Circadian Rhythms Of Clock Gene Expression In The Cerebellum of Serotonin-Deficient
Pet-1 Knockout Mice

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Abstract

Serotonin plays an important role in the central regulation of circadian clock function. Serotonin levels are generally higher in the brain during periods of high activity, and these periods are in turn heavily regulated by the circadian clock located in the suprachiasmatic nucleus. However, the role of serotonin as a regulator of circadian rhythms elsewhere in the brain has not been extensively examined. In this study, we examined circadian rhythms of clock gene expression in the cerebellum in mice lacking the *Pet-1* transcription factor, which results in a developed brain that is deficient in serotonin neurons. If serotonin helps to synchronize rhythms in brain regions other than the suprachiasmatic nucleus, we would expect to see differences in clock gene expression in these serotonin deficient mice. We found minor differences in the expression of *Per1* and *Per2* in the knockout mice as compared to wild type, but these differences were small and of questionable functional importance. We also measured the response of cerebellar clocks to injections of the serotonin agonist 8-OH-DPAT during the early part of the night. No effect on clock genes was observed, though the immediate-early gene *Fos* showed increased expression in wild type mice but not the knockouts. These results suggest that serotonin is not an important mediator of circadian rhythms in the cerebellum in a way that parallels its regulation of the circadian clock in the suprachiasmatic nucleus.

Keywords

circadian; serotonin; cerebellum; period; clock; Fos

1. Introduction

In mammals, the suprachiasmatic nucleus (SCN) within the ventral hypothalamus acts as an endogenous clock, generating circadian rhythms of physiology and behavior (Moore and Eichler, 1972; Stephan and Zucker, 1972). The neurotransmitter serotonin (5-HT) plays an important role in circadian rhythms, modulating photic input to the SCN as well as playing a role in non-photic shifts (Morin, 1999). Induced wheel-running increases 5-HT release in the SCN (Dudley et al., 1998) and causes phase advances along with associated downregulation of *Per1* and *Per2* expression (Maywood et al., 1999). ($\pm$)-8-hydroxy-2-(dipropylamino)tetralin hydrobromide (8-OH-DPAT), a 5-HT_{1A/7} receptor agonist, also causes phase advances during the subjective day (Cutrera et al., 1994; Cutrera et al., 1996; Edgar et al., 1993; Horikawa et al., 2000; Horikawa and Shibata, 2004; Mintz et al., 1997; Smith et al., 2008; Tominaga et al., 1992) that are associated with reductions in *Per1* and *Per2* expression (Horikawa et al., 2000). Taken together, these results suggest that *Per1* and *Per2* expression can be affected by 5-HT signals that reset the clock.

In addition to the SCN, circadian oscillations of clock genes are also present in other areas within the brain and in peripheral tissues within the body (Abe et al., 2002; Damiola et al., 2000; Lamia et al., 2008; Tosini and Menaker, 1996; Yamazaki et al., 2000; Zylka et al., 1998). Studies both *in vivo* (Zylka et al., 1998) and *in vitro* (Abe et al., 2002; Yamazaki et al., 2000) have shown that rhythms generated in extra-SCN brain areas and peripheral tissues lag in phase behind the SCN, suggesting that signals from the SCN synchronize extra-SCN oscillators by a mechanism that is mostly yet unknown (Abe et al., 2002; Damiola et al., 2000; Pando et al., 2002; Yamazaki et al.,

2000). One brain site that expresses functional circadian clocks is the cerebellum. *Per1*, *Per2*, *Clock*, and *Bmal1* are rhythmically expressed (Farnell et al., 2008; Rath et al., 2012) in the cerebellum, though one study did not find a rhythm in *Clock* (Shieh et al., 2005). Administration of tandospirone, a 5-HT_{1A} receptor agonist, reduces *Per1* expression in the cerebellum (Akiyama et al., 1999) suggesting that circadian clock function in the cerebellum is sensitive to serotonergic input. Serotonin concentrations in the cerebellum peak in the first half of the night in rats (Sanchez et al., 2008), consistent with the pattern seen in the SCN in hamsters (Dudley et al., 1998). The functional importance of circadian oscillations in the cerebellum is not known, however, there is some evidence that circadian function in the cerebellum contributes to the appearance of food-anticipatory activity during daily timed restricted feeding (Mendoza et al., 2010).

