

Daily *Socs1* rhythms alter with aging differentially in peripheral clocks in male Wistar rats: therapeutic effects of melatonin

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Abstract Suprachiasmatic nucleus (SCN) in synchronization with the peripheral clocks regulates the temporal oscillations leading to overt rhythms. Aging leads to attenuation of such circadian regulation, accompanied by increased inflammatory mediators prevalently the cytokines. Suppressors of cytokine signaling (SOCS) family of proteins such as SOCS 1, 3 and cytokine-inducible SH2-containing protein (CIS) negatively regulate the cytokine signaling pathway. The role of SOCS1 in aging and circadian system is obscure. We therefore studied the daily rhythms of *rSocs1* mRNA expression at Zeitgeber time (ZT) –0, 6, 12 and 18 in peripheral clocks such as liver, kidney, intestine and heart of 3, 12 and 24 months (m) old male Wistar rats. Interestingly the peripheral clocks studied displayed a rhythmic *rSocs1* gene expression in 3 months. In 12 months group, 12 h phase advance in liver and 12 h phase delay in kidney and heart was observed with abolition of rhythms in intestine. Aging (24 months group) resulted in a phase advance by 6 h in liver and heart with abolition of rhythms in intestine in 24 months group. Kidney was also significantly

affected upon aging with significant decrease in the *rSocs1* levels and abolition of rhythms. The decrease in melatonin levels with aging is associated with decreased immunity and increased oxidative stress. The exogenous administration of melatonin has been linked to play a role in re-synchronization of circadian rhythms, reducing oxidative stress and enhancing immune properties. We therefore had studied the effect of exogenous melatonin upon age induced changes in daily *rSocs1* gene expression patterns. Melatonin treatment partially restored the rhythms and daily pulse (ratio of maximum:minimum levels) in liver and intestine in 12 months group. Melatonin administration resulted in a significant increase in mean 24 h *rSocs1* expression in intestine and heart of 24 months group compared to that of 3 months. The melatonin administration resulted in differential restoration of *rSocs1* rhythms and levels in various tissues of 24 months old group. The sensitivity of 24 months old animals to melatonin found in the present study is a step towards endorsing melatonin as an important anti-aging therapeutic drug.

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Introduction

In mammals, the physiological and behavioral processes follow an approximately 24 h cycles known as circadian rhythms, which are regulated by a master

clock located in suprachiasmatic nucleus (SCN) (Jagota 2012; Erion et al. 2016). At molecular level a transcriptional-translational feedback loop consisting of *Per*, *Cry*, *Bmal1*, *Rev-erb α* and *Ror α* genes and their protein products regulate circadian rhythmicity (Mohawk et al. 2012; Lee et al. 2015). This clock machinery was discovered in several other regions of brain and peripheral organs known as peripheral clocks. Though these organs possess their self-sustained clock machinery they are under the control of master clock (Dibner and Schibler 2015; Hurley et al. 2016). The SCN sends its outputs to various peripheral clocks by regulating release of neuropeptides, secondary messengers, nitric oxide (NO), glucocorticoids (GC's), neurohormones like melatonin, etc. and thus synchronizes with the peripheral oscillators. This synchronization ensures appropriate internal synchronization between different physiological, metabolic and behavioral systems (Golombek et al. 2004). Several researchers have reported the regulation and synchronization of immune system to circadian system (Carter et al. 2016). Interestingly, the clock genes when suppressed or knocked out were reported to result in compromised immune functions leading to increased inflammation (Liu et al. 2007; Hashiramoto et al. 2010; Gibbs et al. 2012; Curtis et al. 2014). However, the inflammatory cell derived mediators if overexpressed or suppressed resulted in the circadian dysfunction (Palomba et al. 2008; Monti 2010; Duhart et al. 2013).

Rev-erb α regulates the expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), which is a key molecule involved in controlling the expression of several cytokines and other pro-inflammatory markers (González-Hernández et al. 2006). Most of the circadian-immune relationships studied were on pro-inflammatory markers like cytokines, chemokines, prostaglandins, etc. The pro-inflammatory cytokines are regulated by a family of proteins known as suppressors of cytokine signaling (SOCS). SOCS family comprise of eight intracellular proteins SOCS 1–7 and cytokine-induced STAT inhibitor (CIS) which participate in inhibiting the JAK/STAT pathway. SOCS1, SOCS3 and CIS are majorly expressed when cytokines are expressed. Both SOCS1 and 3 inhibit the kinase activity of JAK, whereas CIS competitively binds to SH-2 domains of JAK blocking the sites for Signal transducer and activators (STAT). This targets the cytokine signaling complex for ubiquitination and proteasomal degradation

(Yoshimura et al. 2007; Dalpke et al. 2008). Some researchers demonstrated the expression of all the SOCS genes in various porcine tissues with SOCS1 and SOCS3 showing abundant expression and functional similarity in suppressing the cytokine signaling (Delgado-Ortega et al. 2011).

