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Feeding-elicited cataplexy in orexin knockout mice

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Abstract

Mice lacking orexin/hypocretin signaling have sudden episodes of atonia and paralysis during active wakefulness. These events strongly resemble cataplexy, episodes of sudden muscle weakness triggered by strong positive emotions in people with narcolepsy, but it remains unknown whether murine cataplexy is triggered by positive emotions. To determine whether positive emotions elicit murine cataplexy, we placed orexin knockout (KO) mice on a scheduled feeding protocol with regular or highly palatable food. Baseline sleep/wake behavior was recorded with ad lib regular chow. Mice were then placed on a scheduled feeding protocol in which they received 60% of their normal amount of chow 3 hr after dark onset for the next 10 days. Wild-type and KO mice rapidly entrained to scheduled feeding with regular chow, with more wake and locomotor activity prior to the feeding time. On day 10 of scheduled feeding, orexin KO mice had slightly more cataplexy during the food-anticipation period and more cataplexy in the second half of the dark period, when they may have been foraging for residual food. To test whether more palatable food increases cataplexy, mice were then switched to scheduled feeding with an isocaloric amount of Froot Loops, a food often used as a reward in behavioral studies. With this highly palatable food, orexin KO mice had much more cataplexy during the food-anticipation period and throughout the dark period. The increase in cataplexy with scheduled feeding, especially with highly palatable food, suggests that positive emotions may trigger cataplexy in mice, just as in people with narcolepsy. Establishing this connection helps validate orexin KO mice as an excellent model of human narcolepsy and provides an opportunity to better understand the mechanisms that trigger cataplexy.

Keywords

orexin; hypocretin; scheduled feeding; food anticipation; positive emotion

INTRODUCTION

Cataplexy is sudden muscle weakness triggered by strong, generally positive emotions. In people with narcolepsy, cataplexy is typically triggered by laughter, mirth, and pleasant surprise, and in narcoleptic dogs, cataplexy is induced by play, excitement, and presentation of tasty food (Nishino and Mignot, 1997; Krahn et al., 2005; Siegel and Boehmer, 2006). Like

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hypnagogic hallucinations and sleep paralysis, cataplexy is thought to be an inappropriate intrusion of rapid eye movement (REM) sleep-like behavior into wakefulness. Consciousness is preserved during most episodes of cataplexy, but with long episodes, individuals with narcolepsy may fall into non-REM (NREM) or REM sleep. The neural mechanisms of cataplexy are not well understood, but many recent clinical studies have shown that narcolepsy with cataplexy is caused by a loss of orexin/hypocretin signaling (Peyron et al., 2000; Thannickal et al., 2000; Mignot et al., 2002).

Mice lacking the orexin peptides or the orexin-producing neurons also have sudden episodes of atonia, but it is controversial whether these events are truly cataplexy (Chemelli et al., 1999; Hara et al., 2001; Willie et al., 2003; Mochizuki et al., 2004; Zhang et al., 2007). The atonia occurs in the midst of active wakefulness during the dark (active) period and is often accompanied by rhythmic EEG theta activity much like that seen during REM sleep in rodents (Willie et al., 2003; Mochizuki et al., 2004; Zhang et al., 2007). Therefore, these episodes look like a rapid transition from active wake to REM sleep, and then back to wake. Because the nature of these events has been unclear, researchers have described them using a variety of terms: narcoleptic episodes (Chemelli et al., 1999), abrupt behavioral arrests (Hara et al., 2001; Willie et al., 2003), cataplexy (Mochizuki et al., 2004) and direct REM sleep transitions from wake (DREM) (Zhang et al., 2007). In some studies, these episodes were scored as REM sleep despite occurring in the midst of wakefulness (Chemelli et al., 1999; Hara et al., 2001; Willie et al., 2003; Zhang et al., 2007). Overall, researchers have been reluctant to call these episodes cataplexy because so far, there is little evidence that the atonia is triggered by emotions or that consciousness is preserved.

