Oxytocin reduces reward-driven food intake in humans

Short title: Oxytocin and human energy metabolism

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Experiments in animals suggest that the neuropeptide oxytocin acts as an anorexigenic signal in the central nervous control of food intake. In humans, however, research has almost exclusively focused on oxytocin’s involvement in the regulation of social behavior. We investigated the effect of intranasal oxytocin on ingestion and metabolic function in healthy men. Food intake in the fasted state was examined 45 min after neuropeptide administration, followed by the assessment of olfaction and reward-driven snack intake in the absence of hunger. Energy expenditure was registered by indirect calorimetry and blood was repeatedly sampled to determine concentrations of blood glucose and hormones. Oxytocin markedly reduced snack consumption, restraining in particular the intake of chocolate cookies by 25%. Oxytocin moreover attenuated basal and postprandial levels of adrenocorticotropic hormone and cortisol, and curbed the meal-related rise in plasma glucose. Energy expenditure and hunger-driven food intake as well as olfactory function were not affected. Our results indicate that oxytocin, beyond its role in social bonding, regulates non-homeostatic, reward-related energy intake, hypothalamic-pituitary-adrenal axis activity and the glucoregulatory response to food intake in humans. These effects can be assumed to converge with oxytocin’s psychosocial function and imply possible applications in the treatment of metabolic disorders.
The hypothalamic nonapeptide oxytocin is released into the circulation by axonal terminals in the posterior pituitary and moreover acts directly on central nervous receptors. Oxytocin, which has been highly preserved during mammalian evolution, regulates physiological functions related to reproduction and mother-infant interaction such as lactation, and in recent years has been shown to modulate affiliative behavior (for review, see ref. 1). Research in humans has almost exclusively focused on the role of oxytocin in the regulation of prosocial behavior including trust, attachment, and sexual behavior (2-5), largely ignoring potential effects of the neuropeptide on ingestive behavior and metabolism. In fact, evidence from rodent studies indicates that the neuropeptide acts as a strong inhibitor of food intake and affects energy expenditure and glucose homeostasis (6-9). Oxytocinergic neurons in the hypothalamic paraventricular nucleus are assumed to mediate the food intake-limiting effect of leptin, an adipokine that provides the brain with negative feedback on body fat stores and sensitizes caudal brainstem nuclei to satiety factors such as cholecystokinin (10). Hypothalamic oxytocin signaling moreover mediates anorexigenic effects of the satiety factor nesfatin-1 in a leptin-independent manner (11). Importantly, oxytocin reduces food intake not only in normal-weight rodents but also in animals with diet-induced obesity (8,12,13), so that oxytocinergic pathways might be a promising target of clinical interventions in obese patients.

The direct manipulation of neuropeptidergic central nervous signaling pathways can be achieved via the intranasal administration of peptides, which is known to bypass the blood-brain barrier and result in significant cerebrospinal fluid elevations in substance levels within 40 min without the need for systemic infusion (14; for review see ref. 15). This approach has been validated, among others, for vasopressin, a close homologue of oxytocin (14), and intranasal oxytocin administration has been shown to reliably modulate neuropsychological functions in a series of studies (2-5) in the absence of relevant side effects (16). Surprisingly, however, the impact of intranasal oxytocin on energy metabolism including ingestive behavior has not been investigated in humans so far. The assessment of respective effects of
iv. oxytocin (17) is hampered by the fact that peripheral oxytocin is not readily transported across the blood-brain barrier (18).

In the present experiments, we studied the contribution of oxytocin signaling to the control of ingestive behavior and energy expenditure in normal-weight, healthy men, with a particular view to endocrine regulators of metabolism such as ghrelin and insulin as well as hypothalamic-pituitary-adrenal (HPA) axis secretory activity. Ingestive behavior is not only regulated homeostatically, i.e. by central nervous pathways that respond to energy depletion, but also by non-homeostatic brain circuits that process the reward-related, ‘hedonic’ qualities of food intake (19). Therefore, we applied a twofold assessment of food intake that relied on the one hand on a large breakfast buffet following an overnight fast to investigate homeostatic, primarily hunger-driven energy intake (20-22), and on the other hand on a collection of snacks of varying palatability offered after breakfast intake for the measurement of reward-driven food intake (22-24).