Aging is a steady and an unidirectional phenomenon where most of the biological systems including the circadian and the immune systems, were known to get compromised in their functions (Gibson et al. 2009; Jagota 2005, 2012). We had reported that aging affects serotonin metabolome, leptin, antioxidant rhythms and clock gene expression patterns (Manikonda and Jagota 2012; Mattam and Jagota 2014; Reddy and Jagota 2014, 2015). It was reported earlier by many workers that the inflammatory parameters are altered significantly in aged individuals (Coogan and Wyse 2008). Now to understand the effects of aging on synchronization of the circadian and immune systems, we have studied the age induced alterations in daily *rSocs1* rhythms and levels in peripheral clocks liver, kidney, intestine and heart. The neurohormone melatonin, an important regulator of circadian rhythms, a multitasking molecule, an antioxidant, anti-aging, cytokine modulator, etc. (Jagota 2012; Reiter et al. 2014) has been reported to decrease with aging (Karasek 2007; Reddy and Jagota 2015). Many researchers have demonstrated the beneficial effects of exogenous administration of melatonin in decreasing the oxidative stress and enhancing immunity in age induced cardiovascular diseases, neurodegeneration, oxidative stress, etc. (Nelson 2004; Paredes et al. 2014; Su et al. 2015; Ganie et al. 2016). From our laboratory we had earlier reported the differential effects of melatonin in restoration of daily rhythms of serotonin (Jagota and Kalyani 2010) and various components of serotonin metabolism, antioxidant enzymes and lipid peroxidation, NO (Manikonda and Jagota 2012; Reddy and Jagota 2015; Vinod and Jagota 2016), clock genes *rPer1*, *rPer2*, *rCry1*, *rCry2* and *rBmal1* expression (Mattam and Jagota 2014). Therefore we further studied therapeutic effects of melatonin on age induced *rSocs1* rhythms and levels in various peripheral clocks.

Methodology

Male Wistar rats of age groups 3 months (m)-A, 12 m-B and 24 m-C (n = 72 in each group) were

maintained at 23 ± 1 °C under LD, 12:12 [lights on: 06:30 am (Zeitgeber time (ZT)-0) and lights off: 6:30 pm (ZT-12)] for 2 weeks prior to experiment. Each animal was housed separately in polypropylene cages. Food and water were provided ad libitum. Cage changing was done at random intervals.

The animals in A, B and C were divided into three subgroups (A1, A2, A3, B1, B2, B3, C1, C2 and C3) with $n = 24$ in each subgroup.

Groups A1, B1 and C1 did not receive any treatment.

The animals in groups A2, B2 and C2 were administered with 10% ethanol in physiological saline subcutaneously at 1 h before the onset of darkness (ZT-11) for 11 days (Jagota and Kalyani 2010; Manikonda and Jagota 2012).

Groups A3, B3 and C3 the animals were administered melatonin (30 µg/500 µl of 10% ethanol in physiological saline/kg body weight of the animal subcutaneously at ZT-11 for 11 days (Manikonda and Jagota 2012).

The animals in all the groups were sacrificed at different zeitgeber time points (ZT-0, 6, 12 and 18) with $n = 6$ at each time point. For handling the animals in darkness dim red light was used. Peripheral tissues (kidney, intestine, liver and heart) were collected. The tissues were snap frozen and stored at -80 °C until use.

Semi quantitative gene expression of *rSocs1*

50 mg of tissue was taken and 10% homogenate was prepared using Tri reagent (Sigma). To each sample 150 µl of chloroform was added, vortexed and incubated at room temperature (RT) for 10 min. This homogenate was centrifuged at 12000g at 4 °C for 15 min. The aqueous solution was separated and subjected to equal volumes of isopropanol and incubated in ice for 10 min. The samples were then centrifuged at 12000g at 4 °C for 10 min. The supernatant was discarded and the pellet was washed with 75% ethanol, centrifuged at 7500 g at 4 °C for 5 min. The supernatant was discarded, the pellet was air-dried and dissolved in diethyl pyrocarbonate (DEPC) treated water. The RNA was quantitated using nano-drop spectrophotometer (Thermo Fischer 2000) (Chomczynski and Sacchi 2006). First strand cDNA synthesis was performed using Thermo Fischer first strand cDNA synthesis kit. The cDNA of pro-inflammatory 1:10 dilutions were taken. The PCR

master mix of 2x (Thermo Fisher) was added to the reaction tube. 2 µl of primers with a concentration of 10 pro-inflammatory pmol per 20 µl total reaction volume were used for amplification. Specific primers for *rSocs1* were selected as per Gagnon et al. 2007 and for internal control and normalization *β-actin* was selected as per Willem et al. 2005.

<i>rSocs1</i> Forward Primer	TCC TCG TCC TCG TCT TCG TC
Reverse Primer	AAG CCA TCT TCA CGC TGA GC
<i>rβ-actin</i> Forward Primer	AGC CAT GTA CGT AGC CAT CC
Reverse Primer	CTC TCA GCT GTG GTG GTG AA

The amplicons were then run on 2% agarose gel and the images were documented using Bio-Rad gel documentation system. Densitometry of the amplicons was done by using Image J software. The *rSocs1* densities were normalized to the band intensity of *rβ-actin* and were expressed as integrated density (Tang et al. 2009).

Statistical analysis

Data was analyzed using Jandel Scientific Sigma stat software. The *rSocs1* daily pulse (ratio of maximum:minimum levels), mean 24 h levels and daily rhythms in and between the age groups and the treatments were compared by one way ANOVA followed by Post hoc Duncan's test.

In addition Pearson correlation analysis was done between various tissues of all the age groups and conditions (Manikonda and Jagota 2012; Vinod and Jagota 2016). Correlation analysis was done for expression levels of *rSocs1* in mean light (ZT-0, 6 and 12) and dark (ZT-12, 18 and 24/0) phases.

Results

Age induced alterations in daily *rSocs1* rhythms and levels in peripheral clocks

All the age groups demonstrated daily rhythms in *rSocs1* mRNA expression in the hepatic peripheral clock. The maximum and minimum *rSocs1* levels were found at ZT-12 and 0 in 3 months, ZT-0 and 6 in 12 months, ZT-6 and 18 in 24 months groups

respectively. The maximum *rSocs1* levels in 12 and 24 months groups phase advanced by 12 and 6 h respectively (Fig. 1). The daily pulse was decreased by 16.2% from 3 to 12 months and by 38.9% from 3 to 24 months group which is significant ($P_q \leq 0.05$). Mean 24 h *rSocs1* levels were significantly decreased by 17.6% from 3 to 12 months ($P_q \leq 0.05$) and by 5.9% in 24 months group (Fig. 2).