We examined whether murine cataplexy is triggered by scheduled feeding, a stimulus that should evoke strong, positive emotions. In both normal and orexin deficient mutant mice, scheduled feeding increases wakefulness and locomotor activity 1 to 2 hours before the arrival of food, though the increase in food-anticipatory activity was relatively small in orexin deficient mice (Akiyama et al., 2004; Mieda et al., 2004; Kaur et al., 2008). We hypothesized that this anticipation of food should trigger cataplexy in orexin knockout (KO) mice. Because highly palatable food may evoke particularly strong positive emotions, we then repeated the scheduled feeding protocol with Froot Loops, a sweetened cereal often used as a reward in behavioral studies of rodents (Lee et al., 2000; Ahn and Phillips, 2002; Udo et al., 2004; Sevak et al., 2008).

MATERIALS AND METHODS

Animals

Experiment 1 used 15 male orexin KO mice and 14 male wild type (WT) littermates. Subsequently, 8 of the KO mice and 7 of the WT mice were used in Experiment 2. These mice weighed 25–40g and were between 20 and 27 weeks old. Founder orexin KO mice were on a C57BL/6J-129/SvEV background, and their offspring were backcrossed with C57BL/6J mice for 8 generations (Mochizuki et al., 2004). Orexin KO mice were identified using PCR with a neo primer, 5'-TAGTTGCCAGCCATCTGTTG-3' and a genomic primer 5'-ACTCTCCACCCACAGACAGG-3'. WT mice were identified using two genomic primers: 5'-GACGACGGCCTCAGACTTCTT-3' and 5'-TCACCCCTTGGGATAGCCCT-3'. All experiments were approved by the Institutional Animal Care and Use Committees of Beth Israel Deaconess Medical Center and Harvard Medical School.

Surgery and EEG/EMG recordings

Mice were anaesthetized with ketamine-xylazine (100 and 10 mg/kg, i.p.) and implanted with electroencephalogram (EEG) and electromyogram (EMG) electrodes as described previously

(Mochizuki et al., 2004). EEG signals were recorded using two ipsilateral stainless steel screws (1.5 mm to the right of the sagittal suture; 1 mm anterior to bregma and 1 mm anterior to lambda). EMG signals were acquired by a pair of multistranded stainless steel wires inserted into the neck extensor muscles. A telemetric temperature and locomotor activity (LMA) transmitter (TA-F20, Data Sciences International, St. Paul, MN) was placed in the peritoneal cavity. Ten days after surgery, mice were transferred to individual recording cages lined with woodchips. They had ad lib access to food and water and acclimated to the recording cables for another 4 days before baseline recordings. Recordings were performed in a sound-attenuated chamber with a 12:12 hr light:dark (LD) cycle (30 lux; lights on at 07:00 and off at 19:00) and a constant temperature of about 23°C.

The EEG/EMG signals were acquired using Grass Model 12 amplifiers (West Warwick, RI) and digitized at 128 Hz using a sleep scoring system (Sleep Sign, Kissei Comtec, Matsumoto, Japan). The behavior of the animals was simultaneously video-recorded (cage top view) by Sleep Sign. The EEG/EMG signals were digitally filtered (EEG: 0.3–30 Hz, EMG: 2–50 Hz) and semi-automatically scored in 10 sec epochs as wake, NREM, or REM sleep. This preliminary scoring was visually inspected and corrected when appropriate. We operationally defined cataplexy to begin with an abrupt transition from active wake to atonia with EEG theta (4–9 Hz) activity, and to end with an abrupt return from atonia and theta to active wake (Mochizuki et al., 2004). Immobility during these episodes was confirmed using the video recordings. Video recordings also showed that during cataplexy the mouse often collapsed prone or on its side anywhere in the cage, whereas during normal sleep the mouse assumed a curled posture in its nest area. We also required that at least 40 seconds of wakefulness preceded each episode to exclude any REM sleep interrupted by a brief awakening (Fujiki et al., 2006). Details of these scoring criteria are reviewed in a recent consensus definition of murine cataplexy (Scammell et al., 2009).

