Circadian Food Anticipation in Dopamine-1 Receptor Knockout Mice

by

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Abstract

Restricted daily feeding schedules induce circadian rhythms of food anticipatory activity (FAA) in mice and other species. The entrainment pathway(s) and location(s) of circadian oscillators driving these rhythms have not been definitively established. An important role for dopamine signaling and the dorsal striatum is suggested by a confluence of observations, including shifting of FAA rhythms by dopamine receptor agonists, and attenuation by antagonists and D1 receptor knockout (D1R KO). The dopamine reward system exhibits sexual dimorphisms in structure and function; if FAA rhythms are regulated by this system, then FAA may also be sexually dimorphic. To assess this prediction, disc running and general activity were recorded continuously in male and female C57BL/6J mice with food available ad libitum and then restricted to a 4 h daily meal in the middle of the light period. Compared to male mice, FAA in female mice was significantly reduced in duration, total counts, peak level and ratio relative to nocturnal activity. To determine if these differences were mediated by D1 receptors, male and female homozygous D1R KO mice were examined. Compared to wildtype and heterozygous mice, female and male D1R KO mice exhibited a marked attenuation of FAA parameters. The magnitude of the attenuation was greater in females. These results confirm an important role for dopamine D1 receptors in the circadian mechanism by which mice anticipate a daily meal, and reveal a previously unreported sexual dimorphism in the expression of food anticipatory rhythms that appears amplified by D1R KO.

Keywords: Circadian Rhythms; Dopamine; Food Anticipation; Sex Differences

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List of Acronyms

DA	Dopamine
D1R	Dopamine-1 Receptor
FAA	Food Anticipatory Activity
FEO(s)	Food Entrainable Oscillator(s)
KO	Knockout
LD	Light-Dark
RF	Restricted Feeding
SCN	Suprachiasmatic Nucleus
ZT	Zeitgeber Time

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Chapter 1.

Introduction

Mice, rats, and many other species can anticipate feeding opportunities that recur at circadian (~24h) intervals. In mice, anticipation typically emerges within a few days as a bout of locomotor activity that begins 1-3 h prior to mealtime and increases to a peak at mealtime. The ability to time daily meals is thought to be regulated by food-entrainable circadian oscillators (FEOs) that are distinct from the master light-entrainable circadian pacemaker in the suprachiasmatic nuclei (SCN) (Boulos & Terman, 1980; Aschoff 1986; Mistlberger 1994; Stephan 2002). Though substantial evidence indicates that daily food-anticipatory activity (FAA) is governed by a bona fide circadian clock, the neural and molecular mechanisms of this timing system remain to be clarified (Mistlberger, 2011).

Hypothalamic circuits involved in regulating appetite and energy metabolism have attracted attention as possible substrates for FEOs regulating FAA (Acosta-Galvan et al, 2011; Davidson 2009; Escobar et al, 2011; Gunapala et al, 2011; Mistlberger 1994, 2011; Sutton et al 2008; Verwey & Amir, 2009). Rats and mice can also anticipate daily access to rewarding stimuli, such as receptive mates, palatable foods and psychomotor stimulants, without calorie restriction (Angeles-Castellanos et al, 2008; Hsu et al, 2010a, 2010b; Jansen et al, 2012; Landry et al., 2012; Mistlberger and Rusak, 1987; Mohawk et al 2013; Webb et al 2009a; Challet & Mendoza, 2010). This suggests that FEOs may be entrainable by any salient reward, and may reside in neural circuits that mediate reward. Of special interest is the neurotransmitter dopamine, as its presence is critical for the expression of reward seeking behavior (Ungerstedt, 1971; Szczypka et al, 1999) and multiple elements of dopamine signaling are under circadian control (Abarca et al, 2002; Falcón & McClung, 2009; Ferris et al, 2014; ; Mendoza & Challet, 2014; Webb et al, 2009a,b).

Several lines of evidence support an important role for dopamine transmission in the regulation of FAA. Dopamine D1 and D2 receptor agonists administered systemically prior to a daily meal can increase FAA independent of general activity (Liu et al 2012) and can induce anticipatory activity independent of restricted feeding (Shibata et al, 1995; Gallardo et al, 2014). A D2 receptor agonist can shift the onset of FAA in rats (Smit et al. 2013). Dorsal and ventral striatal regions innervated by midbrain dopamine neurons show circadian variations in neuronal activity, extracellular dopamine tone and circadian clock gene expression, and these rhythms can be shifted by restricted daytime feeding schedules (Baltazar et al, 2013; Ferris et al, 2014; Hood et al, 2010; Mendoza et al, 2005; Natsubori et al, 2013; Wakamatsu et al, 2001). The rhythm of clock gene expression can also be shifted by D2 agonists and by drugs of abuse and are eliminated by depletion of striatal dopamine (Hood et al 2010; Falcón & McClung 2009). In addition, chronic systemic administration of methamphetamine can induce a quasi-circadian rhythm in rats and mice, a phenomenon that could represent an appropriation of the food-entrainable circadian system (Blum et al, 2014; Honma & Honma, 2009; Mohawk et al, 2013; Pendergast et al, 2012).

Dopamine-deficient mice do not forage or attempt to eat (Szczypka et al, 1999), and thus cannot exhibit anticipatory activity to a scheduled daily feeding opportunity. We recently showed that dopamine expression limited to the dorsal striatum is permissive for FAA in dopamine-deficient mice, and that FAA is significantly attenuated in D1 but not D2 receptor knockout (KO) mice (Gallardo et al, 2014). D1R KO was also associated with loss of circadian expression of the clock gene Per2 in the dorsal striatum of food-restricted KO mice (Gallardo et al, 2014). These results converge on a hypothesis that FAA is regulated by dopamine sensitive circadian FEOs in the dorsal striatum.

Sex differences have been described in the structure and functional properties of midbrain dopamine neurons and striatal reward circuits in rodents and humans (Becker and Hu, 2008; Becker et al, 2012). These differences, which may underpin sex differences in addiction, are thought to reflect organizational and activational effects of steroid hormones, and hormone-independent chromosomal mechanisms, acting on multiple sites within the reward system (Becker and Hu, 2008; Bobzean et al, 2014; Carroll & Anker, 2010; Fattore et al, 2014). If circadian rhythms of FAA are regulated by a sexually dimorphic reward system, then FAA may also be sexually dimorphic. Sex

differences in light-dark (LD) entrained circadian rhythms driven by the circadian pacemaker in the SCN have been described (Bailey & Silver, 2014; Krizo & Mintz, 2015), but circadian rhythms of FAA are not generated by the SCN (Stephan, 2002; Mistlberger, 2011) and, to our knowledge, a comparison of FAA in male and female rodents is not available in the published literature (Krizo & Mintz, 2015). We provide here the first evidence for sex differences in multiple parameters of FAA rhythms in C57BL/6J mice, and further show that these differences are magnified in mice lacking dopamine D1 receptors.

Chapter 2.

General Method

2.1. Animals and Apparatus

This research was approved by the University Animal Care Committee at Simon Fraser University (protocol #1116). D1R KO mice were bred in-house by heterozygous intercrosses (D1R +/-) of mice originally generated at NIH (Drago et al, 1994). Pups were left with mothers for 4 weeks before weening. D1R KO mice have been previously reported to weigh approximately 30% less than their wildtype and heterozygous littermates and to be noticeably less robust (Drago et al, 1994). Previous research with D1R KO mice has found that survival rates after weaning improve dramatically when chow is mixed with water and provided on the cage floor as opposed to the tops of the cages in whole pellets. To ensure that the D1R KO mice were able to access and ingest available food, rodent chow pellets (5001, LabDiets, St. Louis MO) were made available to all mice on the cage floor during ad libitum feeding periods. During periods of restricted food access, pellets were ground up and mixed in water to facilitate ingestion and prevent hoarding.

DNA was extracted from ear punches, and the *Drd1* locus was genotyped using the following primers: *neomycin* CACTTGTGTAGCGCCAAGTGC, *drd1* TCCTGATTAGCGTAGCATGGAC, and *d1* GGTGACGATCATAATGGCTACGGG. After weening, D1R KO mice were present at lower than expected Mendelian ratios in our facility. For all births, D1R KO mice only made up 16% of mice that survived past weening (the time at which they could be genotyped) compared to 26% and 58% for wildtype and heterozygous mice respectively. Ratios were slightly more Mendelian in female pups (19% D1R KO compared to 13% in males, and 26% wildtype compared to

35% in males). Others have seen ratios at Mendelian rates and there is purportedly some variability from facility to facility (A. D. Steele, personal communication May 2015).