Daily rhythms in expression of *rSocs1* were demonstrated in kidney in 3 and 12 months groups. The maximum and minimum *rSocs1* levels were found at ZT-6 and 18 in 3 months but at ZT-18 and 0 in 12 months respectively. In 24 months groups the *rSocs1* rhythms were abolished. A phase delay of 12 h in the maximum *rSocs1* levels was observed in 12 months (Fig. 1). Upon aging the daily pulse was increased by 19.8% from 3 to 12 months and decreased by 15.5% in 24 months group. The mean 24 h levels were increased significantly from 3 to 12 months by 11.5% ($P_q \leq 0.05$) and were significantly reduced in 24 months group by 31.2% ($P_q \leq 0.05$) (Fig. 2).

A rhythmic pattern of expression appeared in *rSocs1* in intestine of 3 months group. The maximum and minimum *rSocs1* levels were found at ZT-0 and 6 (Fig. 1). The rhythms in both 12 and 24 months were abolished. Upon aging the daily pulse decreased significantly by 42.5 and 47.1% in 12 and 24 months groups respectively as compared to 3 months ($P_q \leq 0.05$). The mean 24 h levels did not show any significant alterations between 3, 12 and 24 months groups (Fig. 2).

All the age groups in heart displayed daily rhythms in *rSocs1* expression. The maximum and minimum *rSocs1* levels were found at ZT-6 and 0 in 3 months, ZT-18 and 6 in 12 months, ZT-6 and 12 in 24 months groups respectively. A phase delay of 12 h was observed in maximum *rSocs1* levels in 12 months group (Fig. 1). There was no significant alteration in mean 24 h levels and daily pulse of *rSocs1* expression with aging (Fig. 2).

The onset of the *rSocs1* rhythm was found at ZT-6 in intestine, at ZT-18 in kidney, at ZT-0 in liver and heart. In all the tissues studied both onset and offset were phase delayed by 6 h in 12 months group. In 24 months group, the daily onset and offset in liver appeared phase advanced by 6 h in comparison to 3 months group but in kidney the onset and offset time was similar to that of 3 months group, whereas, a

Fig. 1 Effect of melatonin on age induced alterations in *rSocs1* expression daily rhythms in liver, kidney, intestine and heart of 3, 12 and 24 m old male Wistar rats. $P_a \leq 0.05$, $P_b \leq 0.05$, $P_c \leq 0.05$ and $P_d \leq 0.05$ (where a, b, c and d refers to comparison with ZT- 0, 6, 12 and 18 respectively within same age group). $P_w \leq 0.05$ (where w refers to comparison of *rSocs1* levels at a time point between control and melatonin treated groups of same age)

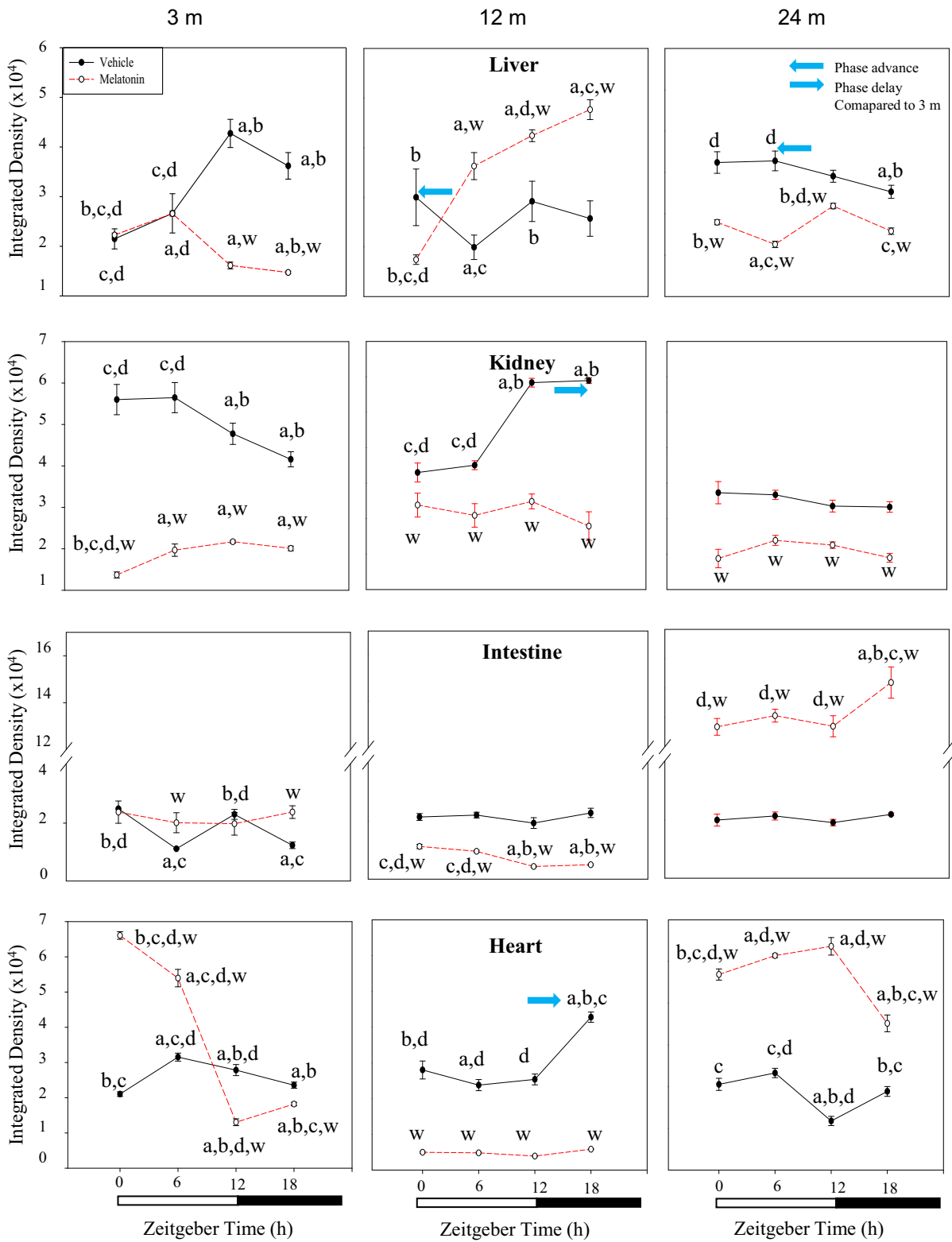
phase delay of 12 and 6 h was found in heart and intestine respectively (Fig. 3).