Pet-1 is an ETS domain transcription factor that is essential for the proper differentiation and development of serotonergic neurons. Mice lacking the Pet-1 gene show a significant disruption of the 5-HTergic system with a 70% loss of 5-HT cell bodies in adult animals (Hendricks et al., 2003). We have previously shown that serotonin-deficient Pet-1 knockout (KO) mice show a lengthening of period in constant darkness and redistribution of peak locomotor activity to the late portion of the night (Paulus and Mintz, 2012) suggesting that depletion of 5-HTergic input may lead to an endogenous clock with a longer free-running period. However, a separate study showed a similar redistribution of activity but a shortening of free-running period (Ciarleglio et al., 2014). Pet-1 KO mice also show alterations in response to photic stimuli, showing a smaller amplitude phase response curve to light that causes smaller phase delays (Paulus and Mintz, 2013). Since little is known about the signals that

drive rhythms in extra-SCN neural clocks, *Pet-1* mice provide a convenient model to investigate the potential role of 5-HT in synchronizing these rhythms. The cerebellum receives significant serotonergic input from the dorsal and median raphe (Hornung, 2003; Jacobs and Azmitia, 1992), structures with a significant influence on the central circadian clock in the SCN. Such inputs would provide an opportunity for information about arousal state to be communicated to cerebellar clocks. Therefore, we hypothesized that daily rhythms in serotonin release contribute to circadian rhythms of gene expression in the cerebellum. In this study we examine the temporal pattern of clock gene expression within the cerebellum, as well as the acute response of clock genes in the cerebellum to injections of the serotonin agonist 8-OH-DPAT.

2. Results

2.1 Circadian Gene Expression in the Cerebellum

Expression of the clock genes *Per1*, *Per2*, *Clock*, *Bmal1*, *Npas2*, and the immediate-early gene *Fos* across the 24-hour cycle was examined in the cerebellum of WT and *Pet-1* KO animals. A summary of statistical findings is shown in Table 1, with the expression profile for each gene shown in Figure 1. Cerebellar *Per1* expression showed rhythmic expression in both genotypes with peak expression occurring at ZT 16 in both WT and KO animals. An additional peak is seen at ZT 8 in WT but not in KO. There were also significant effects of genotype and an interaction indicating that the difference between genotypes at ZT 8 was statistically significant. *Per2* expression also showed a rhythm in both genotypes with peak expression occurring at ZT 16. There was a significant effect of genotype but no interaction indicating that there is significantly reduced *Per2* expression overall in KO animals compared to WT, but that this reduction

is not specific to particular times of day. *Clock*, *Npas2*, and *Bmal1* expression also showed rhythmic expression in the cerebellum of both genotypes. *Clock* and *Npas2* levels were highest during the day while *Bmal1* levels were highest during the late night. *Fos*, however, is not rhythmically expressed in the cerebellum in either genotype. There were no significant effects of genotype and no interactions for *Clock*, *Npas2*, *Bmal1*, and *Fos* indicating that expression of these genes within the cerebellum is unaffected by deletion of the *Pet-1* gene. Cerebellar expression of serotonin transporter (*Sert*) was also assayed. Expression levels were very low but there appeared to be no rhythm of *Sert* expression and no differences between genotypes, however, due to the low expression levels these data are not definitive (data not shown).

2.2 Effects of 8-OH-DPAT on Cerebellar Clock Genes

We next measured selected gene expression responses in the cerebellum to injection of 8-OH-DPAT (Figure 2). For *Per1*, there was no significant effect of 8-OH-DPAT administration ($F_{(1,19)} = 3.67$, $p = 0.07$) or genotype ($F_{(1,19)} = 1.54$, $p = 0.23$) and no interaction ($F_{(1,19)} = 0.11$, $p = 0.75$). Similar to *Per1*, *Per2* expression revealed no significant effect of 8-OH-DPAT administration ($F_{(1,19)} = 0.09$, $p = 0.76$) or genotype ($F_{(1,19)} = 0.02$, $p = 0.89$) and no interaction ($F_{(1,19)} = 0.11$, $p = 0.74$). *Fos* expression showed no effect of treatment ($F_{(1,19)} = 1.91$, $p = 0.18$) and no effect of genotype ($F_{(1,19)} = 4.21$, $p = 0.054$) but a significant interaction ($F_{(1,19)} = 5.73$, $p = 0.027$) with 8-OH-DPAT administration causing increased *Fos* expression in WT animals ($197\% \pm 32\%$ of WT saline mean) but not in *Pet-1* KO animals ($83\% \pm 16\%$ of WT saline mean). Finally, we analyzed the expression of the 5-HT₇ receptor (*Ht7r*). There was no significant effect of

8-OH-DPAT administration ($F_{(1,19)} = 0.81$, $p = 0.38$) or genotype ($F_{(1,19)} = 0.31$, $p = 0.58$) and no interaction ($F_{(1,19)} = 0.06$, $p = 0.81$) in the cerebellum.