Female (n=11) and male (n=6) D1R KO mice as well as control male (n= 15) and female (n=10) littermates (D1R+/- and D1R+/+) were single-housed in standard clear plastic mouse cages with bedding, horizontal running discs (Mouse Igloo Fast-Trac, Bioserv, USA) and unrestricted access to water. Cage lighting was maintained with overhead white LED lights (~ 70 lux) with a 12:12 light-dark (LD) schedule. Disc running was detected by magnetic sensors. General activity was monitored using passive infrared motion sensors mounted above each cage.

2.2. Restricted Feeding

After at least 10 days of activity, recording food was restricted to a daily meal beginning 6-h after lights-on (Zeitgeber Time (ZT) 6, where ZT0 is lights-on by convention). The duration of food availability was gradually reduced from 11-h to 4-h over the first 10 days of food restriction. Powdered chow was mixed with water just prior to mealtime, and placed inside the cage in a plastic weigh boat which was removed after the mealtime. Mice were weighed daily at the beginning of the mealtime. Restricted feeding was maintained for at least 32 days.

To determine whether any FAA evident in D1R KO mice exhibits properties consistent with mediation by an entrained circadian clock, meal time in a subset of mice was delay shifted by 6-h, to ZT12.

2.3. Data Analysis

Activity sensors were monitored continuously using the Clocklab data acquisition and analysis system (Actimetrics, IL). Data were averaged in 10 min bins for visual inspection in the form of actograms and average waveforms generated by Circadia (Dr. Tom Houpt, legacy software, Florida State University) and Prism 6.0 (GraphPad Software, San Diego CA). Nocturnality was quantified as the percent of total daily activity occurring during lights-off. FAA was quantified as the amount of activity during the 4 h

before mealtime (ZT2-6), expressed as total counts and as a ratio of FAA counts to total daily activity excluding hours ZT2-10 (4 h premeal and the 4 h mealtime). FAA duration was quantified using the Clocklab algorithm for detecting the onset of activity prior to mealtime, and averaging this across 10 day blocks of restricted feeding. There were no significant differences between D1R+/+ and D1R+/- in body weight, activity levels or FAA parameters, therefore wildtype and heterozygous mice were pooled into a single 'control' group. The significance of differences between KO and control mice, and between males and females of both genotypes, was evaluated using independent samples t-tests and ANOVA (Prism 6.0).

Chapter 3.

Results

3.1. Activity rhythms with food ad libitum: female vs male control mice

Activity records from representative male and female control and D1R KO mice are illustrated in Figure. 3.1. During ad libitum food access, female control mice exhibited significantly more disc running counts per day than male mice (+147%, t=3.726, p< 0.004; Figs. 3.2A; 3.3A). Females also showed slightly more activity as measured by motion sensors, but the sex difference was not statistically significant (+28 %, t=0.93 p=.37; Figs. 3.2B; 3.3B). There was no evidence for a 4-5 day periodicity in the amount or timing of activity in the females.

Running and general activity were both highly nocturnal. The degree of nocturnality tended to be greater in females but the differences were not statistically significant for either running (95 + 1 % Vs. 91 + 2%, t=1.46, p=.08; Fig. 3.3C) or general activity (90 + 1 % Vs. 87 + 2%, t=.96, p=.18; Fig. 3.3D).

3.2. Activity rhythms with food ad libitum: D1R KO vs control mice

When food was available ad libitum, the D1R KO mice (pooling males and females) showed 36% less disc running (t= 2.323, p<.0.05) than control mice, but 87% more general activity detected by motion sensors (t= 2.849, p<0.01). The differences were in the same direction for males and females considered separately (Figs. 3.2; 3.3A,B). However, with the smaller group sizes, the difference was statistically significant for running in females (D1R KO 48% < control, t=2.78, p=.0178) but not in males (D1R

KO 44% < control, t=1.36, p=.19), while for general activity, the differences were only weak trends for both females (D1R KO 72% > control, t= 1.58, p=.14) and males (D1R KO 67% >, t=1.95, p=.07).

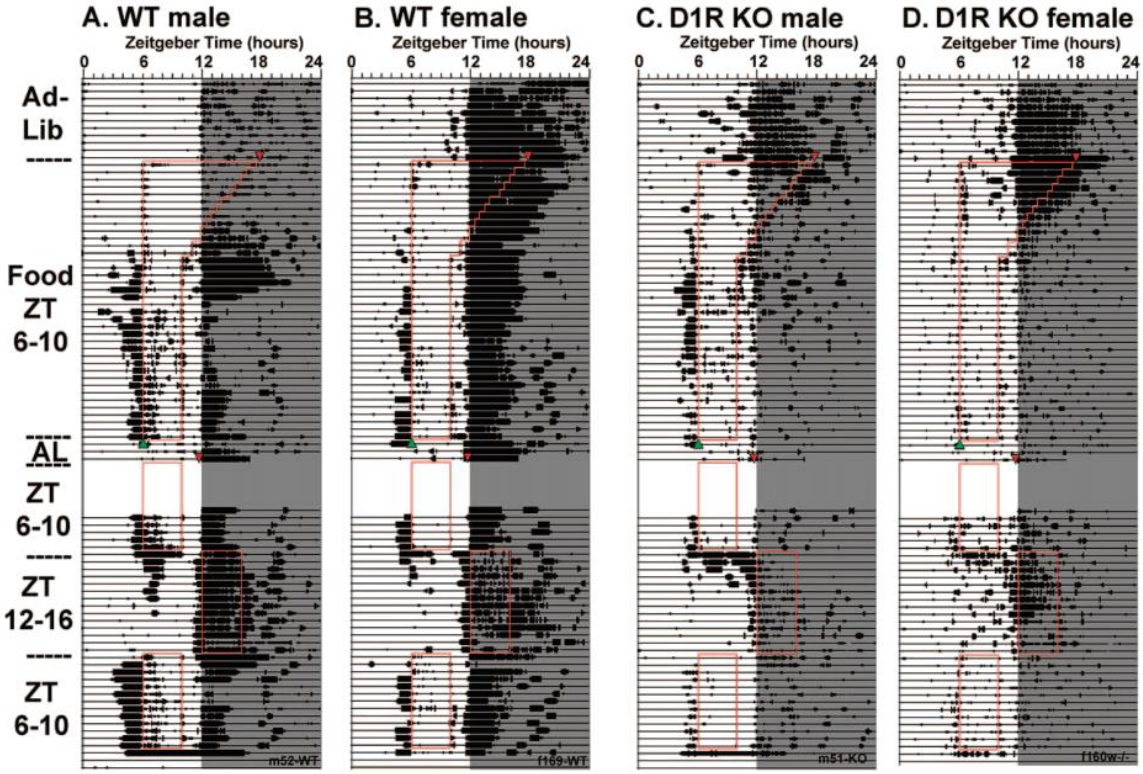

Figure 3.1. Locomotor activity of representative D1R-/- and D1R+/+ (WT) mice during ad libitum food access and restricted feeding.

Each line represents 24 h, plotted in 10 min bins from left to right. Consecutive days are aligned vertically. Bins during which disc running counts were registered are presented by vertical deflections, which create heavy bars when running is continuous over multiple time bins. Mealtime during restricted feeding is demarcated by the red boxes. Lights off is denoted by grey shading. Abbreviations: ZT, Zeitgeber Time; AL, ad libitum.

Nocturnality ratios tended to be lower in D1R KO mice compared to control mice. For disc running, this decrease was significant in females (81 + 4 % Vs 95 + 1 %, t=2.39, p=.017; Fig. 3.3C) but was only a trend in males (85 + 3 % Vs 91 + 2 %, t=1.44, p=.089). For general activity (Fig. 3.3D), the decrease was evident only as a trend in females (82 + 4 % Vs 90 + 1 %, t=1.58, p=.071) and not in males (85 + 3 % Vs 87 + 2 %, t=1.44, p=.25).

3.3. Activity rhythms with food ad libitum: female vs male D1R KO mice

Sex differences in activity levels within the D1R KO group were in the same direction as in the control mice, but statistically these difference were significant only as a trend for disc running (+129% more in females, t=2.037, p= 0.064; Fig. 3.2C; 3.3C) and not for general activity (+32 % more in females, t=0.96, p= 0.35; Fig. 3.2D; 3.3D).

Sex differences in nocturnality within the D1R KO group were in the opposite direction compared to the control mice. Nocturnality ratios in female D1R KO mice, compared to males, were 14.8% lower for disc running (t=2.37, p=.017; Fig. 3.3C) and 6 % lower for general activity (t=1.436, p=.089; Fig. 3.3D).