The correlation analysis demonstrated a significant negative correlation between heart and intestine in 3 months light phase (LP). In dark phase (DP) a significant positive correlation existed between kidney and intestine, liver and heart. In 12 months LP a significant positive correlation was found between liver and heart. The negative correlation between heart and intestine was changed to positive and the positive correlation between kidney and heart was changed to negative. In DP the negative correlations observed between kidney and heart, liver and intestine, heart and intestine were changed to positive correlations compared to 3 months. In 24 months LP a significant positive correlation appeared between kidney and intestine. This correlation was negative in earlier age groups. The correlation between kidney and liver was also changed to positive. The positive correlation between liver and intestine was changed to negative. In DP a significant negative correlation was observed between liver and intestine. The negative correlations between kidney and heart, kidney and liver, heart and intestine were changed to positive correlations when compared to 3 months (Fig. 4).

Effect of melatonin on age induced changes of daily *rSocs1* rhythms and levels in peripheral clocks

There were no significant differences between control and vehicle treated animals in daily *rSocs1* rhythms and levels in all the tissues studied.

Upon melatonin treatment, a rhythmicity in *rSocs1* levels was demonstrated in liver in all the age groups. The maximum and minimum *rSocs1* levels were observed at ZT-6 and 18 in 3 months, at ZT-18 and 0 in 12 months, at ZT-12 and 6 in 24 months groups respectively. Upon melatonin treatment the maximum *rSocs1* levels phase advanced by 6 h in 3 and



12 months, whereas a phase delay by 6 h in 24 months group was demonstrated (Fig. 1). The melatonin treatment resulted in a significant increase by 56.4% in *rSocs1* daily pulse in 12 months ($P_p \leq 0.05$). In 12 months group the mean 24 h levels were increased by 37.2% ($P_p \leq 0.05$). However the melatonin treatment resulted in significant decrease of the mean 24 h *rSocs1* levels in 3 months by 1.6 fold and in 24 months group by 1.75 fold from ($P_p \leq 0.05$) compared to vehicle control animals. When compared to 3 months vehicle control animals, 24 months melatonin treated groups demonstrated a significant decrease in mean 24 h *rSocs1* levels ($P_q \leq 0.05$) whereas 12 months melatonin treated group demonstrated restoration in *rSocs1* levels (Fig. 2).

In kidney of melatonin treated animals, *rSocs1* expression was arrhythmic in 12 and 24 months age groups (Fig. 1). The daily pulse was not altered in all the age groups. Upon melatonin treatment *rSocs1* mean 24 h levels were significantly decreased in 3 months by 62.8%, in 12 months by 43.5% and in 24 months by 41.3% ($P_p \leq 0.05$). When compared to 3 months vehicle control animals, 12 and 24 months melatonin treated groups resulted in significant decrease in mean 24 h *rSocs1* levels ($P_q \leq 0.05$) (Fig. 2).

Expression of *rSocs1* in intestine upon melatonin treatment was rhythmic in 12 and 24 months groups but arrhythmic in 3 months group (Fig. 1). The daily pulse decreased significantly by 45% in 3 months and it increased significantly by 2 fold in 12 months group ($P_p \leq 0.05$) whereas no significant alterations were displayed in 24 months group. 12 months melatonin treated group demonstrated a significant increase and 24 months melatonin treated group a significant decrease compared to 3 months vehicle control group ($P_q \leq 0.05$). The *rSocs1* mean 24 h levels were found to decrease significantly in 12 months group by 63.4% ($P_p \leq 0.05$). In 24 months group *rSocs1* mean 24 h levels were increased significantly by 6.9 fold ($P_p \leq 0.05$) (Fig. 2). Compared to 3 months vehicle control animals, 12 months melatonin treated group showed a significant decrease in mean 24 h *rSocs1* levels whereas 24 months melatonin treated group, a significant increase ($P_q \leq 0.05$) (Fig. 2). Interestingly this increase in 24 months melatonin treated group was 8.1 folds higher than 3 months vehicle control animals.

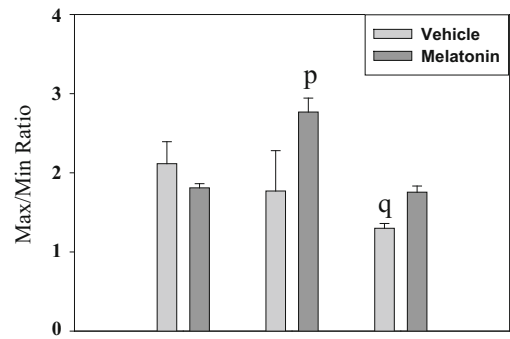
Upon melatonin treatment, the *rSocs1* rhythmicity persisted in heart in 3 and 24 months groups. The

