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Behavioral and hormonal regulation of expression of the clock protein, PER2, in the central extended amygdala

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Abstract

PER2, a key molecular component of the mammalian circadian clock, is expressed rhythmically in many brain areas and peripheral tissues in mammals. Here we review findings from our work on the nature and regulation of rhythms of expression of PER2 in two anatomically and neurochemically defined subregions of the central extended amygdala, the oval nucleus of the bed nucleus of the stria terminalis (BNSTov) and the central nucleus of the amygdala (CEA). Daily rhythms in the expression of PER2 in these regions are coupled to those of the master circadian pacemaker, the suprachiasmatic nucleus (SCN) but, importantly, they are sensitive to homeostatic perturbations and to hormonal states that directly influence motivated behavior.

Key Words: Period2, oval nucleus of the stria terminalis, central nucleus of the amygdala, suprachiasmatic nucleus, glucocorticoid, gonadal hormones, restricted feeding, dopamine, corticotropin-releasing hormone.

Contents

1. Introduction

Circadian rhythms in mammals are known to be modulated by motivational and emotional state. However, the interface between motivational and emotional state and circadian rhythms is not well understood. In earlier studies from our laboratory, we showed that photic induction of the cellular activity marker, Fos, in the suprachiasmatic nucleus (SCN, the master circadian clock) and light-induced phase shifts in free-running activity rhythms are attenuated in rats exposed to light in a context that induces conditioned fear (Amir and Stewart, 1998). Conditioned fear also attenuates light-induced suppression of melatonin release (Funk and Amir, 1999), supporting the view that limbic forebrain mechanisms involved in emotional regulation can influence mechanisms that mediate the transmission of light to the SCN.

In more recent work we have found that many limbic forebrain nuclei implicated in the regulation of motivational and emotional state exhibit daily rhythms in expression of the circadian clock protein, Period2 (PER2) (Amir et al., 2004; Lamont et al., 2005). This finding, together with evidence that the rhythms in PER2 expression in two regions, the oval nucleus of bed nucleus of the stria terminalis (BNSTov) and central nucleus of the amygdala (CEA) are regulated by corticosterone, led us to propose that motivational events modulate circadian rhythms, not only indirectly via light input mechanisms upstream from the SCN, but also by directly modulating tissue specific circadian mechanisms located downstream from the SCN. In this paper we describe the nature of PER2 rhythms in the BNSTov and CEA and findings concerning the neural, hormonal,

environmental and behavioral mechanisms that regulate and modulate these rhythms.

2. Clock genes and their rhythmic expression

In mammals a light-entrainable master clock located in the SCN regulates circadian rhythms in behavior and physiology by synchronizing networks of subordinate circadian oscillators throughout the brain and periphery. These subordinate oscillators are presumed to control, in a tissue-specific manner, the daily fluctuations in cellular and metabolic activity and their functional output (Green et al., 2008; Hastings et al., 2003; Schibler and Sassone-Corsi, 2002). At the cellular level, circadian rhythmicity is driven by interlocked negative and positive transcription and translation/post-translation feedback loops that involve the products of several clock genes, most of which are rhythmically expressed. These include two genes encoding helix-loop-helix PAS transcription factors (*Clock* and *Bmal1*), three Period genes (*Per1, Per2 and Per3*), two Cryptochrome genes (*Cry1* and *Cry2*), two orphan nuclear receptor genes (*Rev-erb, Rora*), and a gene encoding casein kinase (*Tau*) (Dardente and Cermakian, 2007; Reppert and Weaver, 2001). Circadian rhythms in expression of clock genes and proteins occur in the SCN as well as in many brain regions outside the SCN and in most peripheral tissues (Abe et al., 2002; Balsalobre, 2002; Bittman et al., 2003; Portman, 2001; Sakamoto et al., 1998; Shieh, 2003; Yamamoto et al., 2001; Zvonic et al., 2006). In the SCN these rhythms are intrinsically synchronous and self-sustaining, whereas in most other tissues they dampen in

the absence of synchronizing input (Abe et al., 2002; Balsalobre, 2002; Sakamoto et al., 1998). The primary input for synchronization of subordinate oscillators comes from the SCN. Both neural projections and diffusible peptides from the SCN, as well as neural (such as the sympathetic nervous systems), endocrine (such as corticosterone, melatonin, epinephrine) and behavioral (such as locomotor activity, feeding) processes under SCN control have all been proposed as possible mediators (Cheng et al., 2002; Hastings and Maywood, 2000; Holzberg and Albrecht, 2003; Kalsbeek and Buijs, 2002; Mrosovsky, 1996; Oishi et al., 1998; Okamura, 2003; von Gall, 2003). Of particular importance for this review, however, are recent findings demonstrating that both the levels and patterns of expression of clock genes in brain and periphery can be modulated directly, downstream from the SCN. Thus, stress and drugs of abuse (Ammon et al., 2003; Iijima et al., 2002; Takahashi et al., 2001; Yuferov et al., 2003), scheduled restricted feeding (Damiola et al., 2000; Hara et al., 2001; Stokkan et al., 2001; Verwey et al., 2007; Waddington Lamont et al., 2007; Wakamatsu et al., 2001), exercise (Zambon et al., 2003), and periodic absence of nursing mothers (Ohta et al., 2003) have all been shown to either induce or shift the phase of clock gene expression in a number of brain structures and peripheral tissues in rodents.