3. Discussion

The SCN is believed to coordinate rhythms of physiology and behavior (Ralph et al., 1990; Rusak and Zucker, 1979) and has been shown to have the ability to generate these rhythms in the absence of neural connectivity (Silver et al., 1996). Extra-SCN oscillators show rhythmic expression of clock genes (Abe et al., 2002; Damiola et al., 2000; Farnell et al., 2008; Lamia et al., 2008; Stokkan et al., 2001; Yamazaki et al., 2000; Zylka et al., 1998) which lag in phase behind the SCN and eventually dampen without SCN input (Abe et al., 2002; Damiola et al., 2000; Yamazaki et al., 2000) suggesting the SCN entrains rhythms in extra-SCN clocks via an unknown signal or signals. For extra-SCN clocks located in the brain, rhythms may be synchronized via direct neural input from the SCN, or by inputs from other brain regions that are secondary to behavioral rhythms driven by the SCN. For example, the SCN may drive rhythms of sleep and wakefulness, which in turn would drive serotonergic input to many brain regions. *Pet-1* KO mice, therefore, provided a convenient model to examine 5-HT as a synchronizing factor in the cerebellum. Our results suggest that the clocks in the cerebellum are relatively insensitive to a loss of serotonergic input, but reveal new data on clock gene rhythms in the cerebellum.

Our experiment examining the temporal pattern of clock gene expression shows the expression of core clock genes within the cerebellum and provides support for the notion of the cerebellum as an extra-SCN oscillator. Our data confirms numerous studies showing that *Per1* and *Per2* are widely expressed outside the SCN including the

cerebellum (Abe et al., 2002; Shieh, 2003; Sun et al., 1997). Additionally, *Clock* is also expressed widely throughout the brain (King et al., 1997) and within the cerebellum (Shieh, 2003), and *Bmal1* expression has also been shown in the cerebellum (Farnell et al., 2008).

We show that *Per1*, *Per2*, *Clock*, *Npas2*, and *Bmal1* all display rhythmic patterns of expression within the cerebellum of both WT and Pet-1 KO animals. Despite the fact that rhythmic expression of the immediate early gene, *Fos*, has previously been reported within the cerebellum (OHara et al., 1997), our experiments did not find *Fos* to be rhythmically expressed. However, the cerebellum is a heterogeneous structure, and our methodology produces an average of expression across the entire structure. If *Fos* is expressed rhythmically in some regions, it might not be detectable because such rhythmic expression is being averaged together with regions that lack rhythmic expression. Rhythmic expression of cerebellar *Per1* and *Per2* has been shown previously (Farnell et al., 2008; Sun et al., 1997). Our studies show peak levels of cerebellar *Per1* and *Per2* expression occurring at ZT 16 and lower levels of expression throughout the day in both genotypes. This pattern of expression is similar to those seen in other studies with the exception that previous reports show peak expression of *Per* genes around ZT 12 (Farnell et al., 2008; Sun et al., 1997). A separate study that examined the temporal pattern of clock gene expression in liver and skeletal muscle, sites of peripheral oscillators, found peak *Per1* and *Per2* expression at ZT 15, which is more similar to our results (Zylka et al., 1998). Although the cerebellum was not examined in Zylka et al. (1998), peak expression of *Per1* and *Per2* in cerebellum and liver has been shown to occur at the same time within the day (Farnell et al., 2008).

Therefore, the discrepancy between our results and those of Sun et al. (1997) and Farnell et al. (2008) could be due to the differences in time points examined. Both previously published reports examined clock gene expression using samples collected at 6 hour intervals throughout the day while our samples were collected at 4 hour intervals. Since ZT 16 was not a time point examined in previous studies, it is possible that the time of maximal expression of *Per* genes was missed in previous studies.