Fig. 2 Effect of melatonin on age induced alterations in *rSocs1* expression daily pulse and mean 24 h levels in liver, kidney, intestine and heart of 3, 12 and 24 m old male Wistar rats. $P_p \leq 0.05$ and $P_q \leq 0.05$ where p indicates significant difference between vehicle control and melatonin treated groups of same age and q indicates significant difference in comparison to 3 m vehicle control group. Each value is mean \pm S.E. (n = 6) in daily pulse, but (n = 24) in mean 24 h levels

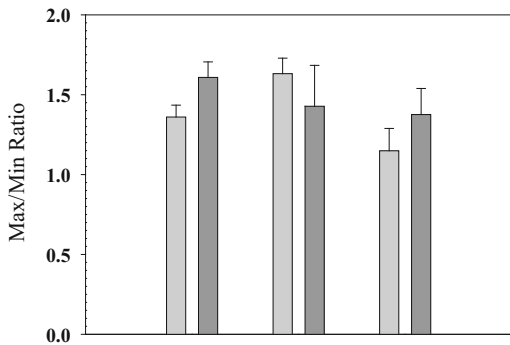
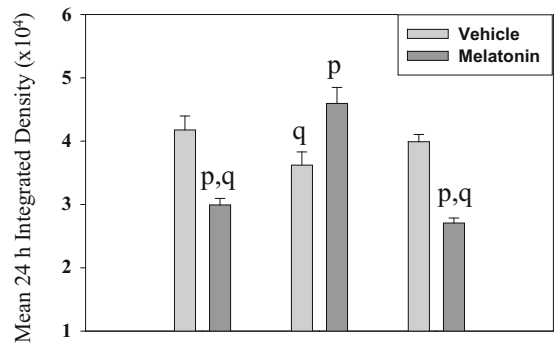
maximum and minimum *rSocs1* levels were observed at ZT-0 and 12 in 3 months, at ZT-12 and 18 in 24 months groups respectively. Upon melatonin treatment the maximum *rSocs1* levels showed a phase advance by 6 h in 3 months and a phase delay of 6 h in 24 months group (Fig. 1). The melatonin treatment in 3 months resulted in a significant increase by 3.45 fold in daily pulse ($P_p \leq 0.05$). The melatonin treatment significantly increased the mean 24 h *rSocs1* levels in 3 months by 45.5% and in 24 months group by 98.4% ($P_p \leq 0.05$) compared to vehicle control. In 12 months group the mean 24 h levels were decreased significantly by 83.8% ($P_p \leq 0.05$) (Fig. 2). A significant decrease by 5.3 folds in 12 months melatonin treated groups but significant increase in 24 months by 54.2% in mean 24 h *rSocs1* levels ($P_q \leq 0.05$) on comparison to 3 months vehicle control group was demonstrated.

The onset time of rhythm in 12 months melatonin treated group was similar to 3 months vehicle group in liver. The onset time was similar to that of a 3 months group in kidney but the offset time showed a phase delay. In intestine and heart with melatonin treatment the onset time in 12 months group was phase delayed by 6 and 12 h respectively to that of 3 months vehicle group. In kidney and heart of 24 months group the onset of rhythm was restored by 6 h with melatonin treatment (Fig. 3).

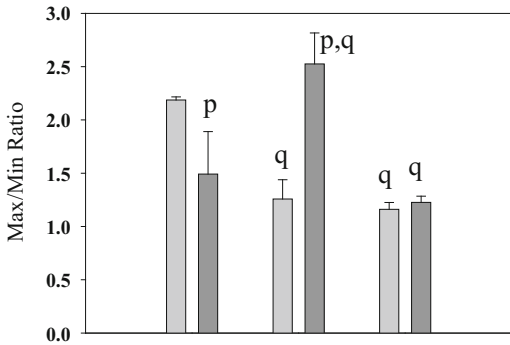
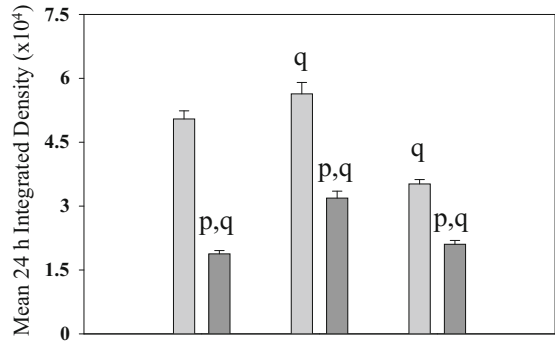
Upon melatonin treatment, the correlation analysis of *rSocs1* expression demonstrated a significant negative correlation between kidney and heart and a significant positive correlation between kidney and liver in LP of 3 months group whereas negative correlation between kidney and heart in addition to a significant negative correlation between liver and heart persisted in DP. In 12 months LP, a significant negative correlation between liver and heart, liver and intestine and a significant positive correlation between intestine and heart was observed. The correlation between kidney and heart was changed to positive and



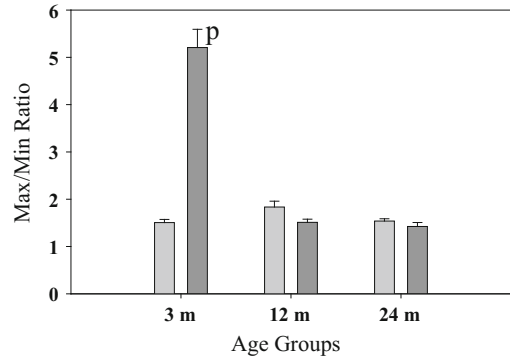
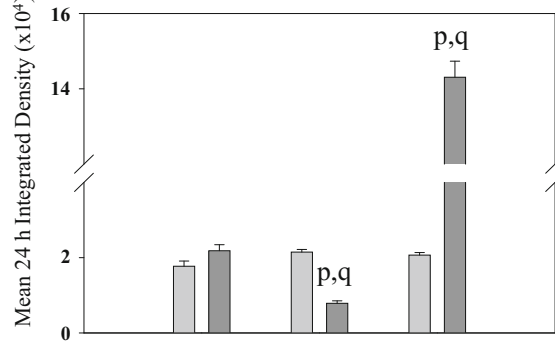
Liver