3. PER2 rhythms in the central extended amygdala

In our recent studies of the expression of clock genes in rat brain, we found daily rhythms in the expression of the quintessential circadian clock protein,

PER2 (Bae et al., 2001; Zheng et al., 1999), in two regions of the extended amygdala known to participate in the regulation of motivational and emotional state, the oval nucleus of the bed nucleus of the stria terminalis (BNSTov), and the central nucleus of the amygdala (CEA) (Amir et al., 2004; Lamont et al., 2005). Significantly, we found that the expression of PER2 in these regions is maximal around the time of transition from day to night, and is uniquely in phase with the PER2 rhythm of the SCN (see Fig. 1). In other brain regions, such as the basolateral amygdala, dorsal striatum, and hippocampus, the rhythms of PER2 expression are typically opposite in phase with that in the SCN, peaking during the transition from night to day (Amir et al., 2006; Amir et al., 2004; Lamont et al., 2005). Furthermore, we found that bilateral SCN lesions, or prolonged housing in constant light (LL), which eliminate PER2 rhythms in the SCN and disrupt circadian behavioral rhythms, abolish the rhythm of PER2 in the BNSTov and CEA (Amir et al., 2004; Lamont et al., 2005), confirming the subordinate nature of these rhythms. Finally, in another experiment in this series, we found that unilateral SCN lesions, which do not affect circadian behavioral rhythms, blunt the rhythm of PER2 in BNSTov ipsilateral, but not contralateral to the lesioned side, emphasizing the importance of neural connections between the SCN and PER2 oscillations in these limbic forebrain areas (Amir et al., 2004).

4. BNSTov and CEA PER2 rhythms are uniquely sensitive to circulating hormones

4.1 Glucocorticoids

Basal rhythmic secretion of glucocorticoids from the adrenals is under the control of the SCN (Szafarczyk et al., 1983). In turn, there is evidence that glucocorticoids (GC) induce clock gene expression in peripheral tissues and in cultured cells (Balsalobre et al., 2000a; Balsalobre et al., 2000b). The BNSTov and CEA are rich in both types of glucocorticoid receptors (MR and GR) (Honkaniemi et al., 1992; Lechner and Valentino, 1999; Roozendaal et al., 2001) and glucocorticoids have been shown to modulate the expression of various neuropeptides and neuropeptide receptors and to affect other cellular parameters within these regions (Makino et al., 1994; Makino et al., 1995; Pompei et al., 1995; Sanchez et al., 1995; Schulkin et al., 1998; Stamp and Herbert, 2001; Watts and Sanchez-Watts, 1995).

Based on these observations, we carried a series of experiments to examine the role of glucocorticoids in the regulation of PER2 in the BNSTov and CEA. We first found that adrenalectomy blunts the rhythmic expression of PER2 in the BNSTov and CEA without affecting PER2 rhythmicity in the SCN. Adrenalectomy had no effect on PER2 rhythms in other limbic forebrain regions such as the basolateral amygdala and hippocampus, indicating that glucocorticoids play a selective role in the regulation of PER2 expression in the BNSTov and CEA (Amir et al., 2004; Lamont et al., 2005). In a second study we sought to determine the nature of the interaction between glucocorticoids and PER2 rhythms, asking whether it was the mere presence or the daily circadian rhythm of circulating glucocorticoids that was critical. We found that in the

absence of the adrenals, corticosterone replacement via the drinking water, which restores daily fluctuations in corticosterone levels, restores the rhythm of PER2 in the BNSTov and CEA, whereas corticosterone replacement via subcutaneous constant-release pellets has no effect (Segall et al., 2006b). Finally, we found that in a conditional mutant mouse devoid of glucocorticoid receptors in the brain (Tronche et al., 1999), PER2 rhythms in the BNSTov and CEA are absent (Segall et al., 2006a). These data demonstrate the importance of circadian glucocorticoid signaling in PER2 rhythms in the BNSTov and CEA and are consistent with the idea that the effect of circulating corticosterone on PER2 rhythms in these regions is mediated by central glucocorticoid receptors.

