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Research Report

Daily methylphenidate and atomoxetine treatment impacts on clock gene protein expression in the mouse brain

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ABSTRACT

Circadian rhythms are repeating patterns of physiological and other parameters that recur with periods of approximately 24 h, and are generated by an endogenous circadian timekeeping mechanism. Such circadian rhythms, and their underlying molecular mechanisms, are known to be altered by a number of central nervous system acting pharmacological compounds, as well as becoming perturbed in a number of common psychiatric and neurological conditions. The psychostimulant methylphenidate and the non-stimulant atomoxetine are used in the pharmacotherapy of attention deficit hyperactivity disorder, a common condition in which circadian rhythms have been reported to be altered. In the present study we have examined the effects of daily methylphenidate or atomoxetine treatment across 7 days on circadian clock gene product expression across numerous brain regions in the male mouse to test the potential impact of such compounds on circadian timing. We report drug, brain region and molecular specific effects of such treatments, including alterations in expression profiles in the suprachiasmatic nucleus, the master circadian pacemaker. These results indicate that drugs used in the clinical management of attention deficit hyperactivity disorder can alter molecular factors that are believed to underpin circadian timekeeping, and such effects may be of importance in both the therapeutic and side effect profiles of such drugs.

Abbreviations: AcbC, core of the nucleus accumbens; AcbSh, shell of the nucleus accumbens; ADHD, attention deficit hyperactivity disorder; ATO, atomoxetine; BLA, basolateral amygdala; CC, cingulate cortex; CEA, central nucleus of the amygdala; CPu, caudate putamen; DAT, dopamine transporter; DG, dentate gyrus; DMH, dorsomedial nucleus of the hypothalamus; ILC, infralimbic cortex; MPD, methylphenidate; NET, noradrenaline transporter; PER, PERIOD; PLC, prelimbic cortex; PVN, paraventricular nucleus of the hypothalamus; SAL, saline; SCN, suprachiasmatic nucleus; ZT, zeitgeber time

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1. Introduction

Attention deficit-hyperactivity disorder (ADHD) is a common psychiatric condition of both childhood and adulthood, characterised by the core symptoms of impulsivity, inattention and hyperactivity (Coogan et al., 2012). The psychostimulant methylphenidate (MPD) and the non-stimulant atomoxetine (ATO) are used for the management of attention deficit hyperactivity disorder (ADHD) in both children and adults (Biederman and Faraone, 2005). Both drugs exert their therapeutic action through manipulation of the catecholaminergic systems, with MPD increasing the synaptic concentration of both dopamine and noradrenaline, though inhibition of the dopamine transporter (DAT) and the norepinephrine transporter (NET), whilst ATO is an inhibitor of the NET and increases synaptic noradrenaline levels (Madras et al., 2005).

Sleep deficits are commonly observed in ADHD (Sobanski et al., 2008), and both beneficial and adverse effects of MPD and ATO on various aspects of sleep have been documented in ADHD (Boonstra et al., 2007). Circadian disturbances in adult ADHD at the behavioural, molecular and endocrine levels have also been shown (Baird et al., 2011) as well as genetic associations between ADHD and clock gene polymorphisms (Kissling et al., 2008). The circadian clock is responsible for the generation of circadian rhythms, which are recurring patterns of behaviour and physiology on a near twenty-four period base and plays a key role in determining the sleep/wake cycle (Dibner et al., 2010; Reppert and Weaver, 2002). The master circadian clock is located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus with other oscillators present throughout the brain and periphery (Guilding and Piggins, 2007). The molecular basis of circadian rhythm generation consists of positive and negative transcriptional/translational feedback loops of "clock" genes and their protein products (Dibner et al., 2010).

Numerous pharmacological agents are known to impact upon the molecular circadian clock (e.g. Uz et al., 2005) and the dopaminergic and noradrenergic systems are implicated in circadian clock functioning (McClung et al., 2005; Wongchitrat et al., 2009). MPD and ATO have both also been shown to exert effects upon the mammalian circadian system (chronic MPD treatment produces a circadian locomotor rhythm in arrhythmic SCN-lesioned rats (Honma and Honma, 1992)) as well as on rodent diurnal rhythms (Algahim et al., 2009,2010), whilst time-of day effects on behavioural sensitization to MPD have also been reported (Gaytan et al., 2000).