**Research Design and Methods**

**Subjects.** Twenty healthy, male non-smokers who were free of medication participated in the study (age, 26.3 ± 0.89 years; body mass index, BMI, 22.66 ± 0.36 kg/m2). All relevant illness was excluded by medical history and clinical examination. Subjects were kept unaware of hypothesized treatment effects on food intake and were informed that the experiments concerned the effect of oxytocin on taste preferences and energy expenditure. Participants gave written informed consent to the study that conformed to the Declaration of Helsinki and was approved by the local ethics committee.

**Design and procedure.** Experiments were carried out in a double-blind, cross-over, within-subject comparison. Each subject participated in two experimental sessions, oxytocin and placebo. The order of conditions was balanced across subjects and the two sessions were
spaced at least 10 days apart. Participants were instructed to abstain from the intake of food as well as of caffeinated and alcoholic beverages after 2000 h on the day preceding each session.

After the subject’s arrival at the lab around 0800 h, a venous cannula was inserted into the subject’s non-dominant arm to enable drawing of venous blood (see Figure 1 for experimental procedure). Thereafter, blood was sampled for baseline assessments of hormonal parameters. Mood, hunger and thirst were rated and energy expenditure was measured by indirect calorimetry. At 0942 h, six 0.1-ml puffs (three per nostril) of oxytocin (Syntocinon; Defiante Farmaceutica, Funchal Madeira, Portugal) and vehicle, respectively, were intranasally administered at 30-sec intervals, amounting to a total dose of 24 IU oxytocin (0.6 ml). Forty-five minutes after administration, subjects were presented with a breakfast buffet from 1030-1100 h. Olfactory function was tested at 1155 h. Mood, hunger and thirst were rated and energy expenditure was measured by indirect calorimetry at baseline, after substance administration, and after the breakfast buffet (Figure 1). At 1240 h, casual snack intake was assessed under the pretext of a snack taste test. Sixty minutes before as well as 35 and 120 min after substance administration, subjects rated the experimenter’s general trustworthiness. Heart rate and blood pressure were monitored throughout the experiment. At the end of experiments, subjects were asked to indicate their account of the study purpose.

**Assessments of food intake, hunger, thirst, mood and olfaction.** The free-choice ad libitum test buffet comprised a variety of food choices (Table 1) from which subjects could eat undisturbed for 30 min. They were not aware that their food intake was measured by weighing buffet components before and after breakfast. This procedure has been repeatedly shown to enable the precise assessment of primarily hunger-driven food intake in the fasted state (20-22). Reward-related eating in the absence of hunger was assessed using a snack test validated in a series of previous studies (22-24). Subjects were presented with three types of snacks of different taste but comparable calorie content and macronutrient composition (Table
2), each on a separate plate and labelled Snack A, B, and C, respectively. The three types were, “TUC Cracker Classic” (salty taste; Griesson-de Beukelaer, Polch, Germany), “Rice waffles” (bland taste; Continental Bakeries B.V., Dordrecht, The Netherlands), and “Double Chocolate Cookies” (EDEKA, Hamburg, Germany). Of each variety, 15 snacks broken into bite size pieces were provided, allowing for a considerable amount to be eaten without the plates appearing empty to ensure that participants would not restrict snack intake based on whether the experimenter could see how much had been consumed. The participant was instructed to taste and rate each type of snack on a visual analogue scale (VAS) anchored by 0 (not at all) and 100 mm (very palatable/sweet/salty). The importance of giving accurate ratings was emphasized and subjects were informed that during and after completion of the rating task they could eat as many snacks as they liked, because any remaining food would be discarded, and were left alone for 10 minutes. Snack intake was covertly measured by weighing the snacks before and after the test.