4.2 Thyroid hormones

The finding that the rhythms of PER2 in the BNSTov and CEA, and not those in other limbic forebrain regions, are sensitive to circulating adrenal hormones led us to ask whether these particular limbic regions might be uniquely sensitive to other types circulating hormones. One class of hormones previously implicated in the regulation of behavioral and physiological circadian rhythms are the thyroid hormones. For example, it has been shown that surgical removal of the thyroid and parathyroid glands or chemical induction of hypothyroidism blunt the daily fluctuations in circulating corticosterone and prolactin levels and alter circadian locomotor activity rhythms (Beasley and Nelson, 1982; McEachron et al., 1993; Murakami et al., 1984).

In our experiments, surgical removal of the thyroid and parathyroid glands disrupted PER2 rhythms in the BNSTov and CEA, again without having effects in other limbic regions (Amir and Robinson, 2006). When considering the mechanisms whereby hormones such as thyroxine (T4) and triiodothyronine (T3) might affect PER2 expression, we speculated that they might act on the transcription of the Per2 gene, indirectly, by modulating the transcriptional activity of REV-ERBalpha and RORa clock components which have been shown to be sensitive to thyroid hormones. Indeed, both have been implicated in the transcriptional regulation of BMAL1, an essential and direct positive regulator of Per2 transcription in mammalian cells (Preitner et al., 2002; Sato et al., 2004). However, because this mechanism is likely to affect clock gene expression in all tissues throughout body, it would not appear able to account for the selective effect of thyroidectomy on PER2 expression in the BNSTov and CEA. Other more likely possibilities would be via their effects on the daily rhythm of plasma corticosterone levels (Murakami et al., 1984), or their effect on neurotransmitters and peptides such as dopamine (DA) and corticotropin-releasing hormone (CRF) (Peterson et al., 2006; Yilmazer-Hanke et al., 2004), that appear be involved in the regulation of PER2 expression in the BNSTov and CEA (see below).

4.3 Ovarian hormones

It is well established that the release of ovarian hormones is influenced by the circadian system (Wiegand and Terasawa, 1982; Wiegand et al., 1978) and, in turn, circadian rhythms of locomotor activity are influenced by circulating levels

of gonadal hormones (Albers, 1981). Given these reciprocal relationships and the importance of the limbic forebrain in reproductive physiology and behavior, it was of interest to examine the role of ovarian hormones in the regulation of PER2 rhythms in BNSTov and CEA of female rats. PER2 rhythms in BNSTov and CEA were found to be strongly affected by the estrous cycle and by estrogen. Specifically, the patterns of PER2 expression observed in BNSTov and CEA varied as a function of day of the estrous cycle, such that on proestrus and estrus it was similar to rhythm seen in the SCN and previously reported in male rats, whereas on the metestrus and diestrus days of the cycle there was a marked blunting of the rhythm of PER2 expression in BNSTov and CEA. Rhythms in the SCN, basolateral amygdala and hippocampus were unaffected. It was also found that in ovariectomized females the patterns of expression of PER2 in limbic forebrain were similar to those in intact males. Treatment of ovariectomized females with injections of estradiol, aimed at mimicking levels seen across the estrous cycle, restored the normal pattern of PER2 expression found previously in BNSTov and CEA (Perrin et al., 2006).

These findings on the role gonadal hormones, taken together with those on glucocorticoids and thyroid hormones, indicate that the oscillations of PER2 expression in BNSTov and CEA are unique in their sensitivity to circulating hormones. Furthermore, they underscore the complexity and diversity of mechanisms involved in regulation of PER2 expression in the brain. Such findings provide an important clue to understanding how normal fluctuations in circulating hormones that affect motivational and emotional states can modulate

normal circadian rhythms within specific regions of the limbic forebrain and affect the synchrony between rhythms in different regions. Similarly, such findings point to ways in which alterations in emotional and motivational states that disrupt hormonal outputs, such as stress, feeding disorders and exposure to drugs of abuse could disrupt synchrony between circadian oscillations in different brain regions and, importantly, uncouple them from the rhythm in the SCN.

5.0 Dopamine, CRF and PER2 expression in BNSTov and CEA

5.1 Dopamine

Dopamine has been implicated in the regulation of circadian rhythms in the fetal SCN (Weaver and Reppert, 1995; Weaver et al., 1992; Weaver et al., 1995) and in the regulation of the expression of retinal clock genes in rodents (Dorenbos et al., 2007; Yujnovsky et al., 2006). Furthermore it has been shown that drugs of abuse that stimulate the release of dopamine or block dopamine reuptake, such as amphetamine and cocaine, respectively, induce the expression of clock genes such as Per1 and Per2 in the dorsal striatum in rats (Lynch et al., 2008; Nikaido et al., 2001).

The BNSTov and CEA receive dense dopaminergic innervations (Hasue and Shammah-Lagnado, 2002) from cells in ventral tegmentum. We asked, therefore, whether dopamine might be involved in the regulation of PER2 expression in these regions. Consistent with this possibility, we found that unilateral denervation of the dopaminergic input to BNSTov and CEA reduced the levels of expression of PER2 in these limbic forebrain regions, whereas

injections of amphetamine increased PER2 expression in these regions (Verwey et al., 2006). Importantly, the dopaminergic input to BNSTov and CEA is activated in response to stressors (Inglis and Moghaddam, 1999; Kozicz, 2002) and drugs of abuse (Carboni et al., 2000; Tran-Nguyen et al., 1998) suggesting a mechanism through which stressors and drugs of abuse could affect patterns of PER2 expression in these regions.