Recently it has been shown that ongoing MPD treatment of mice via drinking water produces phase delays and lengthened free running rhythms and also alters electrical discharge rhythms of SCN neurons (Antle et al., 2012). Further, acute ATO treatment has been shown to phase-shift the rodent locomotor rhythm and to alter circadian clock gene product expression (O'Keeffe et al., 2012).

Given the deficits of the circadian clock in ADHD and the interactions of ADHD medication with sleep, it could be postulated that the therapeutic properties and/or the adverse side effects of these drugs in part could involve modulation of the circadian clock. Our hypothesis for the current study was that in the light of data concerning phase-shifting effects of both MPD and ATO in rodents and the key role of the

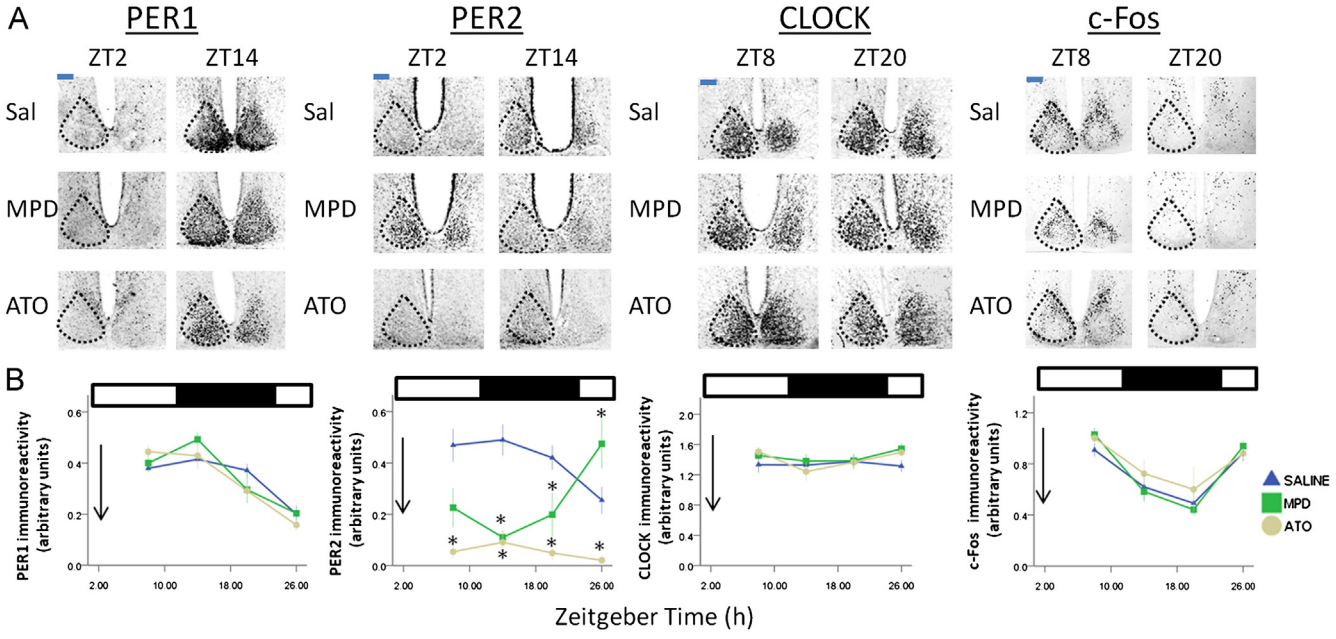

Fig. 1 – *Modulation of clock gene products and c-Fos in the SCN by methylphenidate and atomoxetine.* A: Photomicrographs illustrating the expression of PER1, PER2, CLOCK,and c-Fos in the SCN. The SCN is delineated by the dashed line and the scale bar is 100 μm. B: Expression profiles of CLOCK, PER1, PER2 and c-Fos in the SCN in the saline (SAL), methylphenidate (MPD) and atomoxetine (ATO) groups. * represents $P<0.05$ for pairwise comparison between the value in the appropriate treatment group at that time-point compared to the value in the saline control group at the same time-point. The arrow represents the time of the daily injection.