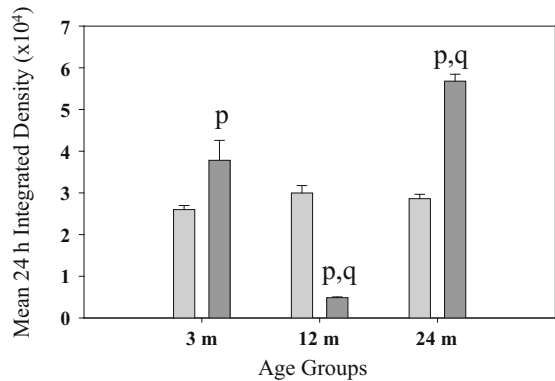
Kidney



Intestine



Heart



correlation between liver and intestine was changed to negative when compared to 3 months group. In DP the correlation between liver and intestine persisted, though negative correlation between kidney and intestine, heart and liver were changed to positive, however, the positive correlations in 3 months between kidney and liver, liver and intestine were found to be changed to negative. In 24 months LP the negative correlations between kidney and intestine, kidney and heart, heart and liver were changed to positive correlations compared to 3 months. The positive correlation between the intestine and liver in 3 months was changed to negative whereas in DP a significant positive correlation was observed between kidney and heart, heart and liver whereas, these correlations were negative in 3 months group. A significant negative correlation was observed between liver and intestine, intestine and heart though These correlations were positive in 3 months group (Fig. 4).

Discussion

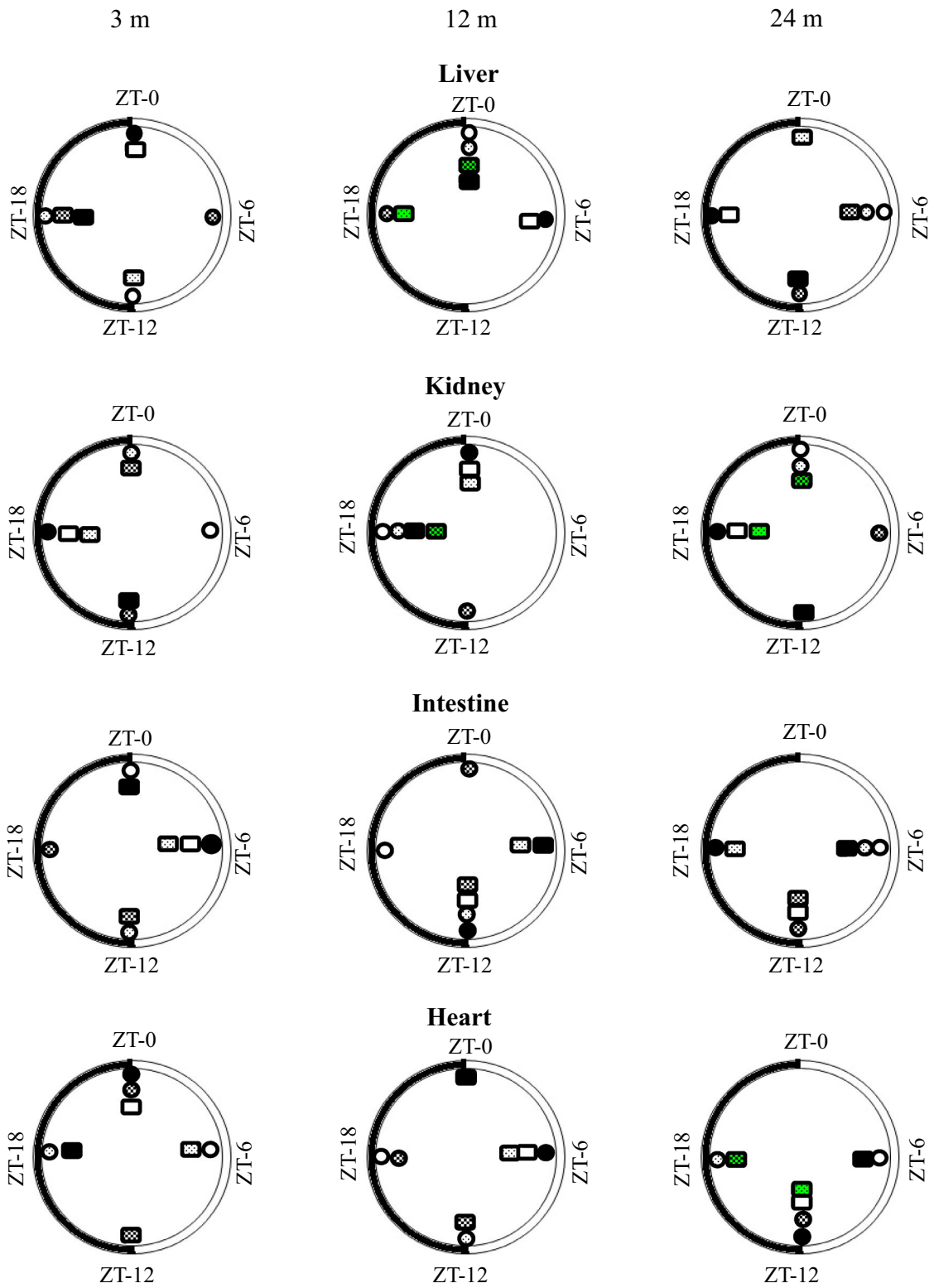
Several physiological systems including the circadian system and cellular defenses compromise in their functions during aging (Gibson et al. 2009; Jagota 2005, 2012). There are several reports which denote an increased inflammatory molecules in aging. The immune system and circadian systems were reported to regulate in a bidirectional fashion where one system modulates the other. The immune mediators primarily the cytokines were extensively studied with respect to their circadian activities and age induced activities (Cermakian et al. 2013; Mate et al. 2014; Altara et al. 2015). The increased IL-6, TNF- α , IFN, etc. were shown to affect the photic responses in SCN (Cayetanot et al. 2009; Oxenkrug 2011; Paladino et al. 2014; Trufakin and Shurlygina, 2016). However the information available on the regulators of the cytokine signaling with aging and circadian perspective is obscure.