5.2 Corticotropin-releasing factor

Many cells in the BNSTov and CEA of rodents express the neuropeptide, corticotropin-releasing hormone (CRF) and we have found that, in addition to the effect on PER2 expression, lesions of the dopaminergic input substantially decrease CRF immunoreactivity in both BNSTov and CEA (Stewart et al., 2008) (see Fig. 2) confirming a major role for dopamine in the regulation of the CRF expression in these regions (Day et al., 2002).

Importantly, like the dopaminergic inputs to these regions, the CRF-containing neurons in BNSTov and CEA are activated in response to stressors (Merali et al., 2004; Merali et al., 1998) and mediate a wide variety of physiological and behavioral responses to stress including fear and anxiety (Davis, 2006; Schulkin et al., 2005), as well as responses that support appetitive behavior, such as increased locomotion (Cador et al., 1993; Cador et al., 1992; Kalivas et al., 1987) and facilitation of responses to incentive stimuli (Merali et al., 1998; Pecina et al., 2006). Furthermore, CRF within the limbic forebrain plays an important role in stress-induced relapse to drug seeking (Erb et al., 1998; Shaham et al., 1997; Heilig and Koob, 2007; Liu and Weiss, 2002;

Spealman et al., 2004; Stewart, 2003). Together these findings suggest CRF as another mediator of the effects of stressors and drugs on PER2 expression in the BNSTov and CEA.

To explore this idea, we studied the effect of targeted silencing of the CRF gene in BNSTov on PER2 expression using long double-stranded RNA-mediated RNA interference (Bhargava et al., 2004). Microinfusions of dsRNA against CRF given into the BNSTov suppressed CRF expression and reduced the level of PER2 (Fig. 3), suggesting that CRF containing cells participate in the regulation of PER2 expression and may mediate the effects of dopamine as well as of stress (and possibly of corticosterone (Makino et al., 1994; Makino et al., 1995) on rhythms of PER2 expression in this structure (Bhargava et al., 2006). The nature and mechanism of this regulation is not at all clear. It is likely, however, to be indirect, inasmuch as we also found that CRF and PER2 are not co-localized in cells in these regions (Fig 4).

In summary, we have shown that circadian oscillations in PER2 expression that we have identified in the BNSTov and CEA, although subordinate to and normally in phase with the SCN, are sensitive to circulating hormones and modified by the activity of transmitter and peptide systems of the brain that do not affect the rhythms of the SCN, itself. Because these systems are themselves subject to circadian modulation by the SCN, it is possible that they provide a means whereby the SCN communicates with downstream circadian oscillators that in turn transmit signals to different effector systems in brain and body. It is well known, however, that many of these hormonal and neurotransmitter systems

are themselves responsive to environmental events and to changes in behavioral states, and, as such, provide a means whereby experience and behavioral states could directly affect these subordinate oscillators downstream from the SCN.

6. Modulation of PER2 expression by environmental perturbations

Successful adaptive functioning of all organisms depends on stable synchronization of endogenous circadian rhythms with the rhythmic events in the environment. For most organisms the most powerful synchronizers are the environmental light cycle and feeding time. Below we describe how perturbations in the environmental light cycle and schedules of feeding affect the synchrony between the rhythms of PER2 expression in the BNSTov and CEA and that in the master clock of the SCN.

6.1 Perturbations of the light cycle

Disorders of sleep, mood, cognition, attention and appetite have been linked to disruptions of circadian rhythms associated with shift-work, and travel across time zones (jet lag) (Hastings et al., 2003; Moore-Ede et al., 1983a; b). In rodents, large abrupt shifts in the entraining light cycle, that mimic travel across time zones, have been shown to transiently disrupt the normal patterns of behavioral and physiological rhythms and to uncouple the rhythms of expression of SCN clock genes from those in subordinate oscillators in some brain regions (e.g., arcuate nucleus, paraventricular nucleus and pineal gland) and in peripheral tissues (e.g., liver, lung and skeletal muscle) (Abe et al., 2002; Reddy et al., 2002; Yamazaki et al., 2000). This temporary uncoupling of clock gene

rhythms results from the fact that re-entrainment to the novel light cycle occurs more quickly in the SCN than in subordinate oscillators. We have shown that in rats subjected to a single large (8-h) delay or advance shift in the entraining 12-h:12-h light-dark (LD) cycle, the rate of re-entrainment of the expression of PER2 is faster in the SCN than it is in the BNSTov, suggesting weak coupling between the BNSTov and the master SCN clock (Amir et al., 2004). Given the importance of the central extended amygdala to emotional and motivational processes, it is likely that the disruption of the synchrony between rhythms of clock gene expression in these regions and the SCN underlies some of the physiological, emotional and behavioral consequences of shift-work and jet lag.