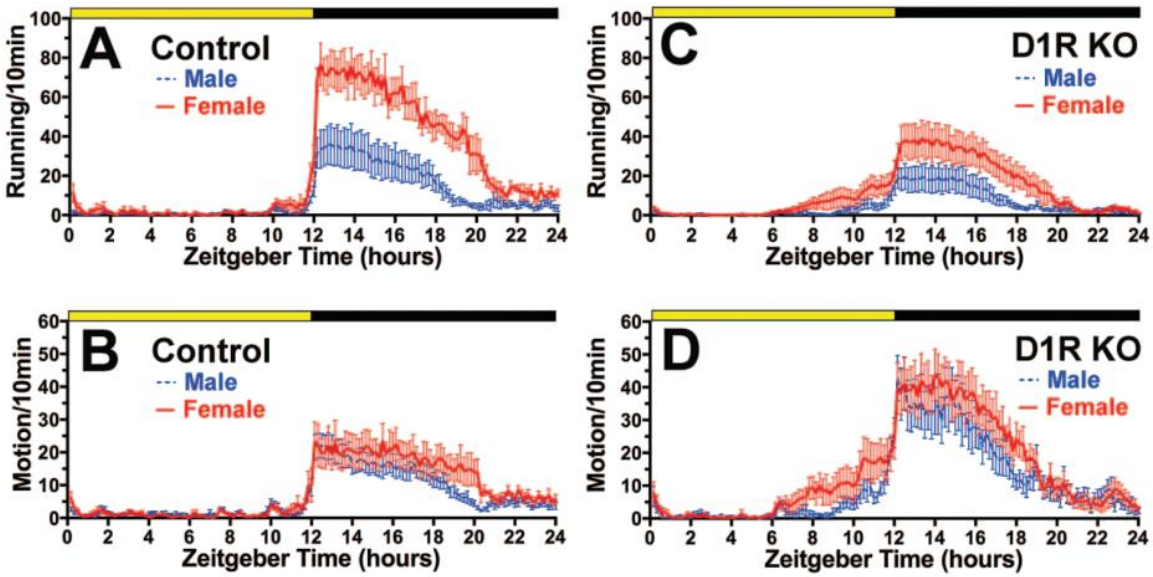

Figure 3.2. Group mean (+SEM) waveforms of disc running (A,C) and general activity (B,D) in control (A,B) and D1R KO (C,D) mice.
Dashed blue curves denote male mice and solid red curves denote females. The 12h daily light period is indicated by the horizontal yellow bar, and the dark period by the black bar.

3.4. Food anticipatory activity: female vs male control mice

Food availability was gradually reduced from 11h/day to 4h/day over the first 10 days of restricted feeding and during that time FAA was minimal in control mice of both

sexes (e.g., Fig. 3.1A,B). FAA began to emerge within the first few days of 4 h daily meals. In the disc running measure, female control mice, compared to male control mice, exhibited a 32% shorter FAA bout duration (t=2.69, p=.0065), 68% fewer FAA counts (t=1.99, p=.029), a 16% lower peak level (t=.99, p=.11) and a 45% lower FAA ratio (t=3.00, p=.003), (Figs. 3.4A, 3.5A-D). In the motion sensor measure, female mice exhibited a 30% shorter FAA bout duration (t=2.48, p=.010), 35% fewer FAA counts (t=1.46, p=.079), a 25% lower peak level (t=1.60, p=.062) and a 40% lower FAA ratio (t=2.62, p=.007). (Fig. 3.4B, 3.5E-H).

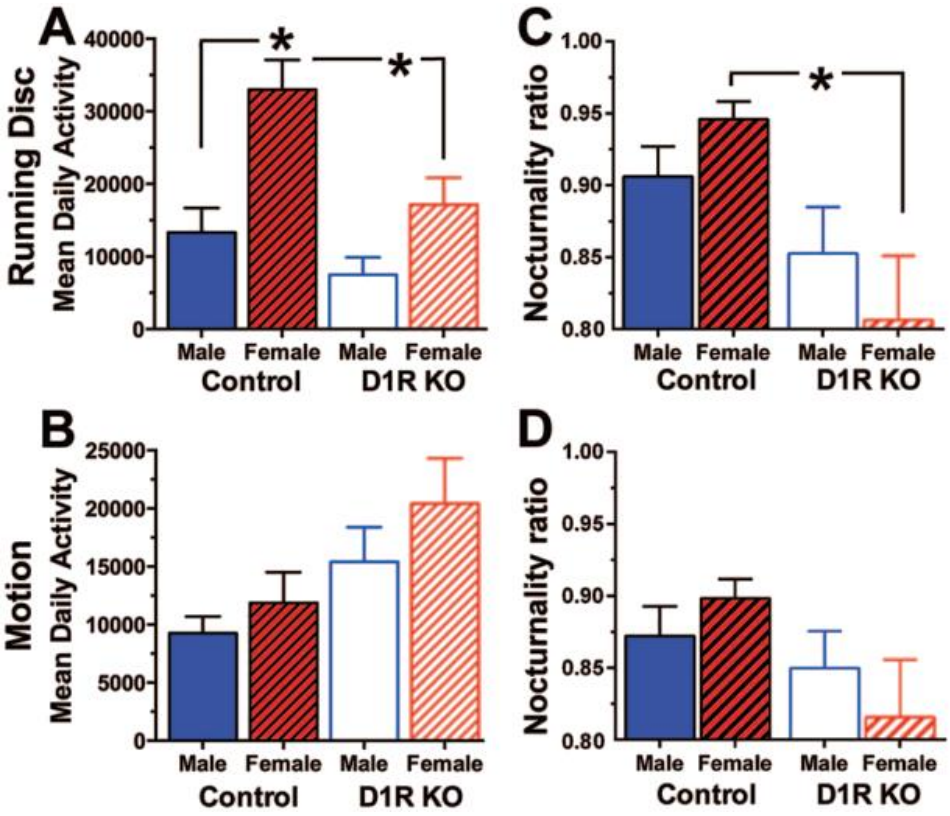

Figure 3.3. Group mean (+SEM) daily activity counts and nocturnality ratios during ad libitum food access.

(A) Disc running. (B) General activity detected by motion sensors. (C) Nocturnality ratios for disc running. (D) Nocturnality ratios for general activity. Significant differences (p<.05) are denoted by an asterisk.

3.5. Food anticipatory activity: D1R KO vs control mice

Compared to control mice, FAA was markedly decreased in D1R KO mice of both sexes (Fig. 3.4C,D; 3.5). FAA duration for disc running was 64% shorter in females (t=3.77, p=.0006) and 41% shorter in males (t=3.86, p=.0005). FAA duration for general activity was 53% shorter in females (t=3.16, p=.0027) and 38% shorter in males (t=3.85, p=.0005). Total FAA counts for running were 95% lower in females (t=4.19, p=.0002) and 86% lower in males (t=3.864, p=.0005). Total FAA counts for general activity were 86% lower in females (t=2.61, p=.0086) and 68% lower in males (t=3.22, p=.0023). FAA peak level for running was 89% lower in females (t=6.28, p<.0001) and 73% lower in males (t=4.11, p=.0003). FAA peak level for general activity was 73% lower in females (t=4.09, p=.0003) and 29% lower in males (t=2.89, p=.0047). The ratio of FAA to nocturnal activity in female D1R KO mice was 48% lower for general activity (t=2.30, p=.018) and 27% lower for disc running (t=1.10, p=.14). In the male D1R KO mice the FAA ratios were not significantly reduced in either general activity (-2%, t=0.08, p=.93) or disc running (25%, t=1.00, p=.32), due to a significant decrease in nocturnal activity (Fig. 3.4C,D; 3.5G,H).

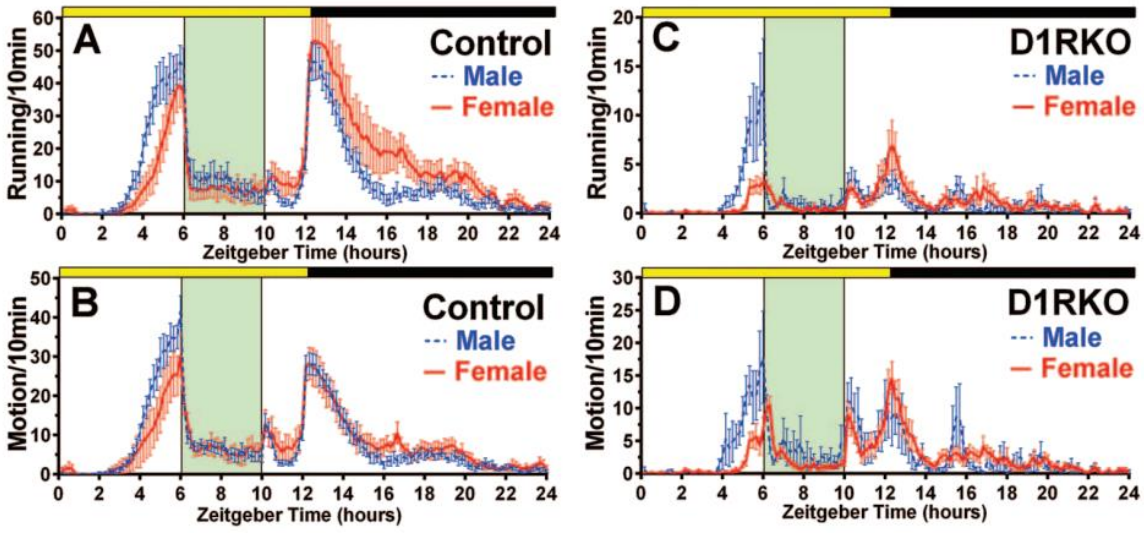

Figure 3.4. Group mean (+SEM) waveforms of disc running (A,C) and general activity (B,D) in control (A,B) and D1R KO (C,D) mice, separated by sex (male=blue dashed curves, female = red curves).
Data for each mouse were averaged over days 23-32 of restricted feeding. The 12h daily light period is indicated by the horizontal yellow bar, and the dark period by the black bar.