Hunger, thirst and also trustworthiness of the experimenter were rated on VASs (0-100). Self-reported mood was assessed with 5-point scales covering the categories good/bad mood, alertness/sleepiness and calmness/agitation (MDBF; ref. 25). Olfactory function was tested 60 min after the test buffet with a validated commercial test kit (Sniffin’ Sticks; Burghart Elektro- und Feinmechanik GmbH, Wedel, Germany) that allows for the separate characterization of the three dimensions olfactory threshold, discrimination, and identification (26).

**Measurement of energy expenditure, plasma glucose, and hormonal parameters.** Energy expenditure (expressed as kcal/d) was measured via indirect calorimetry using a ventilated-hood system (Deltatrac II, MBM-200 Metabolic Monitor; Datex-Engström Deutschland, Achim, Germany). Before each use, the device was calibrated with Quick Cale calibration gas to 5% CO2 and 95% O2. Calorimetric measurements took place from 0900 h to 0930 h.
(baseline), immediately after intranasal substance administration from 0945 h to 1015 h to assess effects of intranasal oxytocin alone, and again between 1105 h and 1145 h, i.e., after the ad libitum test buffet to register postprandial energy expenditure. The rise in energy expenditure between the fasting state (baseline) and the postprandial state reflects diet-induced thermogenesis, i.e., the energy that is emitted as heat during metabolization of food and thus does not contribute to the production of adenosine triphosphate (27).

Blood samples for the assessment of serum insulin, C-peptide, cortisol, growth hormone, leptin as well as plasma glucose, glucagon, total and active glucagon-like peptide-1 (GLP-1), adrenocorticotropic hormone (ACTH), and total ghrelin were centrifuged, and samples were stored at -80°C. Blood for the measurement of glucagon and total/active GLP-1 was pre-treated with aprotinin (370 kIU/ml; Roth GmbH, Karlsruhe, Germany) and DPP-IV-inhibitor blocking reagent (50 µM, Millipore, St. Charles, MO, U.S.A), respectively. Routine assays were used to determine concentrations of plasma glucose (measured in fluoride plasma according to the hexokinase method; Aeroset, Abbott Diagnostics, North Chicago, IL), insulin, C-peptide, ACTH, cortisol (all Immulite, DPC, Los Angeles, CA, USA), total ghrelin, leptin, total and active GLP-1 (all RIA, Millipore, Billerica, MA, USA), and glucagon (RIA, IBL International, Hamburg, Germany).

**Statistical analysis.** Analyses were based on analyses of variance (ANOVA) with the within-subject factors ‘Treatment’, ‘Time’, ‘Nutrient’ and ‘Snack type’ as appropriate. Degrees of freedom were corrected using the Greenhouse-Geisser procedure. Significant ANOVA effects were specified by pairwise t-tests. For blood parameters and energy expenditure, baseline adjustment was achieved by subtracting individual baseline values from individual post-intervention measurements. Supplementary analyses of snack intake and blood glucose peak values relied on analyses of covariance (ANCOVA) including as covariates the differences
between conditions in overall calorie and carbohydrate consumption during breakfast intake. All data are presented as means ± SEM. A P-value < 0.05 was considered significant.

**Results**

**Oxytocin inhibits reward- but not hunger-driven eating.** Oxytocin administration did not affect food intake from the breakfast buffet in the fasted state. Overall food consumption as well as the proportion of ingested macronutrients were nearly identical between conditions (all $P>0.6$; Table 3). Accordingly, hunger ratings ($P>0.2$, two-sided t-test for baseline values; Figure 2A) were not altered by oxytocin ($P>0.9$) and fell to comparably low values of around 15% of the maximal score during breakfast ($P>0.2$; $F(2,36)=74.91$, $P<0.0001$ for Time; $P>0.5$ for treatment effects), indicating that subjects in both conditions were satiated by breakfast intake. Thirst ratings as well as self-rated mood were likewise unaffected by oxytocin ($P>0.12$ for all comparisons).