Our experiments show rhythmic expression of *Bmal1* within the cerebellum of both genotypes, with peak expression occurring around ZT 20 in WT animals. *Bmal1* has also previously been shown to be rhythmically expressed in the cerebellum with peak levels occurring at ZT 18 (Farnell et al., 2008). Again, the discrepancy between times of peak expression in the cerebellum between our experiments and those previously published could be due to differences in times of sample collection as ZT 20 was not a time point examined in the Farnell et al. (2008) study. Separate studies have shown *Bmal1* expression peaks around ZT 20 - 22 in the liver (Lamia et al., 2008; McDearmon et al., 2006). Additionally, unlike the phase lag seen in expression of *Per* genes between the SCN and extra-SCN oscillators (Abe et al., 2002; Damiola et al., 2000; Yamazaki et al., 2000), peak expression of *Bmal1* occurs at approximately the same time in SCN as well as extra-SCN clocks (Farnell et al., 2008) and peak *Bmal1* expression within the SCN has been found anywhere between ZT 15 and ZT 22 (Shearman et al., 2000).

Our study found rhythmic cerebellar expression of *Clock* in both WT and KO animals. *Clock* has been shown to be constitutively expressed within the SCN (Shearman et al., 1999; Shearman et al., 2000). Several other studies have reported

rhythmic expression of *Clock* in a variety of peripheral tissues suggesting that unlike in the SCN, *Clock* expression may be rhythmic in extra-SCN oscillators (Froy et al., 2009; Liu et al., 2007; Yamamoto et al., 2004).

Npas2 is similar to *Clock* in amino acid sequence (King et al., 1997) and the protein product, NPAS2, forms heterodimers with BMAL1 (Reick et al., 2001) and functionally interacts with BMAL1 in the SCN to regulate circadian rhythmicity in the absence of CLOCK (DeBruyne et al., 2007). We found that *Npas2* was rhythmically expressed in the cerebellum of both genotypes with peak expression occurring at ZT 4. *Npas2* is rhythmically expressed in numerous peripheral tissues including the heart, lungs, liver, and kidneys with peak expression occurring early in the circadian day, from CT 0 to CT 5 (McNamara et al., 2001; Yamamoto et al., 2004). To our knowledge, this is the first study to examine the temporal pattern of *Npas2* expression in the cerebellum.

Our results show rhythmic expression of *Per1*, *Per2*, *Clock*, *Npas2*, and *Bmal1* in both WT and Pet-1 KO animals. These results suggest that 5-HT does not function as a synchronizing signal for clock gene expression within the cerebellum. However, *Per1* expression was significantly attenuated in the Pet-1 KO animals at ZT 8 as compared to WT. We are unsure of an explanation for this since no other studies examining *Per1* expression in the cerebellum or peripheral tissues have reported similar findings. In addition, cerebellar *Per2* expression, although rhythmic, is significantly attenuated in Pet-1 KO animals. This suggests that 5-HT may play a role in modulation of the level of *Per2* gene expression, however, the differences in expression of *Per2*, though statistically significant, was quite small and it is unclear whether such differences would have any biological relevance. We also note the appearance of a bimodal peak in WT

expression of *Per1* at ZT 8 and ZT 16. This might be a spurious finding, however, the difference between genotypes at ZT 8 is reflected by a similar (but not statistically significant) difference between genotypes in *Per2* expression. Nevertheless, ZT 8 represents a time of day when 5-HT release would normally be low due to inactivity of the animal, so it is difficult to ascribe a functional purpose for the observed pattern of expression.

In examining the effect of systemic 8-OH-DPAT injections at ZT 16, we found no differences between genotypes in expression of *Ht7r*, *Per1*, or *Per2*. However, *Fos* expression was significantly up regulated in WT animals two hours after 8-OH-DPAT administration when compared to KO animals. The consensus in the literature is that 5-HT_{1A} receptors are not present within the cerebellum (Chalmers and Watson, 1991; Kia et al., 1996; Laporte et al., 1994; Pompeiano et al., 1992). 5-HT₇ receptor expression, however, has been reported in the cerebellum (Geurts et al., 2002; Ruat et al., 1993). Our experiments confirm the presence of *Ht7r* expression within the cerebellum of both genotypes and provide evidence for a site of action for 8-OH-DPAT. However, we found no effect of 8-OH-DPAT administration on *Per1* and *Per2* expression in either genotype. We chose the ZT 16 time point because it is the time of day when serotonin release in the cerebellum would normally be high in WT but significantly attenuated in KO mice. Our results cannot distinguish between a cerebellar clock that is insensitive to 8-OH-DPAT vs. one that is insensitive at the particular time point we chose.