3.6. Food anticipatory activity: female vs male D1R KO mice

Direct comparisons of FAA in female and male D1R KO mice revealed sex differences in FAA parameters that matched or exceeded those evident in the control mice (Fig. 3.5). In the disc running measure, female KO mice, compared to male KO mice, exhibited 76% fewer FAA counts (*t*=3.26, p=.005), a 60% shorter FAA bout duration (t=5.10, p=.00001), a 67% lower FAA ratio (t=3.36, p=.004), and a 66% lower peak level (*t*=2.60, p=.02). In the motion sensor measure, female KO mice exhibited 71% fewer FAA counts (*t*=2.95, p=.01), a 47% shorter FAA bout duration (*t*=4.18, p=.0008), a 70% lower FAA ratio (t=2.78, p=.014), and a 59% lower peak level (t=2.36, p=.032).

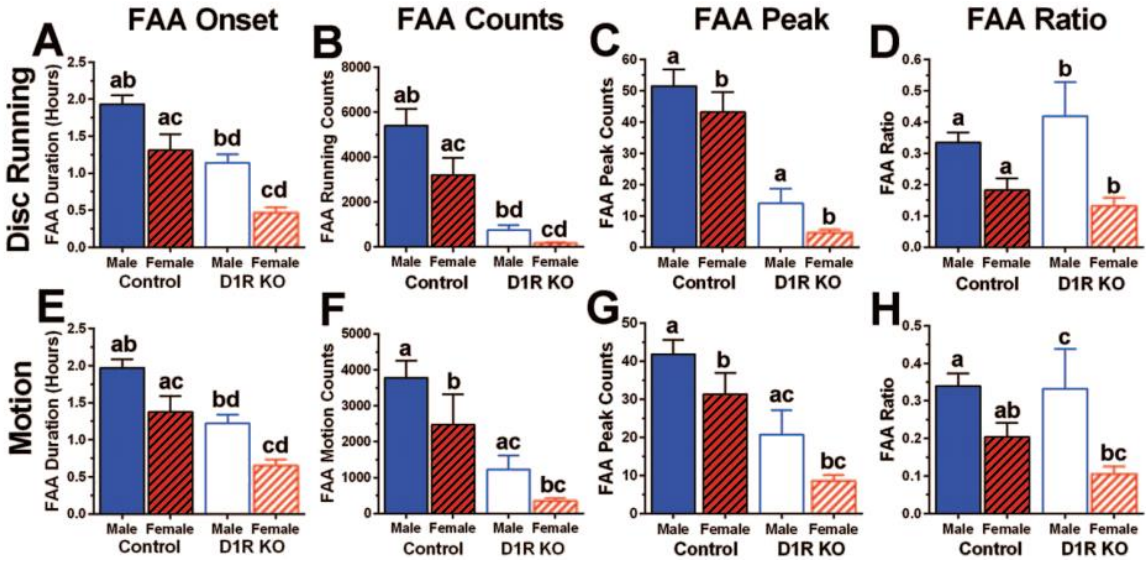

Figure 3.5. Group mean (+SEM) parameters of food anticipatory activity for disc running (A-D) and general activity (motion sensor, E-H) in male and female control and D1R KO mice.

Shared letters denote a significant difference at p<.05 (see text for exact p values).

3.7. Interaction between sex and genotype differences

Two-way ANOVA was carried out to test whether there was a statistical interaction between sex and genotype effects on FAA. In almost all cases, differences between genotypes were larger in female mice than in male mice and the sex

differences was larger in D1R KO mice, however, no interaction effects were detected for running disc FAA counts, duration, peak, or ratio (all p >.05) or for those same parameters in general cage activity (all p >.05).

3.8. Residual food anticipation in D1R KO mice exhibits circadian properties

The emergence of activity prior to a single daily feeding opportunity could be induced by hunger, independent of a circadian oscillator entrainment process. Rest-activity cycles driven by hunger would exhibit properties of hourglass timing, and would reset immediately following a shift of mealtime. By contrast, rhythms generated by self-sustaining circadian oscillators may persist for several cycles or more without an entraining stimulus (e.g., a daily meal), and typically reset gradually following a shift of mealtime. To determine whether residual FAA generated by D1R KO mice was driven by an hourglass timer rather than a circadian oscillator, mealtime was delay shifted by 6 h from ZT6 to ZT12 for 14 days. On the day of the meal shift FAA in D1R KO and control mice persisted through the mealtime before decreasing (Fig. 3.1). On the next two days after the meal shift, activity in control and D1R KO mice of both sexes persisted at the prior mealtime, concurrent with shifting bouts of activity and activity emerging prior to the new mealtime.

3.9. Body weight during restricted feeding

Mice in all groups lost on average 10-20% of their initial body weight over the first ~2 weeks of restricted feeding and began to recover body weight by week three. There was no significant main effect of group on cumulative weight loss ($F_{(3,27)}$ =0.24, p=0.86), and no difference between any pairs of groups.

Chapter 4.

Discussion

The results of this study make two substantive contributions. First, the results confirm and extend our previous report that circadian rhythms of FAA in mice are significantly attenuated by D1R KO (Gallardo et al, 2014). Second, the results provide novel evidence for a previously unreported sex difference in the expression of FAA. A sex difference in FAA is predictable if FAA is regulated by a sexually dimorphic brain system. Dopaminergic reward circuits exhibit sexual dimorphisms in form and function (Becker and Hu, 2008; Bobzean et al, 2014; Carroll & Anker, 2010; Fattore et al, 2014). These results therefore constitute additional, indirect support for a hypothesis that FAA is regulated by the reward system.

4.1. Sex differences in circadian rhythms

When food is available ad libitum, circadian activity rhythms in mice are controlled by a light-entrainable circadian pacemaker in the SCN (Boulos & Terman, 1980; Aschoff 1986). Under these conditions, sex differences have been reported in the timing of activity relative to the light-dark cycle (i.e., phase of entrainment) and in the period and amplitude of activity rhythms free-running in constant dark (Bailey & Silver, 2014; Krizo & Mintz, 2015; Kuljis et al 2013). These differences are believed to reflect both organizational and activational effects of gonadal steroid hormones acting on clock cells in the SCN and possibly elsewhere in the brain. Some effects of sex steroids on circadian rhythm parameters may be mediated indirectly, e.g., by an effect on the level of activity, which by so-called ‘non-photic’ input pathways can alter SCN neuronal activity and modify the phase or period of SCN-dependent activity rhythms (Webb et al 2014).

In the present study, when food was available ad libitum, there was a marked sex difference in the amount of disc running, with female mice running nearly 50% more than males. Most of the additional running occurred at night, as females also tended to be more nocturnal than males. Increased running in females may reflect an activity promoting effect of estrogen, in which case activity would be expected to show a 4-7 day rhythm in synchrony with the estrous cycle. We did not detect regular multiday variations in the activity data during ad libitum (or restricted) food access. This behavioral correlate of the estrous cycle is known to be weaker in mice by comparison with rats, and may be absent in some strains (Kopp et al, 2006).

When food was restricted to a 4 h meal in the light period, control mice of both sexes became active each day 1-2 h in advance of mealtime. The duration and magnitude of this food anticipatory response was significantly greater in the male mice, and especially in disc running. Compared to male mice, the female mice therefore exhibited more nocturnal running when fed ad libitum, and less daytime running when food was limited to the mid-day. Whether these sex differences depend on sex steroids and sexually dimorphic neural circuits remains to be determined. The processes responsible for the sex difference are also an open question. There are at least five possibilities. 1. Sex differences in the duration and amount of FAA could be related to the response of food entrainable circadian oscillators to daily feeding cues, which determines the phase at which those FEOs couple to mealtime (analogous to how differences in the response of the SCN pacemaker to light input determines the timing of activity relative to LD cycles; Pittendrigh and Daan, 1976). 2. FAA parameters could vary if there is a sex difference in the intrinsic period or amplitude of FEOs, at the single cell or population level (assuming that FAA is mediated by a population of intrinsically rhythmic, coupled oscillators). 3. FAA parameters could vary if there is a sex difference in incentive motivation induced by circadian time cues that predict and become conditioned to mealtime (this concept is elaborated further below). 4. FAA parameters could be affected by sex differences in competition between FEOs and light-entrainable oscillators in the SCN. The SCN is thought to actively promote sleep during the daily light period (Mistlberger, 2005), and its output must therefore be overcome or suppressed for FAA to be expressed in the day (Mistlberger, 2006). A higher amplitude SCN or weaker SCN suppression would attenuate the amount of daytime FAA. 5.