6.2 Restricted feeding schedules

In mammals, the SCN clock regulates the time of feeding and, in turn, feeding time can be a powerful synchronizer of behavioral and physiological circadian rhythms and rhythms in expression of clock genes (Mendoza, 2007; Schibler, 2007). In nocturnal rodents, feeding that is restricted to the daytime has been shown to synchronize rhythms of clock gene expression in multiple tissues and organs, including the brain, and to uncouple them from the rhythms of the SCN (Challet et al., 2003; Damiola et al., 2000; Hara et al., 2001; Oishi et al., 2002; Stokkan et al., 2001; Wakamatsu et al., 2001). Furthermore, it is known that such feeding schedules induce characteristic food anticipatory behavioral and physiological rhythms (e.g., rhythms in behavioral arousal and locomotor activity, body temperature, circulating corticosterone) that can be

shown to be independent of the SCN (Mistlberger, 1994). We use restricted feeding schedules to study how perturbation of motivational state and energy balance affect the expression of PER2 in the limbic forebrain. We found that daytime restricted feeding (2 h per day, for 10 days) synchronizes the rhythms of PER2 in BNSTov and CEA and uncouples them from the rhythm of the SCN (Verwey et al., 2007; Waddington Lamont et al., 2007). Specifically, the peak of PER2 rhythms in these regions shifted away from that in the SCN to a time 12 h after daily food presentation. Importantly, such daytime restricted feeding affect PER2 rhythms in other regions of the limbic forebrain in a similar manner. This fact that the effect of restricted feeding was not restricted to BNSTov and CEA stands in contrast to the selective effects of hormonal manipulations, which affect only BNSTov and CEA, and points to a critical role for signals arising disruptions of energy balance in the maintenance and integration of circadian rhythms in the brain.

Significantly, unlike the effect of restricted feeding schedules, which involve daily disruptions of energy balance, scheduled restricted access to treats such as sucrose, saccharine, or a highly palatable liquid diet, in the absence of food deprivation, had no effect on PER2 expression in the limbic forebrain (Verwey et al., 2007; Waddington Lamont et al., 2007). Thus, it appears that the critical factor mediating the effect of restricted feeding is the daily alleviation of a negative metabolic state. Furthermore, we have to conclude that rhythms of PER2 in these areas are relatively insensitive signals arising from the incentive properties of the food substances (Verwey et al., 2007; Waddington Lamont et

al., 2007).

6.3 Stress

Stressful events have been shown to affect circadian behavioral and physiological rhythms (Gorka et al., 1996; Meerlo et al., 2002), however, there is only limited evidence concerning the effects of stressors on clock gene expression in the brain. In mice, the acute stressors, restraint and systemic immune challenge (lipopolysaccharide, LPS), induced the expression of the Per1 gene in the hypothalamic paraventricular nucleus; there was no affect acute exposure to these stressors on Per1 expression in the SCN nor was Per2 expression in either the PVN or SCN affected (Takahashi et al., 2001). In neonatal rats, it was shown that periodic absence of nursing mothers, a stressful event, can shift and entrain the rhythm of expression of Per1 and Per2 in the SCN (Ohta et al., 2003). Finally, we have found in a preliminary study carried out in adult rats housed under a normal light/dark cycle that exposure to chronic daily intermittent restraint stress, given in the daytime, can disrupt the rhythms of PER2 expression in BNSTov and CEA without affecting that in the SCN (Robinson et al., 2005).

Summary and conclusions

As discussed by many of the papers in this special issue, the bed nucleus of the stria terminalis (BNST), and its associated structures in the amygdala, represent a complex and neurochemically heterogeneous set of structures that modulate a wide range of physiological and motivational processes. These

include neuroendocrine, autonomic and behavioral responses to stressors and to drugs of abuse, ingestive behaviors, and reproductive and maternal behaviors (Casada and Dafny, 1991; Choi et al., 2007; Davis et al., 1997; Dumont et al., 2005; Dumont et al., 2008; Dumont and Williams, 2004; Epping-Jordan et al., 1998; Erb et al., 2004; Erb et al., 2001; Erb and Stewart, 1999; Figueiredo et al., 2003; Fink and Smith, 1980; Funk et al., 2006; Gray, 1993; Heilig and Koob, 2007; Herman et al., 2005; Loewy, 1991; Nijsen et al., 2001; Stefanova and Ovtscharoff, 2000; Van de Kar and Blair, 1999; Walker et al., 2001; Walker et al., 2003; Walker et al., 2000).