Experiment 1: Scheduled feeding with regular chow

To determine whether anticipation of food triggers cataplexy in orexin KO mice, we placed mice on a restricted feeding schedule (Figure 1). Baseline sleep/wake behavior was recorded with ad lib access to regular chow (PicoLab Rodent Diet 20-5053), and during this period, mice ate about 4.5 ± 0.1 g of chow/day. Then, at the light onset (7:00) on the first scheduled feeding day (SF1), all remaining food was carefully removed and for the next ten days, mice were given 60% of their normal amount of chow (2.7 g, 10 kcal) three hours after dark onset (22:00). The chow was placed on the bedding in a corner of the cage. Although many previous food-entrainment studies shifted meal-time to the light period (Akiyama et al., 2004; Mieda et al., 2004; Kaur et al., 2008), we set the feeding time in the dark period as this is when most cataplexy occurs in orexin KO mice. We also restricted daily calories to 60% of baseline consumption to increase motivation for food. Both WT and orexin KO mice quickly entrained to the feeding schedule in 5 days with more LMA during the food-anticipation period (Supplemental Figure 1). Sleep/wake behavior was then recorded on day 10 (SF10). On day 11, mice were given a regular amount of food (4.5 g) from 22:00 to the end of the dark period to determine if the behavioral effects seen on SF10 were related to hunger; this day is termed Full Food day. All remaining food was removed at the next light onset. Body weights of the mice were measured one day before the baseline day and on day 11.

Experiment 2: Scheduled feeding with highly palatable chow

To determine whether anticipation or consumption of more palatable food increases cataplexy, 8 orexin KO mice and 7 WT mice were then placed on scheduled feeding with Froot Loops (Kellogg, Battle Creek, MI) instead of regular chow. We chose Froot Loops because it is a sweetened cereal often used as a reward in behavioral studies of rodents (Lee et al., 2000; Ahn and Phillips, 2002; Udo et al., 2004; Sevak et al., 2008). In pilot studies, we tested several

palatable foods commonly used in rodent studies and found that both orexin KO and WT mice greatly prefer Froot Loops over regular chow. After Experiment 1, mice were given 10 kcal (2.6 g) of Froot Loops three hours after lights off (22:00) on days 12 through 14; these days are termed FL1 through FL3. The total calorie intake per day was the same as in Experiment 1 (60% of their normal dietary intake). Body weights of the mice were measured one day after FL3.

Foraging for food is a motivated behavior that may be associated with positive emotions. To determine whether cataplexy late in the dark period is associated with foraging, we scored the video-recorded behavior of orexin KO mice as “foraging”, “non-foraging”, or cataplexy in 10 second epochs. Our analysis focused on the last 2 hours of the dark period as this was a unique time when food-restricted mice had substantially more cataplexy without food, especially on FL3. Foraging has been measured using a variety of methods that show increases in locomotion and food-seeking behavior (Jacquot and Baudoin, 2002; Vaughan and Rowland, 2003; Day and Bartness, 2004; Tucci et al., 2006; Sakkou et al., 2007; Vaanholt et al., 2007). We found no established definition of foraging based on observations of spontaneous behavior, so we operationally defined foraging as locomotion, rearing, jumping, or digging or sniffing in the bedding. These behaviors often occurred in a series that were clustered together prior to eating. Behaviors that constituted non-foraging were eating, drinking, sleeping, quiet wake, grooming, scratching, and any undefined behavior.

Statistical analysis

All results are expressed as means $\pm$ SEM. Hourly changes in behavior, such as amounts of sleep/wake and cataplexy, LMA, and number of cataplexy bouts, were compared between food conditions using two-way, repeated measures ANOVA with a *post hoc*, two-tailed Student's *t* test. Comparisons between different feeding conditions, such as total amounts of sleep/wake/cataplexy and bout analysis of these behavioral states during the dark or light period, were determined using one-way factorial ANOVA with a *post hoc* Fisher's PLSD tests. Correlations between the number of foraging bouts or time spent foraging and the number of cataplexy bouts were determined using linear regression.

RESULTS

After baseline recordings with ad lib chow, WT (n=14) and orexin KO (n=15) mice were switched to a daily allowance of 60% of their normal food intake delivered 3 hours after lights out. All of the mice ate all the food each day, and both WT and orexin KO mice quickly entrained to the feeding schedule in 5 days with more LMA during the food-anticipation period (Supplemental Figure 1). Across the 10 days of scheduled feeding, the weights of WT and orexin KO mice decreased by $4.5 \pm 1.1\%$ and $2.5 \pm 2.1\%$, respectively. Then, body weights were stable between SF10 and FL3 (about 0.3–0.6% gain in both groups). All mice appeared healthy and maintained normal levels of locomotor activity throughout the protocol.