Finally, FAA parameters could reflect sex differences in the inhibitory effect of light on activity. These hypotheses can be tested in future studies by manipulating feeding schedule parameters, SCN integrity and lighting.

4.2. Dopamine D1 receptors regulate FAA but do not explain sex differences

We previously reported that mice lacking dopamine D1 receptors exhibit a marked reduction of FAA as measured by behavior recognition algorithms applied to 24h video snapshots taken at ~weekly intervals (Gallardo et al, 2014). The results reported here confirm and extend phenotyping of FAA in D1R KO mice. We observed a marked decrease in FAA in D1R KO mice relative to control mice, in both measures of behavior, and in multiple FAA parameters (duration, peak level, total counts, and counts relative to nocturnal activity). With a larger sample of both sexes, we found that the decreases in FAA in D1R KO mice were greater in females, thus amplifying the sex difference. If sex differences in FAA in control mice were due to a difference in D1 receptor signaling, then FAA should look more similar in males and females lacking D1 receptors. The sex difference must therefore emerge from some other factor that contributes to the timing and amount of FAA.

The low levels of FAA expressed by D1R KO mice was not secondary to general malaise or metabolic collapse caused by insufficient food intake. Mice that lose weight rapidly may become hypothermic or torpid to save energy. To avoid rapid weight loss mice in this study were introduced to the restricted feeding schedule gradually. This resulted in gradual weight loss and recovery over the first 14-21 days of scheduled feeding, with no differences between KO and control mice. We observed no signs of behavioral torpor, consistent with measures of core body temperature reported previously (Gallardo et al, 2014). When food was provided at ZT6 each day, control and KO mice were mobile and initiated eating with little delay. The reduction in FAA in KO mice was therefore not an expression of ill health secondary to metabolic collapse.

It is tempting to speculate that the D1R KO has impaired a critical element of the timing mechanism responsible for food anticipatory rhythms, resulting in weaker and

variable anticipation. A chronobiological interpretation of the FAA phenotype in D1R KO mice can be developed as follows. Dopamine deficient mice do not spontaneously seek or eat food and thus will not express FAA (Szczypka et al, 1999). Dopamine signaling restricted to the dorsal striatum is sufficient to permit circadian FAA in dopamine deficient mice (Gallardo et al 2014). The dorsal striatum exhibits daily rhythms of clock gene expression that are dopamine-dependent, shifted by dopaminergic compounds and entrained by daily feeding schedules (Hood et al, 2010; Wakamatsu et al, 2001). FAA can also be shifted by dopaminergic compounds (Smit et al, 2013), and anticipation can be enhanced (Liu et al, 2012) or induced by daily injections of D1 or D2 receptor agonists (Shibata et al, 1995; Gallardo et al 2014). Finally, D1R KO flattens the daily rhythm of clock gene expression in the dorsal striatum of food restricted mice (Gallardo et al, 2014). Taken together, these results suggest that D1 receptor signaling plays an important role in the expression of FAA by participating in phase control of FEOs in the dorsal striatum.

Phase control can take two forms. One possibility is that the D1 receptor is in the entrainment pathway from feeding (or reward) related cues to striatal FEOs. A second possibility is that dopamine signaling at D1 receptors functions as a coupling factor necessary for maintaining synchrony among multiple striatal clock cells, analogous to the role of vasoactive intestinal polypeptide and its receptors in the light-entrainable SCN circadian clock (Aton et al, 2005; Hughes et al, 2011; Vosko et al, 2007). In either case, loss of D1 receptor signaling would result in desynchrony and loss of rhythm amplitude at the tissue (striatal) level, and would greatly weaken the effect of scheduled feeding on the circadian organization of food seeking behavior.

4.3. Food entrainable oscillators, dopamine and incentive motivation

There is an extensive literature documenting a critical role for dopamine in a range of processes that together regulate the expression of appetitive behavior (Berridge, 2007; Palmiter, 2008; Wall et al, 2011; Wise, 2009). These processes are obviously intertwined, but can be parsed as learning and memory, motivation, and motor

control. Defects in any of these processes could potentially explain reduced FAA in a dopamine receptor KO model, and so we consider these next.

Animals completely lacking dopamine do not exhibit appetitive behavior in response to deprivation states or conditioned incentive stimuli (Ungerstedt, 1971; Palmiter, 2008). These animals can be described as profoundly unmotivated. Mice lacking only the dopamine D1 receptor exhibit more subtle deficits. D1R KO mice appear less willing to expend effort to obtain food, e.g., if this requires reaching for food in an overhead feeder, but as we and others (e.g., Drago et al, 1994) have observed they do eat and maintain a healthy, albeit reduced weight (~25% below wildtypes) when food is placed on the cage floor. Also, they do respond appropriately to acute food deprivation (e.g., 24h) by becoming hyperactive, and by increased eating when food is available at a time of day when nocturnal mice eat little (Gallardo et al, 2014 and present study). They also exhibit normal preference for palatable foods like saccharin (Wall et al, 2011) and can discriminate normally between a lever that provides food and one that does not (Olsen and Winder, 2009). D1R KO mice thus can process rewards and perform simple discriminations. Deficits appear when food is used to support classical and operant conditioning. D1R KO mice show impaired acquisition of a conditioned anticipatory response to a cue signaling a food reward, and poor performance on lever pressing and T-maze tasks for food reward (Caine et al., 2007; El-Ghundi et al., 2003; Wall et al 2011).

Circadian rhythms of FAA have been conceptualized as the outcome of a simple oscillator entrainment process, whereby a periodic stimulus (food) controls the phase of circadian oscillators that drive a daily rest-activity cycle, which aligns with mealtime according to intrinsic properties of the oscillator (its period and phase resetting characteristics) (Boulos and Terman, 1980; Mistlberger, 1994). An entrained oscillator model can account for FAA without recourse to classical or operant conditioning. Nonetheless, it is conceivable that associative learning processes are recruited when meals recur at predictable, 24h intervals. Circadian oscillators such as those in the dorsal striatum that are entrained by scheduled feeding could regulate FAA in part by emitting circadian time signals that become associated with mealtime and acquire incentive properties that evoke appetitive behavior. These signals could potentially also provide the basis for discrimination and anticipation of two or more unique daily

mealtimes, permitting time-place associations, a circadian function that does not require the SCN circadian clock (Mistlberger et al, 1996; Mulder et al, 2013). If circadian FAA does reflect classical conditioning based on associations between mealtimes and the phase of circadian FEOs, then the greatly diminished FAA in D1R KO mice could represent a deficit in incentive motivation, caused by a degraded circadian signal (failure of striatal FEOs to entrain to meals or couple to each other) and/or an impaired ability to associate circadian phase with food availability.

An alternative explanation for the decreased FAA observed in D1R KO mice could be that the D1R KO mice constitute a depressive phenotype and therefore show less activity directed at pleasurable stimuli. Depression is often accompanied with anhedonia and decreased caloric intake in human populations and animal models of depression, and this symptom is associated with DA transmission (Stein, 2008; Strekalova et al, 2004). Previous research has demonstrated decreased dopaminergic activity is associated with depression like symptoms in mice and rats and that pharmacological antagonism of the D1R receptor can reduce performance in a forced swim test and reverse the effects of drugs that improve performance on the same task (Zheng et al, 2013; Paolo et al, 2010; Yamada, Sugimoto & Yamada, 2004). The effect of D1R inhibition seems independent of the locomotor locomotor effects of these drugs as administration of D1R antagonist SCH 23390 reversed locomotor stimulation associated with two DA reuptake inhibitors but did not reverse increased performance on forced swim tests associated with these drugs (Vaugeois, Pouhé, Zuccaro & Costentin, 1996). Future studies could examine the effects of depression like symptoms on FAA by inducing depression-like symptomology via social defeat or repeated forced swim exposure on mice anticipating a daily meal.