Most if not all aspects of behavior and physiology, including those modulated by the BNST and amygdala, exhibit some degree of circadian rhythmicity under the control of the master circadian clock located in the SCN. Furthermore, it is known that the circadian rhythms driven by the SCN depend on the rhythmic of expression of a number of well characterized clock genes. The work that we have discussed in this paper shows that nuclei within the BNST and amygdala, like the SCN, exhibit circadian oscillations in expression of the clock gene, PER2, an essential component of the mechanism driving circadian rhythmicity. Furthermore, we summarized evidence showing that some of these rhythms, though subordinate to the SCN, are directly sensitive to hormonal and environmental perturbations that normally do not affect the rhythms of gene expression in the SCN.

More specifically, we found robust rhythms of PER2 expression in two related structures, the BNSTov and CEA, that are normally in perfect synchrony

with the rhythms of the SCN. Rhythms in these two regions, though dependent on the functional integrity of the SCN, are selectively sensitive to changes in adrenal, thyroid and gonadal hormones, to changes in dopamine and CRF, as well as to perturbations of motivational state, energy balance and to stressors, none of which affect the rhythm of the SCN. Thus, all of these 'motivational' manipulations can uncouple these tissue-specific subordinate oscillators from the master circadian clock. The specific nature of the consequences of such uncoupling for health and adaptive functioning of the organism is not known, but one might speculate, based on the ideas in the literature on jet lag and shift work, that such uncoupling could give rise to physiological, emotional and behavioral disturbances similar to those seen under these circumstances.

A major task that remains, is to identify the consequences for physiology and behavior of disruptions in the expression of PER2 and its rhythms in the BNSTov and CEA. For example, changes in the rhythmic expression of PER2 could affect the functional integrity of cells in these limbic regions by disrupting local metabolic processes and thereby changing their sensitivity to incoming signals (Green et al., 2008). Consistent with this possibility is the evidence that genetic disruptions of the Per2 gene alters behavioral processes such as sensitization to the behavioral activating effects of cocaine (Abarca et al., 2002) and alcohol preference (Spanagel et al., 2005). We have begun to address these issues more specifically using RNAi-mediated knockdown of Per2 within specific regions of the brain (Gavrila et al., 2008).

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Figure captions

Fig. 1.

Photomicrographs showing examples of PER2 immunoreactivity in the SCN, BNSTov, and CEA of rats perfused at ZT1 or ZT13.

Fig. 2.

Photomicrographs showing examples of immunoreactivity for the dopamine transporter (DAT) and CRF in BNSTov and CEA following unilateral infusions of 6-OHDA into the medial forebrain bundle. Note the absence of DAT staining and the reduction of CRF staining in both BNSTov and CEA on the side of the infusion.

Fig. 3

Photomicrographs showing the effect of unilateral infusions of dsRNA to CRF (left) or control infusions (right) into the BNSTov on local CRF and PER2 expression. Note that infusion of dsRNA led to a decrease in CRF staining and to severe suppression of PER2 expression.

Fig. 4.

Photomicrographs showing examples of CRF expression (left) and of CRF (brown) and PER2 (blue) staining (right) in BNSTov and CEA. Note the lack of coexpression of CRF and PER2 in either region.