In the snack test during the postprandial period, oxytocin in comparison to placebo induced a reduction in total snack intake ($F(1,19)=5.5$, $P<0.03$ for Treatment; Figure 2B) that was driven by a decrease in chocolate cookie consumption by 25% ($P<0.01$, two-sided t-test; Figure 2C and Table 4). These effects remained significant when corrected for overall calorie as well as carbohydrate consumption during the preceding test buffet (both $P<0.04$ for Treatment; $P<0.007$ for the difference in chocolate cookie consumption). Across conditions, intake of chocolate cookies by far exceeded that of the remaining snacks ($F(1,23)=9.50$, $P<0.004$ for Snack type). Also, sweetness and saltiness ratings were highest for chocolate cookies and salt crackers, respectively ($F(2,31)=342.28$, $P<0.0001$, and $F(2,36)=112.18$, $P<0.0001$, for Snack type). Oxytocin did not affect ratings for chocolate cookies and salt crackers ($P>0.3$) and even slightly increased rated palatability of rice waffles ($P<0.05$; Table 4). In the olfactory task, no treatment effects on perceptual thresholds ($P>0.4$), olfactory discrimination ($P>0.6$) and olfactory identification ($P>0.2$) emerged, and oxytocin
administration did not affect the trustworthiness of the experimenter as perceived by the participants ($P>0.6$).

**Energy expenditure is not acutely affected by oxytocin administration.** Energy expenditure assessed by indirect calorimetry was comparable between the placebo and the oxytocin condition during the entire experimental period ($F(1,19)=2.12$, $P>0.16$ for Treatment x Time, $F(1,19)=0.10$, $P>0.75$ for Treatment), averaging $1609\pm41$ vs. $1651\pm37$ kcal/d ($P>0.12$) under baseline fasting conditions, $1615\pm21$ vs. $1633\pm13$ kcal/d ($P>0.46$) after placebo and oxytocin administration, respectively, and $2021\pm48$ vs. $1985\pm34$ kcal/d ($P>0.4$) after breakfast intake, with the latter values reflecting diet-induced thermogenesis approximately 23% above preprandial baseline measurements ($F(1,19)=145.24$, $P<0.0001$ for Time).

**Oxytocin reduces HPA axis activity as well as norepinephrine concentrations and blunts the glucose response to food intake.** During baseline, none of the blood parameters including blood glucose differed between conditions (all $P>0.18$). Oxytocin exerted a sustained suppressive effect on HPA axis activity, reducing serum ACTH and plasma cortisol concentrations during the entire post-administration period ($F(1,18)=4.67$, $P<0.05$, and $F(1,18)=5.15$, $P<0.04$, respectively, for Treatment; Figure 2D/E). The effect on cortisol was particularly pronounced before breakfast intake ($F(2,35)=4.82$, $P<0.02$ for Treatment x Time). In parallel, preprandial circulating concentrations of norepinephrine were reduced by oxytocin treatment ($F(1,19)=5.41$, $P=0.03$ for Treatment x Time; Figure 3F). Supplementary analyses indicated that the oxytocin-induced decreases in cortisol concentrations (AUCi 0930-1145h) and chocolate cookie intake were significantly correlated ($r=0.56$, $P=0.012$, Pearson’s coefficient).
The circulating concentrations of glucose, insulin, C-peptide, and total GLP-1 showed the expected meal-related increase across conditions (all \( P < 0.0001 \) for Time; Figure 3A-D). Whereas levels of insulin, C-peptide and total GLP-1 were not affected by oxytocin administration (all \( P > 0.16 \)), the peak glucose response to breakfast intake (15 min after meal termination) was reduced by 0.57 mmol/l following oxytocin compared with placebo administration (Figure 3A; \( P < 0.02 \), two-sided t-test). This difference was still evident when adjusted for preceding total and carbohydrate-specific breakfast intake (both \( P < 0.03 \)). Total plasma concentrations of ghrelin were suppressed by breakfast intake (\( F(2,29)=31.62, P < 0.0001 \) for Time) without significant treatment effects (\( P > 0.95 \); Figure 3E). Conversely, 15 min after breakfast serum leptin levels were increased by around 28% compared to preprandial levels (\( F(4,80)=28.98, P < 0.0001 \) for Time; Figure 3F). Leptin concentrations did not differ between conditions across the whole experimental period (\( P > 0.38 \)), although there was a trend towards reduced preprandial leptin concentrations after oxytocin administration (\( F(1,19)=3.39, P = 0.08 \) for Treatment). Circulating concentrations of growth hormone and active GLP-1, i.e., the intact form of GLP-1, were likewise comparable between conditions (all \( P > 0.14 \)).