However, we can establish that the 8-OH-DPAT did have physiological effects on the cerebellum. *Fos* expression was significantly elevated in the cerebellum of WT but not KO animals two hours following a systemic injection of 8-OH-DPAT. Our results are

similar to previously published results showing increased *Fos* mRNA in the cerebellum at 30 and 90 minutes following 8-OH-DPAT administration (Tilakaratne and Friedman, 1996). The lack of *Fos* induction in response to 8-OH-DPAT in KO animals may suggest a reduced capacity of 5-HT receptors within the cerebellum to be activated by 5-HTergic compounds. However, induction of *Fos* mRNA peaks within 30 – 45 minutes and rapidly declines, therefore, our study did not examine *Fos* expression at its peak time of induction. It would be of interest to investigate differences in *Fos* induction in response to 8-OH-DPAT along a shorter time course.

Our data suggests that developmental dysfunction of the 5-HT system does not have a large influence on rhythmic expression of clock genes in the cerebellum, indicating that 5-HT is not likely to be an important synchronizing factor for cerebellar clocks. It is possible that a greater change in clock function would be seen in a model with a total loss of serotonin neurons. However, small changes in *Per1* expression and attenuated *Per2* expression were seen in KO animals indicating that 5-HT contributes somewhat to clock gene expression. No effect of 5-HT agonists was seen on core clock genes but there were significant reductions in *Fos* induction in KO animals, indicating that the developmental disruption of the serotonergic system may lead to reductions in the ability of 5-HT receptors to be activated by serotonergic agonists. Since we have already shown lengthening of free-running circadian period and shifts in timing of activity within the 24 hour cycle in *Pet-1* KOs (Paulus and Mintz, 2012) as well as a reduced amplitude of the phase response curve to light leading to smaller phase delays (Paulus and Mintz, 2013), these data indicate that although serotonin plays an important

role in regulating circadian rhythms in the SCN, it is not likely that it plays a similar role in circadian rhythms in the cerebellum.

4. Experimental Procedure

4.1 Animals

Pet-1 KO mice on a mixed 129Sv and C57BL/6 background were obtained from Dr. Evan Deneris (Case Western Reserve University, Cleveland, OH) (Hendricks et al., 1999). Pet-1 heterozygote breeding pairs were used to produce all experimental animals. Experiments were conducted by matching siblings of different genotypes as much as possible to avoid bias from the mixed genetic background. At the time of weaning animals were ear-tagged and a tail-snip was collected for DNA extraction and genotyping. Experimental animals were entrained to a 12:12 light-dark (LD) cycle prior to the start of the experiment, which occurred when the animals were aged 9-12 weeks. Food and water were available *ad libitum* and all experimental procedures were approved by the Kent State University Institutional Animal Care and Use Committee.

4.2 Genotyping

Tail snips from all animals were placed into an extraction buffer containing 20mM Tris pH 8.0, 1 mM EDTA, 400mM NaCl, and 0.5% SDS with 20mg/mL Proteinase K added in order to digest the snip. Samples were maintained at 55°C with slow rotation overnight. Supernatant from each sample was spun down in order to pellet DNA, rinsed with ethanol and resuspended in TE buffer. DNA of interest was amplified using PCR with primers from IDT (3' PET: 5' GCC TGA TGT TCA AGG AAG ACC TCG G 3' 5'

PET: 3' CGC ACT TGG GGG GTC ATT ATC AC 3' 5' LOX: 5' CGG TGG ATG TGG AAT GTG TGC G 3') and thermocycler conditions beginning with a 3 minute hold at 94°C followed by 42 cycles of 94°C for 50 seconds, 62°C for 30 seconds and 72°C for 40 seconds. PCR product was analyzed using gel electrophoresis. Wild-type (WT) animals were identified by DNA fragments of 209 base pairs while Pet-1 knockout (KO) animals were defined by DNA fragments of 361 base pairs.

4.3 Gene expression time course experiment

Adult male WT (n = 36) and KO (n = 30) were housed on a 12:12 light-dark (LD) schedule for at least two weeks prior to collection of samples. Animals were sacrificed by cervical dislocation at four hour intervals throughout the 24 hour day (ZT 0, ZT 4, ZT 8, ZT 12, ZT 16, and ZT 20). The experiment was conducted in a LD cycle, rather than in constant conditions, because the goal of the experiment was to measure the impact of serotonin on rhythms in the cerebellum under entrained conditions, not to evaluate changes in phase that might result in constant conditions. Brains were rapidly extracted, quickly frozen in isopentane that had been cooled on dry ice and stored at -80°C until ready for processing.