4.4. Circadian properties of residual FAA in D1R KO mice

Although FAA was markedly diminished in D1R KO mice, all of the KO mice exhibited at least some FAA on some days. It is conceivable that residual FAA could be mediated by a compensatory non-circadian mechanism. By shifting mealtime, we were able to confirm that FAA in both KOs and control mice persisted for several days at the original mealtime, a property consistent with mediation by a circadian clock, and not

consistent with either a metabolic hourglass process or an interval timer reset by the daily meal. The bouts of persisting FAA in the KO mice, although weak, exhibited evidence of so-called transient cycles, interpreted as gradual shifting of circadian oscillators toward the new mealtime. The control mice, by contrast, showed a surprisingly robust and prolonged persistence of FAA at the prior mealtime for 7-8 days, concurrent with emergence of FAA at the new mealtime. We have observed similar cases of persisting FAA after meal shifts in rats (Smit et al 2014). This may constitute novel evidence in support of a hybrid FEO-classical conditioning model of FAA, whereby multiple phases of a FEO can be conditioned to mealtime, and this process is weakened in D1R KO mice. Whatever the specific mechanism, the results here indicate that FAA in D1R KO mice, although weak, retains circadian properties.

4.5. Interpretive Considerations

A plausible reading of FAA duration data allows for the interpretation that later onset of FAA does not represent impaired anticipation, but timing behavior that is more accurate. An animal that only became active immediately prior to mealtime would certainly be a more impressive example of biological timing than one that became active a few hours before mealtime. Animals that spend less time actively moving around and therefore less energy would have an advantage over animals that was active for hours before each meal opportunity. However, the functional purpose of FAA in a natural environment should be kept in mind. In the wild, animals are in constant competition for food sources, and these food sources may not appear with the same regularity as in laboratory experiments, so a clock that ensures they are out foraging *before* food becomes available increases the probability that they will be have access to that food and not arrive after it has been eaten. Of course, anticipation that lasts too long could also be symptomatic of an impaired timing system. A later average FAA onset may also result from animals that show anticipation only on some days and not others or animals that show variable onset with later average start times, either of which would indicate poorer timing.

4.6. Perspectives

Circadian food restriction schedules are imbedded in the experimental protocols of many studies of classical and operant conditioning. In such studies, food is provided once daily in a limited amount to increase the salience of food as a reward. It is a near certainty that circadian oscillators sensitive to meal timing will be entrained by these schedules. To the extent that food-entrainable circadian oscillators regulate the timing and intensity of reward driven behaviors, apparent deficits in such behaviors may be due to a defect in a food entrainment process. This circadian perspective may provide new insights into the functioning of dopaminergic circuits in normal and abnormal behavior (Webb et al, 2009a; Falcón & McClung, 2009)

A role for dopamine signaling in the expression of FAA may provide a unifying framework for interpreting alterations in FAA associated with other genetic manipulations. FAA has been reported to be attenuated by loss of ghrelin (LeSauter et al, 2009; Blum et al, 2009; Merkestein et al, 2012) and melanocortin 3 (Sutton et al, 2008) receptors, and to be enhanced by loss of leptin (Mistlberger & Marchant, 1999; Ribeiro et al, 2011; but see Gunapala et al 2010) and 5HT2c (Hsu et al., 2010c) receptors. These receptors are expressed in midbrain regions containing dopamine synthesizing neurons. Activity of midbrain dopamine neurons is enhanced by ghrelin (Abizaid et al, 2006) and reduced by leptin (Xu, 2014) and by serotonin acting at 5HT2c receptors (Di Giovanni et al, 2010). We propose that dopamine neurons and striatal D1R signaling may represent a final common pathway by which multiple metabolic and reward-related factors regulate the expression of anticipatory activity rhythms induced by daily schedules of restricted access to food or other rewards. The specific contributions made by D1 receptor expressing neurons in this process, and the mechanisms underlying the sex differences, remain to be fully specified.

4.7. Future Directions

The research presented here reveals several avenues for continued investigation into the role of dopaminergic signalling in regulating FAA. In the future, it will be useful to use more sophisticated gene ablation strategies that allow for site-specific and

temporally restricted deletion of D1R or other dopamine-related genes. Conditional gene knockout of D1R in the striatum and other regions will help clarify the role of signalling at these sites and has the benefit of reducing potential developmental confounds associated with global D1R KO. Further insight could be gained by placing the D1R gene under the control of tetracycline using TET-on/TET-off technology. This would allow for medium- to- long-term control over the production of D1R in individual animals and allow for within-subjects comparisons. Using this approach also enables the ability to change the animals' effective phenotype during continued entrainment to restricted feeding schedules, which may aid in distinguishing between effects associated with acquisition versus maintenance of FAA.

Temporal control over DA receptor production may also help clarify the role dopamine signalling plays in entraining behavior to feeding time. If the primary deficit contributing to low FAA is motivational in nature, reinstating production of D1R (or another DA receptor) after stable entrainment to a meal has been established should result in a rapid increase in premeal activity (this would be the case regardless of the degree to which FAA relies on associative learning or simple oscillator processes). This hypothesis follows from previous research that has shown intact learning in DA deficient mice after DA production is permitted even though no learning was evident during training when DA production was null (Palmiter, 2008). On the other hand, if D1R receptor activation is contributing to entrainment to feeding directly then releasing D1R production during FAA should result in gradual increase in FAA as D1R signalling is released as an available entrainment pathway. The specificity of TET-on/TET-off strategies can be enhanced further by placing the activation of the tetracycline-response elements under control of Cre recombinase to limit activation spatially as well as temporally.

While genetic techniques lend themselves well to examining organizational and medium- to long-term effects of putative components contributing to FAA, acute activation of receptors could more readily reveal potential entrainment pathways governing food anticipation. Previously reported anticipation to daily administration of a D1R agonist (Gallardo et al, 2014) is strong evidence that daily activation of D1 receptors contributes to FAA. More specific investigations of potential dopaminergic entrainment pathways could be accomplished by activation of specific neuronal

populations innervating the striatum using optogenetic or designer-receptor (DREADD) techniques. Of particular interest would be input from the substantia nigra as it is the source of a large portion of striatal input (Palmiter, 2008). More acute activation of neuronal populations also presents the opportunity to mimic patterns of activation analogous to multiple meals, anticipation to which remains poorly understood and required for a complete description of circadian food anticipation.

Isolating the contribution of specific brain structures or molecules to normal FAA timing has been difficult because the apparent distributed nature of the FEOs governing food anticipation (Mistlberger, 1994; 2011) make it possible that compensatory mechanisms mask the normal contributions of those areas or systems. A potential strategy to address this with regard to D1 activation in the striatum is to take advantage of FAA phenotypes associated with a compromised canonical clock gene loop. Animals with deletions of all redundant critical elements in either the positive (*Bmal1* -/-) or negative arm (*Per1/2/3* -/-) of the molecular clock show remarkably plastic limits to FAA entrainment allowing entrainment to schedules as short as 15 hours (Pendergast et al, 2012; Takasu et al, 2012). By introducing such deletions in a site-specific manner and then either pharmacologically, or through optogenetics or DREADD, activating those neurons on a cycle that should only produce entrainment in cells with the knockout, it should be possible to see the contribution of these areas to food anticipatory activity without compensatory systems being recruited.

The lack of persisting FAA in D1R KO mice at the old ZT6 mealtime following the shift to ZT12 feeding is interesting and presents possibilities for clarifying potential contributions of phase learning to anticipation of one or more mealtimes. It has been shown that behavioral flexibility is promoted by D2 activation, whereas D1 activation promotes rigid behavioral responding and habit formation (see Beeler et al, 2014 for review). Intact D2 signalling in D1R KO mice may permit adaptation to changes in meal parameters (including timing) which is evident in the canonical transients to a delayed mealtime seen in D1R KO mice. However, the lack of D1R signalling promoting habit formation prevents continued exploration of old mealtimes like that seen in wildtype mice. Interestingly, D1R KO mice showed increased FAA after another change in mealtime back to ZT6. Previously, we have also shown that D1R KO mice can transiently show robust FAA if they have been previously exposed to a palatable meal.

This change in another meal parameter (this time palatability or nutrient content) and subsequent increase in FAA may represent the same phenomenon. If activity at old mealtimes is mediated by dopaminergic modulation of incentive salience of a specific circadian phase, we may expect that D2R KO mice will show more residual FAA after changes in meal timing.

References

Abarca, C., Albrecht, U., & Spanagel, R. (2002). Cocaine sensitization and reward are under the influence of circadian genes and rhythm. *Proceedings of the National Academy of Sciences, 99*(13), 9026–9030.

Abizaid, A., Liu, Z. W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., … Horvath, T. L. (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *Journal of Clinical Investigation, 116*(12), 3229-3239.

Acosta-Galvan, G., Yi, C. X., Vliet, J. van der, Jhamandas, J. H., Panula, P., Angeles-Castellanos, M., … Buijs, R. M. (2011). Interaction between hypothalamic dorsomedial nucleus and the suprachiasmatic nucleus determines intensity of food anticipatory behavior. *Proceedings of the National Academy of Sciences, 108*(14), 5813–5818.