**Discussion**

We demonstrate that oxytocin inhibits food intake and impacts endocrine regulation in humans. The anorexigenic effect of oxytocin emerged during the postprandial period, when reward-driven eating motivation prevails, whereas energy intake in the fasted state was not affected. Although this pattern could also imply that oxytocin effects on ingestive behavior emerge with a certain delay, this assumption is not supported by previous studies indicating robust central nervous effects of the peptide within 90 min after administration (e.g., 3,4,28). While energy expenditure remained completely unaltered, oxytocin globally attenuated HPA axis activity and blunted the peak glucose response to food intake, suggesting an insulin-
sensitizing action of the peptide. These findings indicate that the oxytocin system contributes to the control of reward-related eating as well as of stress axis regulation and glucose homeostasis in humans.

Oxytocin has been shown in a number of experiments in rodents to inhibit feeding after intracerebroventricular injection (6,8). This effect could be mimicked by the peripheral administration of high oxytocin doses that supposedly trigger hypothalamic oxytocin release in a feed-forward fashion (7,12). Furthermore, oxytocin receptor antagonists have been found to acutely hamper the anorexigenic central nervous impact of hormones like cholecystokinin and corticotropin-releasing hormone (29,30). Vice versa, α-melanocyte stimulating hormone, a crucial player among the catabolic messengers, triggers oxytocin release from supraoptic neurons (31). Oxytocin may moreover induce a satiating effect by modulating distention signals from the stomach (32), but in the present experiments we found no differences between conditions in post-breakfast hunger ratings. Considering that oxytocin’s anorexigenic impact selectively concerned the consumption of palatable snacks, it might rather be speculated that oxytocin acted on receptors expressed in the brain reward circuit, such as in the ventral tegmental area (VTA) and nucleus accumbens (33,34) that contribute to the regulation of palatable food intake (19). This conclusion should be corroborated in more mechanistically orientated experimental approaches and also in behavioral studies applying effort-based tests to assess the reward-driven motivation to obtain palatable food (35).

The attenuating effect of oxytocin on snack intake focused on chocolate cookies that were preferentially eaten by our subjects, which underlines the reward-related component of oxytocin’s anorexigenic impact. Nevertheless, subjective ratings of chocolate and salty snacks differed in sweetness and saltiness but not palatability, suggesting that oxytocin specifically dampens the motivation to consume sweet-tasting food. In accordance, in rodents oxytocin injection into the VTA suppresses sucrose intake (36) and oxytocin signaling is strongly activated by chronic sucrose ingestion (37), while oxytocin knock-out animals display a
preference for sucrose and carbohydrates with sweet taste (38). Oxytocin did not affect the rated palatability of chocolate cookies, which might be taken as an indication that it acted on dopaminergic pathways responding to the incentive salience of food rather than opioidergic/cannabinoid signaling assumed to process the palatability of ingested nutrients (39). Tests of olfactory function indicated that the decrease in snack intake was not mediated by effects on sensory processing. Furthermore, biasing effects on ingestive behavior related to demand characteristics and social desirability were excluded by interviews confirming unawareness of food intake measurements, and by ratings of the perceived trustworthiness of the experimenter. From a clinical perspective, the conclusion that oxytocin acts on reward-processing brain circuits to suppress snack intake is in line with observations in patients with Prader-Willi syndrome who suffer from hyperphagic obesity due to insatiable food craving and have been reported to display a 40% reduction in the number and size of oxytocin neurons (40).