Figure 1

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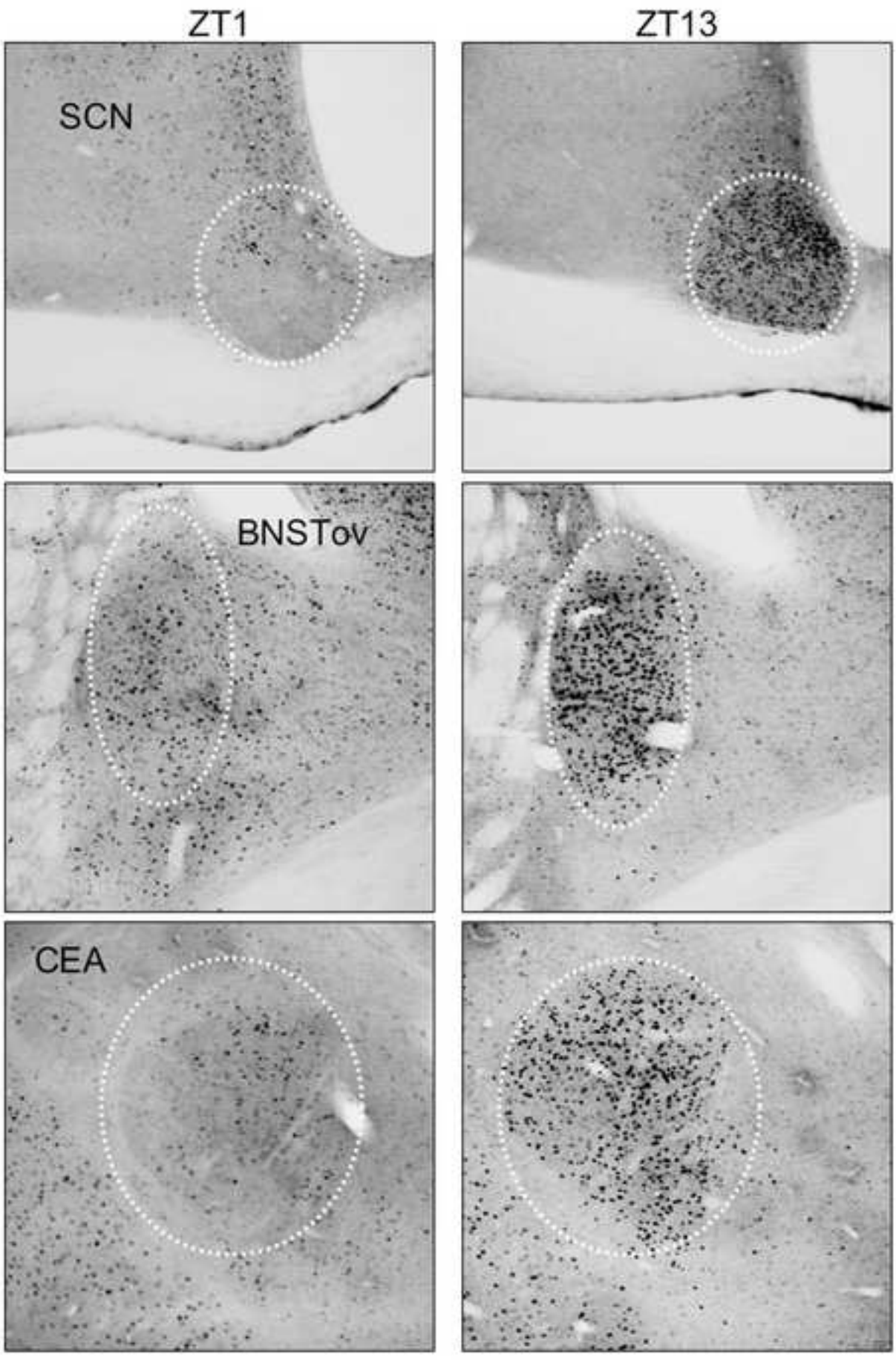

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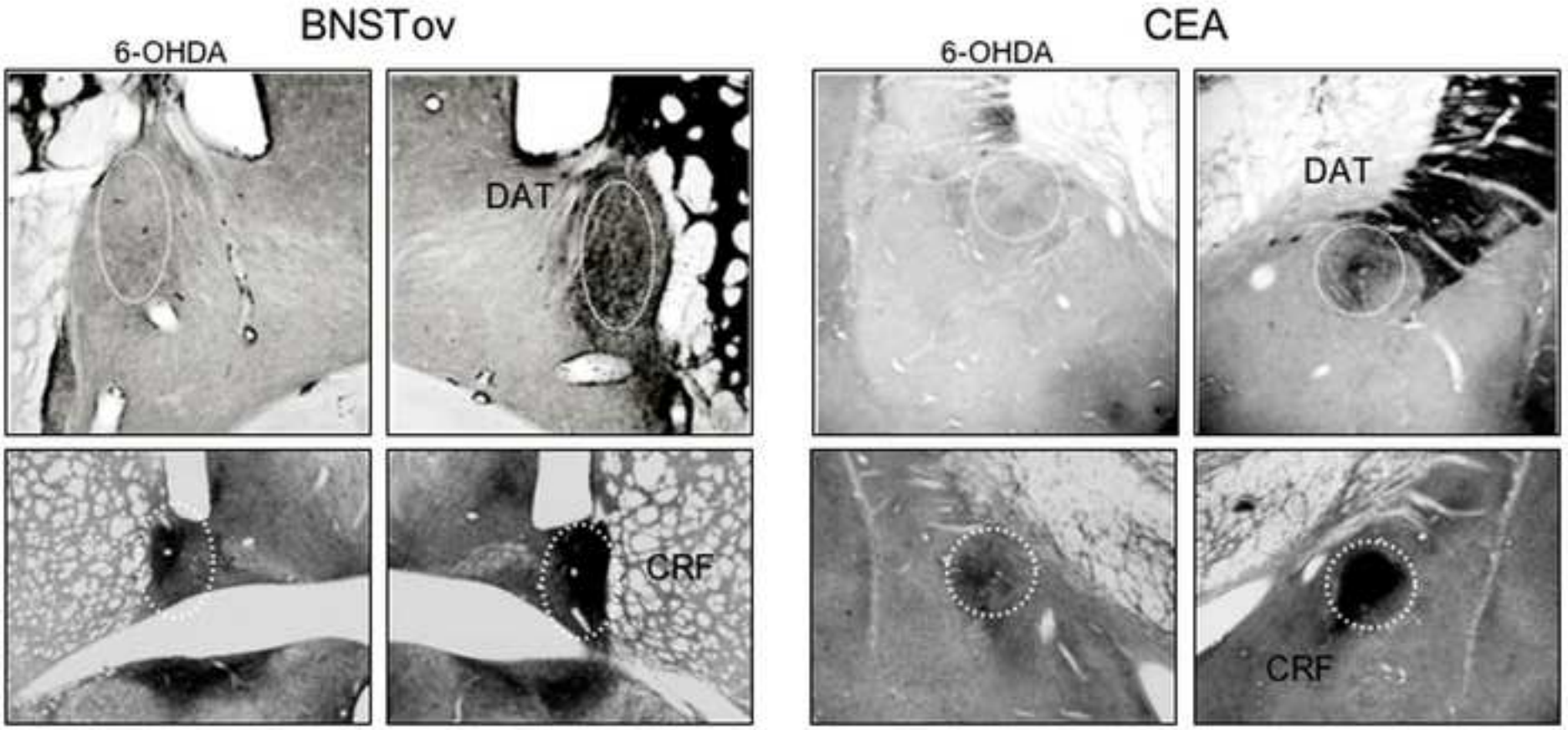

