N, Sakai A, Matsuzaki I, Miwa Y, Goto K, Sakurai T (2000) Orexin-induced food intake involves neuropeptide Y pathway. *Brain Res* 859:404–409
- Yoshida Y, Fujiki N, Nakajima T, Ripley B, Matsumura H, Yoneda H, Mignot E, Nishino S (2001) Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light–dark cycle and sleep–wake activities. *Eur J Neurosci* 14:1075–1081
- Zhang M, Balmadrid C, Kelley AE (2003) Nucleus accumbens opioid, GABAergic, and dopaminergic modulation of palatable food motivation: contrasting effects revealed by a progressive ratio study in the rat. *Behav Neurosci* 117:202–211