Scheduled feeding increases arousal and cataplexy

On the tenth day of scheduled feeding (SF10), WT and KO mice spent more time awake than on the baseline day (WT: $F=5.328$, $P<0.0001$; KO: $F=2.991$, $P<0.0001$) (Figure 2). Over the 2 hr before food presentation, both WT and orexin KO mice were awake nearly 100% of the time, suggesting that they anticipated the timing of food delivery. Video recordings showed that most food was consumed in the 3 hr after delivery of food, and both groups of mice had a second period of increased wake late in the dark period that was probably associated with foraging for any remaining food. LMA in WT mice increased by 115% during the food-anticipation period (19:00–21:00; $F=8.782$, $P<0.0001$ vs baseline). As previously reported (Hara et al., 2001; Mochizuki et al., 2006; Zhang et al., 2007), the absolute amount of LMA was

lower in orexin KO mice, but LMA still increased by 73% prior to food delivery ($F=4.948$, $P<0.0001$). Both group of mice had normal levels of LMA after delivery of food.

Scheduled feeding increased cataplexy in orexin KO mice (Figure 3). Prior to the food presentation (21:00), orexin KO mice had about 50% more episodes of cataplexy on SF10 than on the baseline day, but this was not statistically significant. During the 3 hour period after delivery of food, mice spent much of their time eating, and during this interval cataplexy was not increased. Surprisingly, the frequency of cataplexy roughly doubled in the last 6 hours of the dark period. This late rise in cataplexy may have been related to foraging, because this late peak disappeared when mice received their regular amount of food the next day (Full Food). This late rise in cataplexy was not caused by hunger alone because total food deprivation did not increase cataplexy in a preliminary study (data not shown).

Scheduled feeding consolidated sleep/wake behavior in WT and orexin KO mice during the dark period (Supplemental Figure 2). The amount of wake increased by 15% in both groups. The number of wake bouts decreased by about 20%, and the duration of wake bouts increased in both groups, though wake bouts were still much shorter in orexin KO mice. Both groups had fewer NREM and REM bouts during the dark period with only slight changes in bout durations. As a result, total sleep decreased by 28% in WT and 22% in KO mice during the dark period of SF10.

These results demonstrated that scheduled feeding with normal chow increased wake and locomotor activity, but the increase in cataplexy was small.

Highly palatable food substantially increases cataplexy and arousal

To determine whether cataplexy could be triggered by highly palatable food, we next gave orexin KO mice ($n=8$) and WT mice ($n=7$) an isocaloric amount of Froot Loops 3 hours after dark onset (22:00) for three days. All mice ate the entire amount (60% of the daily calorie intake) of Froot Loops each day. Even on the first night (FL1), Froot Loops substantially increased wakefulness and LMA in both groups, and unlike on SF10, wakefulness and LMA remained high throughout the feeding (3 hr after food delivery) and the post-feeding (the following 6 hr) periods (Figure 4). The food-anticipatory activity was attenuated on FL1, probably because the mice ate their normal amount of food the day before (Day 11; Full Food), but the anticipatory activity resumed completely by FL3. The sleep/wake profiles are summarized in Supplemental Figure 2.

Froot Loops markedly increased cataplexy in orexin KO mice (Figure 5). On FL1, the number of cataplexy bouts increased as usual prior to feeding, and then with delivery of this highly palatable food, frequent episodes of cataplexy continued over the next several hours. On FL3, during the pre-feeding period, cataplexy was even more frequent, and it persisted at this high level throughout the dark period. The hourly amounts of cataplexy were also increased during the feeding and post-feeding periods. The overall increase in cataplexy was caused by more frequent cataplexy episodes (107% more on FL3 than on SF10) (Supplemental Figure 2). WT mice did not have any cataplexy with Froot Loops.