The oxytocin-triggered decrease in ACTH and cortisol as well as norepinephrine concentrations in the basal and postprandial state extends and refines previous findings of an attenuating impact of i.v. oxytocin on basal corticotropic function (41) and of intranasal oxytocin on HPA axis activity in response to social and physical stress (42,43) and supports the assumption that the suppression of HPA axis activity by oxytocin is not only mediated via adrenal (44) but also central mechanisms. Acute and chronic activation of endocrine stress axes favors the intake of ‘comfort food’, i.e., highly palatable food (45). In a negative feedback loop, activation of central nervous reward circuits by consuming sucrose reduces stress-induced HPA axis activity (46). The intake of sugar compared with equicaloric fat solution induces a selective, twofold increase in hypothalamic oxytocinergic neuronal activity, whereas central nervous oxytocin receptor antagonism triggers the intake of sucrose but not fat (47). Oxytocin might impact the crosstalk between reward- and stress-related pathways by modulating VTA and nucleus accumbens dopamine signaling (48) known to
facilitate stress-induced HPA axis activity (49). The conclusion that the inhibition of palatable snack intake by oxytocin involves a stress axis-related component (43) is supported by the positive association between oxytocin’s attenuating effects on cortisol concentrations and chocolate cookie intake.

In addition to its dampening effect on HPA axis activity, oxytocin administration blunted the peak plasma glucose response to breakfast intake. Total calorie and macronutrient uptake from the breakfast buffet were closely comparable in both conditions and, moreover, the reduction in blood glucose concentrations was still evident after correcting the data for slight differences in these parameters. Considering that the circulating concentrations of insulin, C-peptide and both total and active GLP-1, an incretin hormone with insulin-secretory properties, were not affected by oxytocin, this finding suggests a subtle, but discernible improvement in insulin sensitivity after administration of the peptide. Although this conclusion is in need of corroboration in experiments focusing on glucose homeostasis, it is in line with findings that oxytocin enhances insulin sensitivity and glucose tolerance in a rodent model of diet-induced obesity independent of its effects on body weight (7,50).

We found no effect of acute oxytocin administration on fasting and postprandial energy expenditure as assessed by indirect calorimetry. In diet-induced obese rats losing weight due to chronic oxytocin administration, the decrease in energy expenditure normally associated with weight loss was prevented by oxytocin treatment, probably via effects on hypothalamic thermoregulation (7). Vice versa, the ablation of oxytocin neurons favors the development of obesity by reducing energy expenditure (9). Against this background, our finding suggests that rather than exerting acute effects, oxytocin contributes to the regulation of energy expenditure on a long-term basis. Also, in our experiments oxytocin did not affect the circulating concentrations of ghrelin and GLP-1 and induced merely non-significant changes in leptin, hormones known to affect energy expenditure and energy homeostasis (51). Although intranasal oxytocin administration has been previously found to increase plasma
concentrations of the peptide (2), this pattern moreover argues against a peripheral mediation of the observed changes in ingestive behavior.

In sum, our study provides evidence for a significant contribution of oxytocin to the control of reward-related eating behavior as well as endocrine regulation in humans. Further experiments should elucidate the preconditions and ramifications of oxytocin’s anorexigenic effects in humans by exploring the composition and timing of meals as well as the regulation of satiety in dependence of oxytocin administration. Considering recent findings that oxytocin modulates VTA activation in response to cues predicting social reward and punishment (52), its impact on the brain reward system might represent a common denominator of its psychosocial and anorexigenic properties. In concert with the dampening of stress axis activity, these effects might for example optimize maternal behavior during breast-feeding by isolating the mother from distracting food stimuli and preventing stress-induced inhibition of lactation (53). Excessive reward-driven food intake, chronic HPA axis activation and insulin resistance are key factors in the pathogenesis and maintenance of obesity. With most recent clinical pilot data pointing to weight-loss inducing properties of long-term intranasal oxytocin administration in obese humans (54), the potential application of oxytocin in the treatment of metabolic disorders deserves particular attention in future research.