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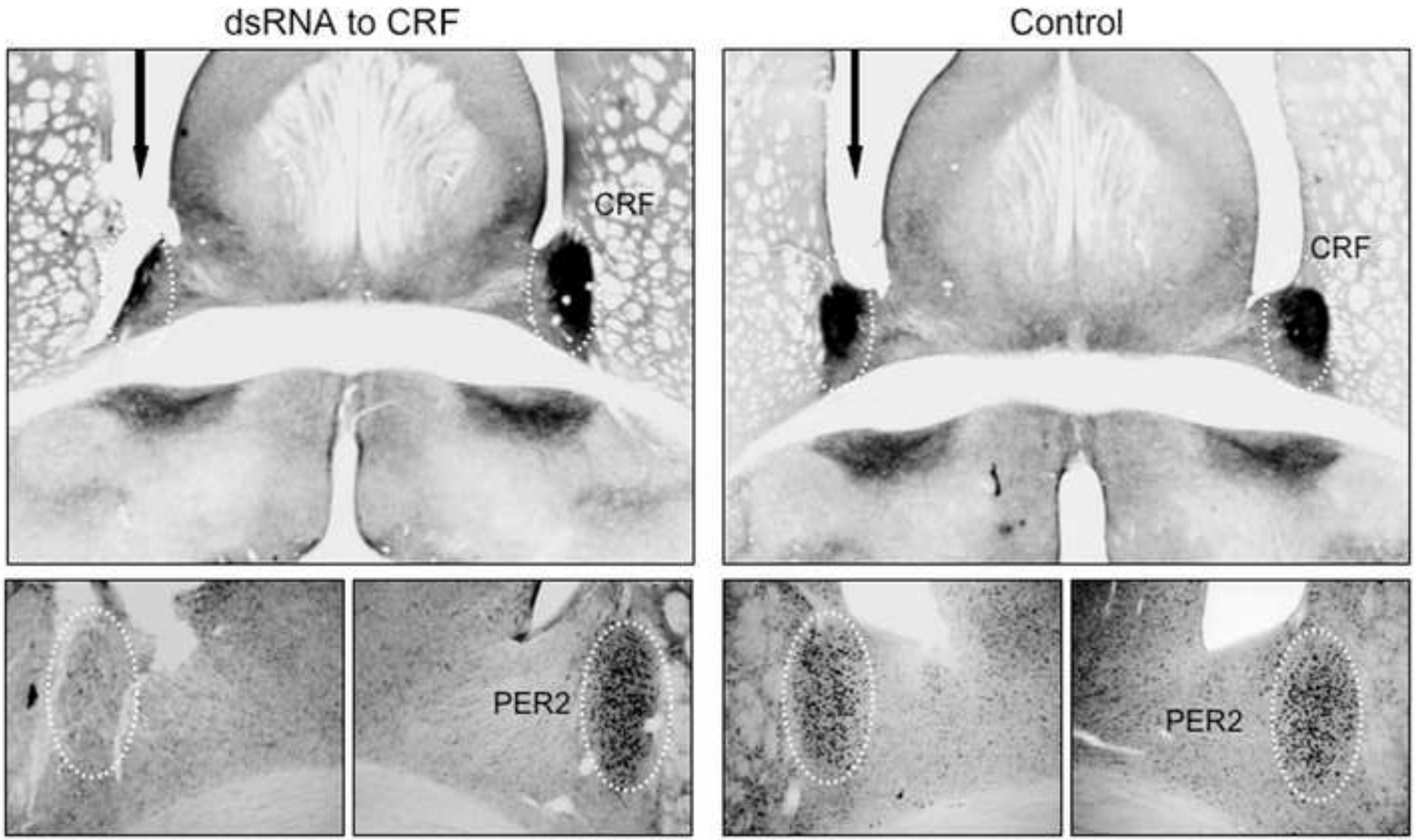

Figure 4

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