To better understand the modulation of cataplexy by feeding, we analyzed the length of cataplexy bouts (Figure 6). On FL3, orexin KO mice had a large increase in short bouts of cataplexy lasting less than 1.5 min. In fact, these short bouts accounted for most of the increase in cataplexy during the pre-feeding, feeding, and post-feeding periods. Short cataplexy bouts also increased on SF10 though to a lesser extent. The feeding protocol had no impact on bouts longer than 1.5 min. This change did not represent fragmentation of a single cataplexy episode into several small bouts because the episodes were not clustered; inter-cataplexy-intervals, as estimated by the mean duration of wake bouts before or after cataplexy, were the same over

the feeding protocol (Supplemental Figure 3). Rather, these observations demonstrate that brief episodes of cataplexy occur frequently when orexin KO mice anticipate, consume, and look for food, especially highly palatable food.

To determine whether cataplexy occurs in association with foraging, we quantified the time spent foraging during the last 2 hr of the dark period on FL3 (Supplemental Figure 4). The number of foraging bouts was linearly correlated with the number of cataplexy episodes ($R^2=0.82$), demonstrating a strong association between this motivated behavior and the triggering of cataplexy.

Because murine cataplexy has similarities with REM sleep such as EEG theta activity and atonia, we also examined changes in REM sleep (Supplemental Figure 5). With scheduled feeding, orexin KO mice had fewer REM bouts of all durations during the dark period. Consequently, the mice had 20% (SF10) and 60% (FL3) less REM sleep than on the baseline night. This reduction was proportionate to the decrease in total sleep time during the dark period (22% on SF10 and 50% on FL3) (Supplemental Figure 2). To compensate for this sleep deficit, WT and KO mice had 10–13% more light period NREM sleep on SF10 and FL3 than on the baseline day, and their NREM sleep was more consolidated. Interestingly, REM sleep did not increase during the light period, suggesting that REM sleep pressure is not likely to contribute to the increase in cataplexy.

DISCUSSION

These studies demonstrate that in orexin KO mice, cataplexy increased slightly with scheduled feeding of regular chow but increased 3-fold with highly palatable food. This robust response to palatable food suggests that murine cataplexy may be triggered by positive emotions just as in human and canine narcolepsy. To our knowledge, this is the first report to describe a feeding-related trigger of cataplexy in a rodent model of narcolepsy.

Positive emotions may trigger murine cataplexy

In people with narcolepsy, cataplexy is often triggered by positive emotions such as laughter, mirth, and pleasant surprise, and in narcoleptic dogs, cataplexy is often elicited by feeding and playing. This strong association with positive emotions is a fundamental aspect of cataplexy, yet it remains unknown whether similar factors trigger cataplexy in mice.

The present results strongly support this idea as scheduled feeding with highly palatable food increased cataplexy in orexin KO mice. With scheduled feeding of regular chow, the increase in cataplexy was relatively small, with a statistically insignificant increase prior to the delivery of food and then about twice as many cataplexy bouts in the post-feeding period. Perhaps, mice experienced little excitement when anticipating regular chow, whereas they were highly motivated to find food later in the dark period because they were calorie-restricted. In contrast, with Froot Loops, orexin KO mice had more cataplexy before the delivery of food, during the feeding period, and in the post-feeding period. Perhaps Froot Loops boosted their motivation for food and caused strong positive emotions that triggered frequent episodes of cataplexy throughout the dark period. Late in the dark period, cataplexy was strongly correlated with foraging, suggesting that this motivated behavior and perhaps associated positive emotions may trigger cataplexy.

These results build upon prior studies of factors that trigger murine cataplexy. Wheel running is very rewarding for mice (Lett et al., 2002; Werme et al., 2002), and we previously found that access to running wheels doubled the amount of cataplexy in orexin KO mice. Specifically, running wheels increased the probability of cataplexy per minute of wakefulness, and high levels of running often preceded cataplexy (España et al., 2007). In addition, mice and rats

make ultrasonic vocalizations with positive social events such as mating and playing with siblings (Panksepp, 2007), and a recent study demonstrated that these vocalizations were positively correlated with cataplexy in orexin KO mice (Burgess et al., 2008). In combination with our present observations, these results strongly suggest that positive emotions can trigger cataplexy in narcoleptic mice.

