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Guest Editorial

A bark in time: Identification of a canine circadian clock marker

Nearly all mammalian physiological systems undergo circadian variation, a consequence of evolution on a revolving planet exposed to continuous ~24 h cycles of light and dark. Of all environmental stimuli, the importance of anticipating the time of daily photostimulation for survival has given rise to the development of endogenous cell molecular clocks. At their core are oscillating auto-regulatory feedback loops consisting of highly conserved core clock genes and their protein products, which act in concert to generate a ticking biological clock mechanism (Reppert and Weaver, 2002). The ability to reliably detect clock gene oscillations in specific tissues will provide important markers of rhythmic downstream physiological events and are the basis for the experiments described by Dr Keitaro Ohmori and colleagues of the Tokyo University of Agriculture and Technology in this issue of *The Veterinary Journal* (Ohmori et al., 2013).

Of considerable importance for veterinary science are the daily rhythms of an animal's immune system and the circadian influences on pharmacokinetic parameters, such as drug absorption, metabolism and elimination. These phenomena are already considered to be highly relevant for practical human medicine (Smolensky and Peppas, 2007). Underlying these physiological fluctuations are circadian oscillations of the core clock genes responsible for driving the cyclic rhythmic expression of subsets of downstream clock-controlled genes that are specific to the functioning of each tissue. In this way, time-of-day signals, perceived by the eye and processed by the master circadian clock in the suprachiasmatic nucleus (SCN), are transmitted throughout the body to keep the entire organism in harmony with its environment.

The horse is an animal of veterinary importance in which significant progress has been made in understanding circadian timing at the molecular, tissue and behavioural level (Murphy, 2009; Martin et al., 2010). Similar to dogs (Ohmori et al., 2013), core body temperature and serum cortisol rhythms have been reported in the horse (Piccione et al., 2002; Murphy et al., 2007, 2011). Of greater interest, is the finding by Ohmori and colleagues that the core clock genes *Per2*, *Bmal1*, *Clock* and *Cry1* do not oscillate in canine peripheral blood mononuclear cells (PBMCs) in vivo (Ohmori et al., 2013). This agrees with our similar observations in healthy horses (Murphy et al., 2006). It is as yet unclear why this tissue does not share the temporally related clockwork mechanism observed in almost all other peripheral tissues (Oishi et al., 1998), but recent evidence indicates a role for non-transcriptional events in sustaining cellular circadian rhythms in blood (O'Neill and Reddy, 2011).

In contrast, and again mirroring work using equine fibroblasts (Murphy et al., 2006), all of the clock genes examined in canine cultured PBMCs responded to a 2 h incubation with adult horse serum (Ohmori et al., 2013), the standard serum shock protocol used to temporarily resynchronise component circadian cell oscillators and investigate temporal expression profiles within the clockwork mechanism (Balsalobre et al., 1998). The observed rise in expression of *Per1, Per2* and *Cry1*, and concomitant reduction in expression of *Bmal1*, following serum shock, clearly echoes the inverse temporal relationships characteristic of the molecular clockwork mechanism in the SCN and peripheral tissues of rodents (Oishi et al., 1998), as well as the temporal patterns of expression reported in the equine circadian clock (Murphy et al., 2006; Martin et al., 2010). Since only a single time point was examined in this initial canine study (Ohmori et al., 2013), it is speculated that sequential sampling of cultured canine PBMCs for >24 h will reveal the temporal relationships between clock genes within the molecular clock mechanism of this species.

A unique finding by Ohmori et al. (2013) is that *Per1* expression rhythmically oscillates in canine blood cells. *Per1* functions as an immediate early gene (Albrecht et al., 1997) and Ohmori et al. (2013) provide further evidence of this in a canine model by its upregulation in response to glucocorticoid stimulation. However, what cannot be explained is its convincing circadian profile over a 24 h sampling period in vivo. As suggested by the authors, the ability to identify oscillating expression of a clock gene in healthy dogs may prove invaluable in future studies examining the circadian clock response to drug administration.

Since a less invasive means of monitoring the circadian cycle in animals is desirable, we recently demonstrated robust oscillations of clock gene transcripts in hair follicle cells from horses (Watts et al., 2012). This highly accessible tissue requiring limited expertise for collection should now provide an ideal way to monitor the phase of the peripheral circadian clock in these and other domestic species.

Circadian regulation of cortisol and the pineal hormone melatonin is key to understanding immune-circadian interaction in mammals and its implications for veterinary interventions. Cortisol, which peaks at mid-morning in dogs (Ohmori et al., 2013), acts as a potent suppressor of pro-inflammatory cytokines (Russo-Marie, 1992; Petrovsky et al., 1998). The immunomodulatory action of melatonin at night is also well established and is due in part to its ability to inhibit expression of tumour necrosis factor α and interleukin 6 (Sullivan et al., 1996; Wu et al., 2001).

There is further evidence in horses that the time-of-day of antigen presentation influences the temporal patterns of cytokine and clock gene expression in whole blood (Mclynn et al., 2010). This supports the theory that the direction and severity of an immune response depends on the cytokine environment at the time-of-

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day of immune stimulation (Petrovsky and Harrison, 1997). This was most clearly demonstrated by Marpegan et al. (2009), who observed significant diurnal variation in endotoxin-induced mortality in mice and improved survival in animals exposed to a cyclic light–dark environment vs. constant photoperiodic conditions following challenge with endotoxin. This suggests that the time-of-day of veterinary surgical intervention may have a significant impact on survival statistics, especially when the risk of post-surgery sepsis is high. Furthermore, from a management perspective, the lighting conditions that post-surgical or sick animals are exposed to could potentially influence recovery (Herdegen, 2002). Disrupted circadian rhythms occur in the absence of rhythmic photoperiod signals and are a well-accepted cause of physical malaise (Karatsoreos, 2012).

In the near future, as the importance of understanding circadian regulation of physiology in domestic species becomes increasingly apparent for veterinary practice, an explosion of papers reporting these findings is anticipated, and not before time.

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