Angeles-Castellanos, M., Salgado-Delgado, R., Rodríguez, K., Buijs, R. M., & Escobar, C. (2008). Expectancy for food or expectancy for chocolate reveals timing systems for metabolism and reward. *Neuroscience*, *155*(1), 297–307.

Aschoff, J. (1986). Anticipation of a daily meal: A process of 'learning' due to entrainment. *Monitore Zoologico Italiano-Italian Journal of Zoology, 20*(2)*,* 195-219.

Aton, S. J., Colwell, C. S., Harmar, A. J., Waschek, J., & Herzog, E. D. (2005). Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. *Nature Neuroscience*, *8*(4), 476–483.

Bailey, M., & Silver, R. (2014). Sex differences in circadian timing systems: Implications for disease. *Frontiers in Neuroendocrinology*, *35*(1), 111–139.

Baltazar, R. M., Coolen, L. M., & Webb, I. C. (2013). Diurnal rhythms in neural activation in the mesolimbic reward system: critical role of the medial prefrontal cortex. *European Journal of Neuroscience*, *38*(2), 2319–2327.

Becker, J. B., & Hu, M. (2008). Sex differences in drug abuse. *Frontiers in Neuroendocrinology*, *29*(1), 36-47.

Beeler, J. A., Cools, R., Luciana, M., Ostlund, S. B., & Petzinger, G. (2014). A kinder, gentler dopamine… highlighting dopamine's role in behavioral flexibility. *Frontiers in neuroscience*, *8*.

Berridge, K. C. (2007). The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology*, *191*(3), 391–431.

Blum, I. D., Patterson, Z., Khazall, R., Lamont, E. W., Sleeman, M. W., Horvath, T. L., & Abizaid, A. (2009). Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice. *Neuroscience*, *164*(2), 351–359.

Blum, I. D., Zhu, L., Moquin, L., Kokoeva, M. V., Gratton, A., Giros, B., & Storch, K.-F. (2014). A highly-tunable dopaminergic oscillator generates ultradian rhythms of behavioral arousal. *eLife*, e05105.

Bobzean, S. A., DeNobrega, A. K., & Perrotti, L. I. (2014). Sex differences in the neurobiology of drug addiction. *Experimental Neurology*, *259*, 64-74.

Boulos, Z., & Terman, M. (1980). Food availability and daily biological rhythms. *Neuroscience and Biobehavioral Reviews, 4*(2), 119–131.

Caine, S. B., Thomsen, M., Gabriel, K. I., Berkowitz, J. S., Gold, L. H., Koob, G. F., … Xu, M. (2007). Lack of self-administration of cocaine in dopamine D1 receptor knock-out mice. *The Journal of Neuroscience*, *27*(48), 13140–13150.

Carroll, M. E., & Anker, J. J. (2010). Sex differences and ovarian hormones in animal models of drug dependence. *Hormones and Behavior*, *58*(1), 44-56.

Challet, E., & Mendoza, J. (2010). Metabolic and reward feeding synchronises the rhythmic brain. *Cell and Tissue Research*, *341*(1), 1–11.

Davidson, A. J. (2009). Lesion studies targeting food-anticipatory activity. *European Journal of Neuroscience*, *30*(9), 1658–1664.

Di Giovanni, G., Esposito, E., & Di Matteo, V. (2010). Role of serotonin in central dopamine dysfunction. *CNS Neuroscience & Therapeutics*, *16*(3), 179–194.

Drago, J., Gerfen, C. R., Lachowicz, J. E., Steiner, H., Hollon, T. R., Love, P. E., … Huang, S. P. (1994). Altered striatal function in a mutant mouse lacking D1A dopamine receptors. *Proceedings of the National Academy of Sciences, 91*(26), 12564–12568.

El-Ghundi, M., O'Dowd, B. F., Erclik, M., & George, S. R. (2003). Attenuation of sucrose reinforcement in dopamine D1 receptor deficient mice. *European Journal of Neuroscience, 17*(4), 851–862.

Escobar, C., Salgado, R., Rodriguez, K., Blancas Vázquez, A. S., Angeles-Castellanos, M., & Buijs, R. M. (2011). Scheduled meals and scheduled palatable snacks synchronize circadian rhythms: Consequences for ingestive behavior. *Physiology & Behavior, 104*(4), 555–561.

Falcón, E., & McClung, C. A. (2009). A role for the circadian genes in drug addiction. *Neuropharmacology*, *56, Supplement 1*, 91–96.

Fattore, L., Melis, M., Fadda, P., & Fratta, W. (2014). Sex differences in addictive disorders. *Frontiers in Neuroendocrinology, 35*(3), 272-284.

Ferris, M. J., España, R. A., Locke, J. L., Konstantopoulos, J. K., Rose, J. H., Chen, R., & Jones, S. R. (2014). Dopamine transporters govern diurnal variation in extracellular dopamine tone. *Proceedings of the National Academy of Sciences*, *111*(26), E2751–E2759.

Gallardo, C., Darvas, M., Oviatt, M., Chang, C., Michalik, M., Huddy, T. F., … Steele, A. (2014). Dopamine receptor 1 neurons in the dorsal striatum regulate food anticipatory circadian activity rhythms in mice. *eLife*, e03781.

Gunapala, K. M., Gallardo, C. M., Hsu, C. T., & Steele, A. D. (2011). Single gene deletions of orexin, leptin, neuropeptide Y, and ghrelin do not appreciably alter food anticipatory activity in mice. *PLoS ONE, 6*(3), e18377.

Honma, K., & Honma, S. (2009). The SCN-independent clocks, methamphetamine and food restriction. *European Journal of Neuroscience, 30*(9), 1707–1717.

Hood, S., Cassidy, P., Cossette, M. P., Weigl, Y., Verwey, M., Robinson, B., … Amir, S. (2010). Endogenous dopamine regulates the rhythm of expression of the clock protein PER2 in the rat dorsal striatum via daily activation of D2 dopamine receptors. *The Journal of Neuroscience*, *30*(42), 14046–14058.

Hsu, C. T., Dollár, P., Chang, D., & Steele, A. D. (2010). Daily timed sexual interaction induces moderate anticipatory activity in mice. *PLoS ONE, 5*(11), e15429.

Hsu, C. T., Patton, D. F., Mistlberger, R. E., & Steele, A. D. (2010). Palatable meal anticipation in mice. *PLoS ONE, 5*(9), e12903.

Hsu, J. L., Yu, L., Sullivan, E., Bowman, M., Mistlberger, R. E., & Tecott, L. H. (2010). Enhanced food anticipatory activity associated with enhanced activation of extrahypothalamic neural pathways in serotonin$_{2C}$ receptor null mutant mice. *PLoS ONE, 5*(7), e11802.

Hughes, A. T. L., Guilding, C., & Piggins, H. D. (2011). Neuropeptide signaling differentially affects phase maintenance and rhythm generation in SCN and extra-SCN circadian oscillators. *PLoS ONE, 6*(4), e18926.

Jansen, H. T., Sergeeva, A., Stark, G., & Sorg, B. A. (2012). Circadian discrimination of reward: evidence for simultaneous yet separable food- and drug-entrained rhythms in the rat. *Chronobiology International*, *29*(4), 454–468.

Kopp, C., Ressel, V., Wigger, E., & Tobler, I. (2006). Influence of estrus cycle and ageing on activity patterns in two inbred mouse strains. *Behavioural Brain Research, 167*(1), 165-174.

Krizo, J. A., & Mintz, E. M. (2015). Sex differences in behavioral circadian rhythms in laboratory rodents. *Frontiers in Endocrinology*, *5*, 234

Kuljis, D. A., Loh, D. H., Truong, D., Vosko, A. M., Ong, M. L., McClusky, R., … Colwell, C. S. (2013). Gonadal- and sex-chromosome-dependent sex differences in the circadian system. *Endocrinology*, *154*(4), 1501–1512.

Landry, G. J., Opiol, H., Marchant, E. G., Pavlovski, I., Mear, R. J., Hamson, D. K., & Mistlberger, R. E. (2012). Scheduled daily mating induces circadian anticipatory activity rhythms in the male rat. *PLoS ONE*, *7*(7), e40895.

LeSauter, J., Hoque, N., Weintraub, M., Pfaff, D. W., & Silver, R. (2009). Stomach ghrelin-secreting cells as food-entrainable circadian clocks. *Proceedings of the National Academy of Sciences, 106*(32), 13582–13587.

Liu, Y. Y., Liu, T. Y., Qu, W. M., Hong, Z. Y., Urade, Y., & Huang, Z. L. (2012). Dopamine is involved in food-anticipatory activity in mice*. Journal of Biological Rhythms, 27*(5), 398-409.

Mendoza, J., Angeles-Castellanos, M., & Escobar, C. (2005). Differential role of the accumbens shell and core subterritories in food-entrained rhythms of rats. *Behavioural Brain Research*, *158*(1), 133–142.