Scheduled feeding increases short bouts of cataplexy

The increase in cataplexy with scheduled feeding was almost entirely due to an increase in short episodes of cataplexy. These bouts typically lasted less than 1.5 minutes, a duration very similar to that seen in dogs and people with narcolepsy (Nishino et al., 2000; Okun et al., 2002; Scammell et al., 2009). In fact, these short bouts may be closest to true cataplexy. Murine cataplexy often shows two phases: an initial arrest phase when the EEG shows a mix of low amplitude fast frequencies and theta activity with variable amplitude, and a second phase when regular amplitude theta activity dominates (Willie et al., 2003; Mochizuki et al., 2004). Though it is speculative, the first phase may represent true cataplexy with atonia and perhaps preserved consciousness, and the second phase may be REM sleep. Some people with narcolepsy report dreaming during prolonged episodes of cataplexy, suggesting that there may be a low threshold to transition from cataplexy into REM sleep (Hishikawa and Shimizu, 1995).

Alternatively, the long episodes (>1.5 min) could be direct transitions from wake to REM sleep with no intervening cataplexy because the long episodes did not increase with scheduled feeding. However, we detected no differences in the initial patterns of EEG and behavioral activity between the short and long episodes. Therefore, we hypothesize that the long episodes are cataplexy possibly transitioning into REM sleep as mentioned above, and the probability that cataplexy transitions into REM sleep is unaffected by positive emotions. Differences between cataplexy and REM sleep could be examined by studying physiologic markers of REM sleep such as saccadic eye movements, pontogeniculooccipital waves (Datta and Maclean, 2007), and autonomic tone (Miki et al., 2003; Yoshimoto et al., 2004). Another major distinction between cataplexy and REM sleep rests on whether consciousness is preserved. Establishing whether mice are conscious during cataplexy will be challenging but might be testable by examining conditioned responses to sounds or other stimuli.

Other perspectives

The increase in cataplexy with highly palatable food was probably triggered by positive emotions, but other interpretations are possible. First, the increase in cataplexy could be a consequence of altered sleep/wake behavior. Cataplexy and REM sleep have many physiologic similarities, and reduced REM sleep might lower the threshold for cataplexy. With scheduled feeding, mice had less REM sleep during the dark period, and this could increase the likelihood of cataplexy. This explanation seems unlikely as there was no compensatory increase in REM sleep during the light period. Another possibility is that cataplexy increased because with scheduled feeding, mice were awake for longer periods and cataplexy is more likely to occur with long wake bouts (España et al., 2007). However, this phenomenon cannot explain the large increase in cataplexy with palatable food as orexin KO mice had wake bouts of similar length on SF10 and FL3. Last, cataplexy tends to occur during period of high locomotor activity, but triggering by locomotion also seems unlikely as on all days of scheduled feeding, cataplexy increased late in the dark period without much coincident increase in LMA. Overall, an increase in positive emotions with scheduled feeding of highly palatable food is the most likely explanation for the increased frequency of cataplexy.

These results also show that orexin KO mice anticipate scheduled feeding normally, suggesting that orexin has little role in the food-entrainable oscillator (Mistlberger et al., 2003; Kaur et al., 2008). This contrasts with prior studies of orexin neuron-deficient mutant mice which had

less food-anticipatory activity before scheduled feeding during the light period (Akiyama et al., 2004; Mieda et al., 2004). Perhaps, food entrainment requires other neurotransmitters released by the orexin neurons (Chou et al., 2001; Crocker et al., 2005), or food-anticipation in the light period may be blunted in narcoleptic mice because they have defective locomotor activity, lower autonomic tone, or less need for food than WT mice (Hara et al., 2001; Kayaba et al., 2003; Hara et al., 2005; Mochizuki et al., 2006; España et al., 2007).

Could metabolic state influence cataplexy?

Scheduled feeding with 60% of normal calories was a moderate metabolic challenge that slightly reduced the weights of mice. One could argue that food restriction increased cataplexy by altering energy status or brain glucose levels. For example, low concentrations of glucose increase the firing of orexin neurons, whereas high levels of glucose reduce neuronal activity (Yamanaka et al., 2003; Burdakov et al., 2006). We did not measure glucose concentrations, but food restriction might reduce glucose availability, whereas the higher sugar content of Froot Loops might increase glucose levels. Still, if glucose levels influence cataplexy, it must occur through non-orexin pathways as orexin KO mice constitutively lack orexin signaling.