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Table 1. Composition of the test buffet.

| Food                  | Weight (g) | Energy (kcal) | Carbohydrate (g) | Fat (g) | Protein (g) |
|-----------------------|------------|---------------|------------------|---------|-------------|
| **Neutral**           |            |               |                  |         |             |
| Whole wheat bread     | 165        | 360           | 71               | 2.3     | 12          |
| Wheat rolls           | 240        | 275           | 122.4            | 3.4     | 6.3         |
| White bread           | 30         | 72            | 14.6             | 0.4     | 2.2         |
| Butter                | 120        | 928           | 0.7              | 99.8    | 0.8         |
| Whole milk            | 750        | 491           | 36               | 26.3    | 24.8        |
| **Sweet**             |            |               |                  |         |             |
| Strawberry jam        | 50         | 147           | 35.8             | 0.1     | 0.1         |
| Hazelnut spread       | 40         | 218           | 21.6             | 12.8    | 2.8         |
| Honey                 | 40         | 123           | 30               | 0       | 0.1         |
| Sugar                 | 24         | 98            | 24               | 0       | 0           |
| Fruit curd            | 125        | 140           | 19.3             | 3.3     | 7.7         |
| Vanilla pudding      | 125        | 134           | 20.8             | 3.8     | 3.5         |
| Strawberry milk       | 200        | 167           | 18.2             | 6.8     | 7.4         |
| Banana                | 179        | 168           | 38.3             | 0.4     | 2           |
| Apple                 | 195        | 104           | 22.2             | 1.2     | 0.6         |
| Pear                  | 140        | 78            | 17.4             | 0.4     | 0.7         |
| Orange                | 180        | 72            | 15               | 0.4     | 1.8         |
| Tangerine             | 80         | 35            | 8.2              | 0       | 0.5         |
| Orange juice          | 400        | 173           | 36               | 1       | 4           |
| **Savory**            |            |               |                  |         |             |
| Poultry sausage       | 40         | 74            | 0.1              | 4.3     | 8.3         |
| Cervelat sausage      | 34         | 120           | 0.1              | 10.2    | 6.1         |
| Sliced cheese         | 100        | 374           | 0                | 29.2    | 25.5        |
| Cream cheese          | 33         | 87            | 0.6              | 7.8     | 3           |
| (natural)             |            |               |                  |         |             |
| Cream cheese (herbs)  | 40         | 124           | 1                | 11.6    | 3.2         |
| **Total**             | **3330**   | **4562**      | **553**          | **226** | **123**     |

All values are rounded to the closest decimal.
Table 2. Snacks offered in the snack test.

|                      | Chocolate cookies | Rice waffles | Salt crackers |
|----------------------|-------------------|--------------|---------------|
| Energy value (kcal/100 g) | 500              | 390          | 486           |
| Carbohydrate (g/100 g)    | 57.2             | 63           | 63            |
| Fat (g/100 g)           | 26.6             | 22           | 22            |
| Protein (g/100 g)       | 6                | 8.6          | 7.8           |

Nutritional values of the snacks offered to the participants during the postprandial period. All values are according to the manufacturers (see Materials and Methods). A glass of still mineral water was provided along with the cookies.
Table 3. Food intake from the test buffet.

| Food intake (kcal) | Placebo  | Oxytocin | $P$-value |
|-------------------|----------|----------|-----------|
| **Total**         | 1180±103 | 1190±105 | 0.84      |
| Carbohydrates     | 517±41   | 540±41   | 0.43      |
| Fat               | 517±54   | 509±57   | 0.84      |
| Protein           | 145±16   | 142±14   | 0.82      |
| **Savory foods**  | 314±38   | 309±29   | 0.91      |
| **Sweet foods**   | 233±44   | 206±40   | 0.22      |

Total food intake, intake of macronutrients and food intake according to taste. Savory and sweet foods contained in the test buffet are listed separately in Table 1. $P$-values are derived from paired, two-tailed $t$-tests. $n=20$. 
Table 4. Calorie intake and snack ratings during the snack test.