Merkestein, M., Brans, M. A. D., Luijendijk, M. C. M., de Jong, J. W., Egecioglu, E., Dickson, S. L., & Adan, R. A. H. (2012). Ghrelin mediates anticipation to a palatable meal in rats. *Obesity*, *20*(5), 963–971.

Mistlberger, R. E., & Marchant, E. G. (1999). Enhanced food anticipatory circadian rhythms in the genetically obese zucker rat. *Physiology & Behavior*, *66*(2), 329–335.

Mistlberger, R. E. (1994). Circadian food-anticipatory activity: Formal models and physiological mechanisms. *Neuroscience and Biobehavioral Reviews, 18*(2), 171–195.

Mistlberger, R. E. (2011). Neurobiology of food anticipatory circadian rhythms. *Physiology and Behavior, 104(4),* 535–545.

Mistlberger, R. E., & Rusak, B. (1987). Palatable daily meals entrain anticipatory activity rhythms in free-feeding rats: Dependence on meal size and nutrient content. *Physiology & Behavior*, *41*(3), 219–226.

Mistlberger, R. E., de Groot, M. H. M., Bossert, J. M., & Marchant, E. G. (1996). Discrimination of circadian phase in intact and suprachiasmatic nuclei-ablated rats. *Brain Research*, *739*(1), 12–18.

Mohawk, J. A., Pezuk, P., & Menaker, M. (2013). Methamphetamine and dopamine receptor D1 regulate entrainment of murine circadian oscillators. *PLoS ONE*, *8*(4), e62463.

Mulder, C. K., Gerkema, M. P., & Van Der Zee, E. A. (2013). Circadian clocks and memory: time-place learning. *Frontiers in Molecular Neuroscience*, *6*, 8.

Natsubori, A., Honma, K. I., & Honma, S. (2013). Differential responses of circadian Per2 expression rhythms in discrete brain areas to daily injection of methamphetamine and restricted feeding in rats. *European Journal of Neuroscience, 37*(2), 251-258.

Olsen, C. M., & Winder, D. G. (2009). Operant sensation seeking engages similar neural substrates to operant drug seeking in C57 mice. *Neuropsychopharmacology*, *34*(7), 1685–1694.

Palmiter, R. D. (2008). Dopamine signaling in the dorsal striatum is essential for motivated behaviors. *Annals of the New York Academy of Sciences*, *1129*(1), 35–46.

Paolo, S. D., Canu, S., Sardella, M., Spanu, C., Serra, G., & Franconi, F. (2010). Dopamine is involved in the antidepressant-like effect of allopregnanolone in the forced swimming test in female rats. *Behavioural Pharmacology*, *21*(1), 21-28.

Pendergast, J. S., Oda, G. A., Niswender, K. D., & Yamazaki, S. (2012). Period determination in the food-entrainable and methamphetamine-sensitive circadian oscillator(s). *Proceedings of the National Academy of Sciences*, *109*(35), 14218–14223.

Pittendrigh, C. S., & Daan, S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents - IV. Entrainment: Pacemaker as clock. *Journal of Comparative Physiology, 106*(3), 291–331.

Ribeiro, A. C., Ceccarini, G., Dupré, C., Friedman, J. M., Pfaff, D. W., & Mark, A. L. (2011). Contrasting effects of leptin on food anticipatory and total locomotor activity. *PLoS ONE*, *6*(8), e23364.

Shibata, S., Ono, M., Fukuhara, N., & Watanabe, S. (1995). Involvement of dopamine, N-methyl-D-aspartate and sigma receptor mechanisms in methamphetamine-induced anticipatory activity rhythm in rats. *Journal of Pharmacology and Experimental Therapeutics*, *274*(2), 688–694.

Smit, A. N., Michalik, M., Patton, D. F., Petersen, C. C., & Mistlberger, R. E. (2014). Meal shift experiments reveal unusual properties of circadian food anticipatory rhythms in rats and mice. *Society for Research on Biological Rhythms,* 14: 170.

Smit, A. N., Patton, D. F., Michalik, M., Opiol, H., & Mistlberger, R. E. (2013). Dopaminergic regulation of circadian food anticipatory activity rhythms in the rat. *PLoS ONE*, *8*(11), e82381.

Stein, D. J. (2008). Depression, anhedonia, and psychomotor symptoms: The role of dopaminergic neurocircuitry. *CNS Spectrums*, *13*(07), 561-565.

Stephan, F. K. (2002). The “other” circadian system: Food as a zeitgeber. *Journal of Biological Rhythms*, *17*(4), 284–292.

Strekalova, T., Spanagel, R., Bartsch, D., Henn, F. A., & Gass, P. (2004). Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. *Neuropsychopharmacology*, *29*(11), 2007-2017

Sutton, G. M., Perez-Tilve, D., Nogueiras, R., Fang, J., Kim, J. K., Cone, R. D., … Butler, A. A. (2008). The melanocortin-3 receptor is required for entrainment to meal intake. *The Journal of Neuroscience*, *28*(48), 12946–12955.

Szczypka, M. S., Rainey, M. A., Kim, D. S., Alaynick, W. A., Marck, B. T., Matsumoto, A. M., & Palmiter, R. D. (1999). Feeding behavior in dopamine-deficient mice. *Proceedings of the National Academy of Sciences*, *96*(21), 12138–12143.

Takasu, N. N., Kurosawa, G., Tokuda, I. T., Mochizuki, A., Todo, T., & Nakamura, W. (2012). Circadian Regulation of Food-Anticipatory Activity in Molecular Clock–Deficient Mice. *PLoS ONE*, *7*(11), e48892.

Ungerstedt, U. (1971). Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiologica Scandinavica. Supplementum*, *367*, 95–122.

Vaugeois, J. M., Pouhé, D., Zuccaro, F., & Costentin, J. (1996). Indirect dopamine agonists effects on despair test: dissociation from hyperactivity. *Pharmacology Biochemistry and Behavior*, *54*(1), 235-239.

Verwey, M., & Amir, S. (2009). Food-entrainable circadian oscillators in the brain. *European Journal of Neuroscience*, *30*(9), 1650–1657.

Vosko, A. M., Schroeder, A., Loh, D. H., & Colwell, C. S. (2007). Vasoactive intestinal peptide and the mammalian circadian system. *General and Comparative Endocrinology*, *152*(2–3), 165–175.

Wakamatsu, H., Yoshinobu, Y., Aida, R., Moriya, T., Akiyama, M., & Shibata, S. (2001). Restricted-feeding-induced anticipatory activity rhythm is associated with a phase-shift of the expression of mPer1 and mPer2 mRNA in the cerebral cortex and hippocampus but not in the suprachiasmatic nucleus of mice. *European Journal of Neuroscience*, *13*(6), 1190–1196.

Wall, V. Z., Parker, J. G., Fadok, J. P., Darvas, M., Zweifel, L., & Palmiter, R. D. (2011). A behavioral genetics approach to understanding D1 receptor involvement in phasic dopamine signaling. *Molecular and Cellular Neuroscience*, *46*(1), 21–31.

Webb, I. C., Antle, M. C., & Mistlberger, R. E. (2014). Regulation of circadian rhythms in mammals by behavioral arousal. *Behavioral Neuroscience, 128*, 304-325.

Webb, I. C., Baltazar, R. M., Lehman, M. N., and Coolen, L. M. (2009a). Bidirectional interactions between the circadian and reward systems: is restricted food access a unique zeitgeber. *European Journal of Neuroscience*, *30*(9), 1739–1748.

Webb, I. C., Baltazar,R. M., Wang, X., Pitchers, K. K., Coolen, L. C., and Lehman, M. N. (2009b). Diurnal variations in natural and drug reward, mesolimbic tyrosine hydroxylase, and clock gene expression in the male rat. *Journal of Biological Rhythms*, *24*(6), 465–476.

Wise, R. A. (2009). Roles for nigrostriatal - not just mesocorticolimbic - dopamine in reward and addiction. *Trends in Neurosciences*, *32*(10), 517–524.

Xu, L. (2014) Leptin action in the midbrain: From reward to stress. *Journal of Chemical Neuroanatomy*, *61*, 256-265.

Yamada, J., Sugimoto, Y., & Yamada, S. (2004). Involvement of dopamine receptors in the anti-immobility effects of dopamine re-uptake inhibitors in the forced swimming test. *European Journal of Pharmacology*, *504*(3), 207-211.

Zheng, M., Fan, Y., Shi, D., & Liu, C. (2013). Antidepressant-like effect of flavonoids extracted from Apocynum venetum leaves on brain monoamine levels and dopaminergic system. *Journal of Ethnopharmacology*, *147*(1), 108-113.

Zhou, Q. Y., Palmiter, R. D. (1995). Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. *Cell, 83,* 1197–1209.