4.4 8-OH-DPAT gene expression experiment

Adult male WT (n = 12) and Pet-1 KO (n = 11) animals were housed on a 12:12 LD schedule for at least two weeks prior to the start of the experiment. At ZT 16, animals received either an injection of saline (n = 11, 6 WT and 5 KO) or 8-OH-DPAT (5 mg/kg) (n = 12, 6 WT and 6 KO) intraperitoneally (i.p.). Two hours after drug or vehicle

administration, animals were sacrificed by cervical dislocation, brains were rapidly extracted, frozen in isopentane that had been cooled on dry ice, and stored at -80°C until ready for processing.

4.5 Sample processing and analysis

Brains were kept in a cooled chamber and the right half of the cerebellum was carefully dissected from the brain. Cerebellar tissue was homogenized using sonication and RNA was purified using a Qiagen RNeasy kit with an on-column DNase treatment (Qiagen) following the manufacturer's protocol. RNA quality and quantity from each sample was measured using a spectrophotometer and 1 µg of total purified RNA was reverse transcribed to make cDNA using an Applied Biosystems TaqMan Reverse Transcription Reagents kit following the standard protocol and using random hexamers. qRT-PCR was performed on an Applied Biosystems Prism 7000 sequence detection system using TaqMan Universal Master Mix and Taqman primers (using the standard protocol) for *Period* genes (*Per1* and *Per2*), *Clock*, *Bmal1*, *Npas2*, *Ht7r*, and the immediate early gene *Fos*, using *gapdh* as a control gene. All samples were assayed in triplicate with each triplicate containing 20 ng of cDNA. Analysis of relative gene expression was performed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). For ease of comparison, data was normalized by reporting levels of gene expression as a percentage of the WT mean value (experiment 1) or the WT saline mean (experiment 2) for each gene. Statistical analysis was performed using a two-way ANOVA with posthoc comparisons performed using a Tukey-Kramer test.

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Table 1.

Gene	Effect of Genotype	Effect of Time	Interaction
<i>Per1</i>	$F_{(1,53)} = 7.16$ p = 0.0099*	$F_{(5,53)} = 41.65$ p < 0.0001*	$F_{(5,53)} = 5.55$ p = 0.0004*
<i>Per2</i>	$F_{(1,53)} = 5.36$ p = 0.025*	$F_{(5,53)} = 17.86$ p < 0.0001*	$F_{(5,53)} = 0.74$ p = 0.60
<i>Clock</i>	$F_{(1,53)} = 0.87$ p = 0.36	$F_{(5,53)} = 2.66$ p = 0.032*	$F_{(5,53)} = 0.36$ p = 0.87
<i>Npas2</i>	$F_{(1,53)} = 0.06$ p = 0.81	$F_{(5,53)} = 12.36$ p < 0.0001*	$F_{(5,53)} = 0.80$ p = 0.56
<i>Bmal1</i>	$F_{(1,53)} = 0.04$ p = 0.84	$F_{(5,53)} = 2.72$ p = 0.029*	$F_{(5,53)} = 1.05$ p = 0.40
<i>Fos</i>	$F_{(1,53)} = 1.23$ p = 0.27	$F_{(5,53)} = 1.09$ p = 0.38	$F_{(5,53)} = 1.42$ p = 0.23

Table 1: Table summarizing the results of *Per1*, *Per2*, *Clock*, *Npas2*, *Bmal1*, and *Fos* expression in the cerebellum of wild type and Pet-1 knockout animals at 4 hour intervals throughout the 24-hour day. Statistically significant p-values are in bold text and denoted by asterisks.

Figure Legends

Figure 1. Gene expression over the 24-hour day in the cerebellum of Pet-1 knockout mice (circles) and wild type mice (squares). The x-axis shows zeitgeber time (lights off at ZT12 and lights on at ZT 0). The y-axis shows expression relative to the wild type 24-hour mean value. *Significantly different from WT, $p < 0.05$.

Figure 2. Relative gene expression for wild type (solid) and Pet-1 KO mice (hatched) after injection of 8-OH-DPAT or saline. *Significantly different from corresponding saline group, $p < 0.05$.

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Figures

Figure 1

Figure 2