| Rating category | Snack type     | Placebo  | Oxytocin |   P-value |
|-----------------|----------------|----------|----------|-----------|
| **Intake (kcal)** | Chocolate cookies | 185±41   | 138±38   | **0.007** |
|                  | Rice waffles    | 18±3     | 13±2     | 0.15      |
|                  | Salt crackers   | 81±19    | 75±16    | 0.75      |
|                  | Total           | 283±44   | 227±44   | **0.03**  |
| **Palatability** | Chocolate cookies | 7.7±0.28 | 7.45±0.26 | 0.45      |
|                  | Rice waffles    | 2.99±0.43| 3.68±0.43| **0.05**  |
|                  | Salt crackers   | 7.11±0.35| 7.31±0.29| 0.59      |
| **Sweetness**    | Chocolate cookies | 8.06±0.16| 7.87±0.25| 0.53      |
|                  | Rice waffles    | 0.95±0.24| 0.90±0.29| 0.88      |
|                  | Salt crackers   | 1.55±0.37| 1.49±0.43| 0.83      |
| **Saltiness**    | Chocolate cookies | 0.92±0.31| 0.92±0.37| 1.00      |
|                  | Rice waffles    | 1.42±0.33| 1.68±0.43| 0.55      |
|                  | Salt crackers   | 6.25±0.44| 6.79±0.44| 0.32      |

Nutritional values of the snacks are listed in Table 2. P-values are derived from paired, two-tailed t-tests. n=20.
**Figure 1. Experimental procedure.** Following baseline assessments of blood parameters, psychological variables and energy expenditure, healthy young men were intranasally administered oxytocin (24 IU) and placebo, respectively, at 0942 h (nose symbol). At 1030 h, 45 min after substance administration, subjects were allowed to eat ad libitum from a free-choice test buffet for 30 min. Around 60 min after termination of the buffet, at 1155 h, olfactory function was assessed and at 1240 h, 100 min after the end of the buffet meal, snack intake was measured under the pretext of a taste-rating task. Throughout the session, mood, hunger and thirst were assessed and blood samples were taken (black triangles).

**Figure 2. Oxytocin inhibits reward-driven eating and reduces HPA axis activity.**

(A) Mean (± SEM) hunger ratings assessed before (averaged across the 0915 h and 0930 h baseline values) and after intranasal administration (upright dotted line) of oxytocin (24 IU; black dots and solid lines) and placebo (vehicle; white dots and dotted lines). Forty-five minutes post-treatment, subjects ate from a test breakfast (1000-1030 h) and 100 min thereafter, they ingested snacks under the pretext of a taste test (1240-1250 h). (B) Mean (± SEM) cumulative snack intake (kcal) in the placebo (white bar) and the oxytocin condition (black bar). (C) Individual chocolate cookie consumption assessed at the same test in the placebo (left) and the oxytocin condition (right). Individual values of both sessions are connected by lines. Lower panels depict mean (± SEM) concentrations of (D) plasma adrenocorticotropic hormone (ACTH), (E) serum cortisol, and (F) norepinephrine assessed before (averaged across the 0915 h and 0930 h baseline values) and after oxytocin (black dots and solid lines) and placebo (vehicle; white dots and dotted lines) administration. Mean baseline values of both conditions are averaged to a common baseline. n=20; * P<0.05, ** P<0.01 for comparisons between conditions (paired t-tests).
Figure 3. Plasma glucose and hormones. Mean (± SEM) concentrations of (A) plasma glucose, (B) serum insulin, (C) serum C-peptide, (D) plasma total GLP-1, (E) plasma total ghrelin, and (F) serum leptin assessed before (averaged across the 0915 h and 0930 h baseline values) and after intranasal administration (upright dotted line) of oxytocin (24 IU; black dots and solid lines) and placebo (vehicle; white dots and dotted lines). Subjects ate from a test breakfast from 1000-1030 h and ingested snacks under the pretext of a taste test from 1240-1250 h. Mean baseline values of both conditions are averaged to a common baseline. n=20; * P<0.05 for comparisons between conditions (pairwise t-tests).
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