One could also argue that hunger or related negative (not positive) emotions may have induced cataplexy. However, mice were entrained to scheduled feeding for many days, and persistent hunger cannot explain the marked increase in cataplexy after switching to Froot Loops. Overall, it seems most likely that the increase in cataplexy was related to positive emotions

Implications and future directions

These experiments demonstrate that scheduled feeding of highly palatable food substantially increases murine cataplexy. Short bouts of cataplexy are the main source of this increase, and it is possible that during some longer episodes, cataplexy transitions into REM sleep. These results help establish the connection between positive emotions and murine cataplexy, validating orexin KO mice as an excellent model of narcolepsy. On a technical level, feeding mice highly palatable food provides an easy method to substantially increase the frequency of cataplexy, enabling easier screening of anti-cataplexy drugs. This approach could also be used in studies to identify the neural pathways that trigger murine cataplexy, and these results would likely provide novel insights into the still-mysterious neurobiology of positive emotions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Experimental design. Sleep/wake behavior was recorded on the baseline day with ad lib food. Mice were then fed 60% of their normal amount of regular chow at 22:00 (3 hr after dark onset) for 10 days. Sleep/wake behavior was recorded on the tenth day (SF10). On the eleventh day, mice were given a regular amount of food (Full Food, 4.5 g) at 22:00 until the end of the dark period. Mice were then switched to an isocaloric diet of Froot Loops for three days (at 22:00). Sleep/wake behavior was recorded on the first (FL1) and third (FL3) days.

Figure 2.

Scheduled feeding of regular chow increases wake and locomotor activity (LMA). *Top panels:* WT and orexin KO mice spent significantly more time awake before food delivery at 22:00 (dashed line) on SF10 compared to the baseline day with ad lib food. *Bottom panels:* LMA also increased in both groups in the hours before food delivery on SF10, but the amounts of LMA were less in orexin KO mice. * $p < 0.05$, ** $p < 0.01$ compared with baseline day.

Figure 3.

Scheduled feeding with regular chow slightly increases cataplexy in orexin KO mice. *Top:* In the hours before delivery of food, the number of cataplexy bouts increased by about 50%, but this change was not statistically significant. Cataplexy also increased toward the end of the dark period on SF10. This late peak was absent when mice were given a regular amount of chow on the full food night. *Bottom:* The hourly amounts of cataplexy were increased in the late dark period on SF10. * $p < 0.05$ compared with baseline day.

Figure 4.

Scheduled feeding with highly palatable food increases wake and LMA before and after the delivery of food. In both groups of mice, Froot Loops caused a long-lasting increase in wake (*top panels*) and LMA (*bottom panels*) after food delivery on the first night (FL1). The anticipatory arousal was blunted on FL1 probably because mice had a regular amount of food on the prior night (Full Food). On the third night (FL3), both groups showed long-lasting increases of wake and LMA from the food-anticipation period to the middle of the dark period.

* p<0.05, ** p<0.01 compared with baseline day.

Figure 5.

Scheduled feeding with highly palatable food markedly increases cataplexy in orexin KO mice. Video recordings showed that most food was consumed in the 3 hours after delivery of food (between the dashed lines), and foraging was common in the post-feeding period. *Top:* Prior to the delivery of Froot Loops on FL 3, the number of cataplexy bouts was increased. Larger increases were seen in the feeding and post-feeding periods on FL1 and FL3. *Bottom:* The hourly amounts of cataplexy were also increased on FL1 and FL3. * $p < 0.05$, ** $p < 0.01$ compared with baseline day.

Figure 6.

Scheduled feeding with highly palatable food markedly increases short bouts of cataplexy during the dark period. *Top:* Orexin KO mice had many more bouts of cataplexy lasting less than 1.5 min on FL3; a smaller increase in short bouts was observed on SF10. *Middle:* The short (up to 1.5 min) episodes of cataplexy occurred more frequently (normalized per hour of time) in the feeding and post feeding periods on FL1 and FL3. The number of bouts in the pre-feeding period was significantly increased only on FL3. *Bottom:* The number of long cataplexy episodes (longer than 1.5 min) was not affected by scheduled feeding. * $p < 0.05$, ** $p < 0.01$ compared with baseline day. † $p < 0.05$, †† $p < 0.01$ compared with the indicated day.