The *rSocs1* expression was rhythmic in all the peripheral clocks studied. The maximum *rSocs1* expression during the day time was observed in heart, kidney and intestine. The *rSocs1* expression obtained in the present study could be phase delayed when compared to cytokine expressions the maximal gene expression were reported during the mid/late dark

Fig. 3 Effect of melatonin administration on *rSocs1* maximum and minimum levels, onset and offset in various tissues. open circle and filled circle indicates the maximum and minimal expression respectively in vehicle treated animals whereas the representation in melatonin treated animals are circles with squares and circles with dots. The onset and offset in vehicle treated animals were represented by open rectangle and filled rectangle respectively whereas in melatonin treated animals they are represented by rectangle with squares and rectangle with dots. Additionally, the green coloured icons indicate the restoration with melatonin treatment. (Color figure online)

phase (González-Hernández et al. 2006; Voderholzer et al. 2012). The diurnal pattern of *rSocs1* indicates that the expression of *rSocs1* follows the cytokines expression. In liver the *rSocs1* pattern was similar to that of the antioxidant enzyme Glutathione peroxidase (GPx) activity (Manikonda and Jagota 2012). The concurrence of daily rhythms of GPx and *rSocs1* in liver may indicate an intricate network of antioxidative and anti-inflammatory systems functioning together to maintain the cellular homeostasis which gets affected with aging. Interestingly, with respect to NO rhythms studied earlier (Vinod and Jagota 2016) the antiphase expression of *rSocs1* and GPx in liver reiterates the interplay between the pro-inflammatory and the anti-inflammatory systems. The daily onset and offset in 3 months group indicates that the immune rhythms in hepatic and cardiac systems could be closer whereas the renal and bowel systems differ in these rhythms. The mean 24 h *rSocs1* levels decreased with aging in both kidney and liver. This is in agreement with the previous studies where the deletion of *Socs1* gene was reported to result in reduction of survivability (Tortorella et al. 2006).

In middle age group *rSocs1* showed a phase advance of 12 h in liver and a delay of 12 h in kidney and heart whereas intestine displayed abolished rhythms. This sensitivity of intestine with aging in the present study is in corroboration with earlier studies where gut homeostasis and the microbiota synchrony seems to get affected as early as in middle age resulting in several gut disorders (D'Souza 2007; Arboleya et al. 2016; Bischoff 2016). The amplitude of *rSocs1* rhythms was reduced in all the peripheral clocks studied except heart in 24 months group. Decreased daily pulse and mean *rSocs1* levels observed in liver in present study as well as decreased antioxidant enzymes activities in liver reported earlier (Manikonda and Jagota 2012) may lead to age related



immune pathologies (Indo et al. 2015; Zhang et al. 2015).

Though the circadian regulation of *rSocs1* has not yet been elucidated, it is noteworthy to observe in 3 months heart and kidney *rSocs1* demonstrated similar pattern of expression as that of *rPer1* in SCN and in liver to that of *rPer2* in SCN (Mattam and Jagota 2014). This could be because of the presence of cAMP-response Element (CRE) in the *Socs1* promoter (Impey et al. 2004) similar to *Period* genes (Travnickova-Bendova et al. 2002). Interestingly, the *Socs1* expression pattern in liver was similar to that of SCN (Sadki et al. 2007). Further investigations could elucidate the possible role of circadian regulation of *Socs1* expression.

The correlation analysis reveals that the heart and liver were always positively correlated in both LP and DP in all the age groups. In the kidney and liver their negative correlation persisted in both DP and LP up to middle age. Thus, the metabolic activities and the circulatory activities could be directly proportional to immune defenses whereas the excretory system could be inversely regulated to hepatic activities.

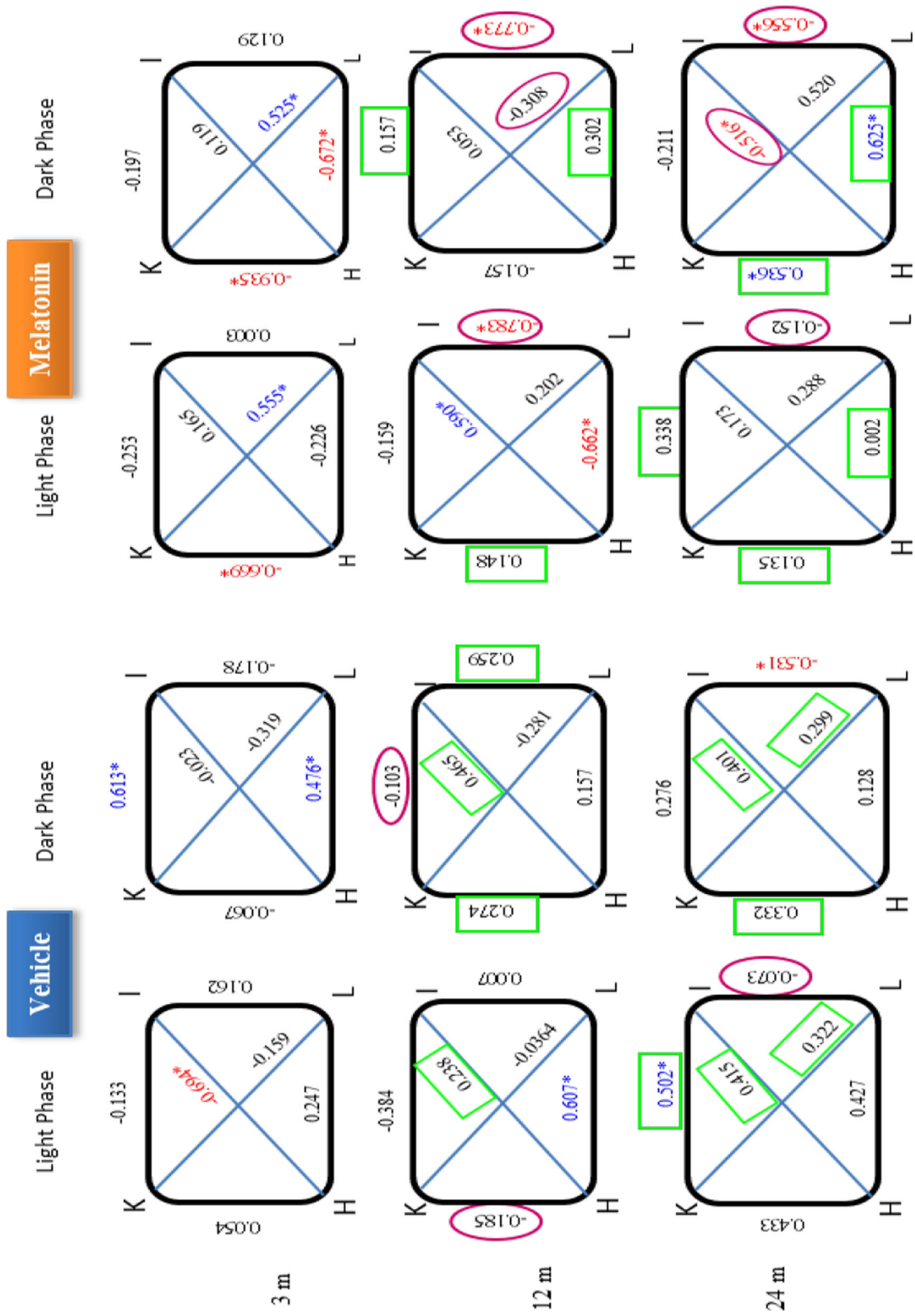
Melatonin has several abilities such as modulation of circadian rhythms, regulation of cytokines, free-radical scavenging activity etc. Due to its decreased availability with aging most of its downstream processes are affected leading to increased risk of several age related disorders (Jagota 2012; Reiter et al. 2014). Exogenous melatonin had been reported to be beneficial in maintaining a healthy living even at old age resulting in longevity by many researchers (Hardeland 2012; Ramis et al. 2015; Gubin et al. 2016; Mukda et al. 2016). In the present study melatonin treatment partially restored the phase of *rSocs1* in both 12 and 24 months liver, in 24 months kidney, intestine and heart. These results are similar to that of our earlier findings with respect to antioxidant enzymes in liver and clock gene *rPer2* in SCN (Manikonda and Jagota 2012; Mattam and Jagota 2014). The restoration of daily pulse of *rSocs1* with melatonin treatment in liver was similar to that of NO (Vinod and Jagota 2016). Increased *rSocs1* expression in 24 months group was measured in intestine and heart. The similar effect of melatonin was observed in previous studies that showed increased expression of *Socs3* in traumatic brain injury (Tsai et al. 2011) and

Fig. 4 Effect of melatonin administration on pair wise correlation between mean light (ZT-0, 6, 12) and mean dark (ZT- 12, 18, 24/0) phase values of *rSocs1* in liver (L), kidney (K), intestine (I) and heart (H) in various age groups of male Wistar rats. Asterisk indicates the statistically significant values between groups. Values in blue colour indicates significant positive correlation and red colour indicates significant negative correlation. Green rectangle indicates negative to positive conversion of a correlation and magenta oval indicates positive to negative conversion of a correlation. (Color figure online)

elevated NO levels in intestine (Vinod and Jagota 2016). Decreased induction of cytokines with melatonin treatment (Choi et al. 2011; Qin et al. 2012; Shin et al. 2014), can be correlated to restoration of *rSocs1* levels in liver and kidney.

With melatonin treatment the correlation between the kidney and liver in 12 months DP was almost similar to that of the correlation observed in DP of 3 months vehicle control group. A partial restoration in correlation was observed between kidney and heart, heart and intestine in LP of 24 months group when compared to that of 3 months vehicle control group. Due to aging we have observed an imbalance in stoichiometry between the tissues for *rSocs1* expression. The correlations between heart and liver, heart and intestine were partially restored with melatonin administration.

In conclusion, we here report that the *rSocs1* expression was rhythmic in adult in all the peripheral clocks studied in male Wistar rats. The *rSocs1* daily rhythms, phase, amplitude and pulse altered differentially with age. Intestine was the most sensitive organ to aging where the rhythms were abolished as early as in middle age. Highest *rSocs1* levels were found in kidney. The daily onset and offset of the *rSocs1* rhythms were differentially altered in the tissues with aging. Thus demonstrating disintegration in the correlation between these stoichiometric interactions. The onset and offset of the *rSocs1* rhythms were partially restored with melatonin treatment in kidney, intestine and heart. The correlation analysis demonstrated that melatonin treatment was effective in differentially restoring the *rSocs1* stoichiometry between the tissues studied. The sensitivity of 24 months old animals observed in present study to melatonin is a step towards endorsing melatonin as a promising and an important anti-aging therapeutic drug.



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