Functional changes of the SCN in spontaneous hypertension but not after the induction of hypertension

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Running Head: The SCN, activity and hypertension

Abstract

The present study investigated the circadian behavior of Spontaneously Hypertensive Rats (SHR) during the pre-hypertensive and hypertensive stage, with the aim to gain insight whether observed **changes in the functionality of SCN** in the hypertensive state are cause or consequence of hypertension. Four types of animals were used in this study: 1) SHRs which develop hypertension genetically; 2) their normotensive controls, Wistar Kyoto rats (WKYs); 3) Wistar rats whereby hypertension was **surgically- induced (2 Kidney 1 Clamp-2K1C-method)** and 4) Sham operated control Wistar rats. Period length and activity levels and amplitude changes of locomotor and wheel running activity were determined, in constant conditions, as a measure of the functionality of the suprachiasmatic nucleus (SCN). Hereto two conditions were used, constant darkness (0 lux) and constant dim (5 lux) light. SHRs showed a shortened period of their locomotor and running wheel activity rhythms in constant darkness during both pre-hypertensive and hypertensive stages and exhibited period lengthening in constant dim light conditions, only during hypertensive stages. **Total amount as well as the amplitude of daily running wheel rhythms showed an inverse correlation with the period length and this relation was significantly different in SHRs compared to WKYs.** None of the aforementioned changes in circadian rhythms were observed after the surgical induction of hypertension. The present findings suggest early functional changes of the SCN in the etiology of spontaneous hypertension.

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3 1 1. INTRODUCTION
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7 3 The mammalian circadian timing system comprises a central and multiple peripheral clocks
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9 4 (Buijs & Kalsbeek, 2001). The central clock of this timing system is located in the
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11 5 suprachiasmatic nucleus (SCN) in the anterior hypothalamus and the peripheral clocks are
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13 6 located in other brain regions and bodily organs (Klein et al., 1993; Moore, 2013). Both
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15 7 central and peripheral clocks run with a molecular transcriptional-translational feedback
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17 8 loop defined by an interplay between heterodimers of the canonical clock genes PER/CRY
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19 9 and CLOCK/BMAL (Gustafson & Partch, 2015; Okamura et al., 2010; Reppert & Weaver,
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21 10 2002). **The rhythmicity of peripheral clocks is affected by SCN driven neuro-humoral-**
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23 **metabolic signals (Su et al., 2016).**
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28 12 Circadian timing system related pathologies are proposed and demonstrated to be at
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30 13 the core of a number of central nervous system and systemic disorders, such as obesity,
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32 14 metabolic syndrome, hypertension, immune system disorders, Alzheimer's disease and
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34 15 neuropsychiatric conditions (Buijs et al., 2016). Accordingly, in relation to hypertension
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36 16 disturbances have been reported both in the central and peripheral clocks (Goncharuk et al.,
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38 17 2001; Sládek et al., 2012). Blood pressure levels are shifted to higher levels without a
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40 18 nighttime fall in essential hypertension (Pickering, 1990; Verdecchia et al., 1990) and
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42 19 postmortem analyses of human SCN revealed significant changes in three major SCN
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44 20 transmitter systems in hypertensive patients (Goncharuk et al., 2001). Spontaneously
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46 21 hypertensive rats (SHRs) are extensively used as an animal model of human primary
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48 22 hypertension and they exhibit cardiovascular and neurochemical pathologies similar to
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50 23 human patients (Avidor et al., 1989; Peters et al., 1994). Moreover, when embryonic
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52 24 hypothalamic tissue containing the SCN was transplanted from SHRs to normotensive rats,
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the latter developed hypertension (Eilam et al., 1991; Stoynev et al., 1996). Besides, systolic blood pressure rhythms are inverted in aged SHR, resembling the non-dipper phenotype of essential hypertension (Minami et al., 1988; Munakata et al., 1990). Together these studies clearly suggest the involvement of the biological clock in the etiology of hypertension.

Pathology associated with hypertension has also been studied in several hypertension models aiming at altering kidney function by modulating the renin-angiotensin system (RAS). Interestingly several of these models also have disturbed circadian functions. Transgenic hypertensive TGR(mREN2)27 rats (TGR), carrying an additional mouse renin gene in their genome, develop hypertension with inverted blood pressure rhythms peaking during the animals' inactive period and have abnormalities in their circadian system and peripheral organ functions (Dzibríková et al., 2011; Lemmer et al., 2003). Other models include salt loading and clamping arterial blood supply to the kidney. In all these models RAS is targeted, however, both central and peripheral nervous system changes are reported (Azar et al., 1981; Katholi et al., 1982; **Lemmer et al., 2000a; Lemmer et al., 2000b**; Pijacka et al., 2016).

In relation to clock genes and peripherally induced hypertension; Cry1, 2 and Per1 knock out animals developed salt-dependent hypertension (Doi, 2012; Solocinski et al., 2016). In DAHL hypertensive rats, Per2, Bmal1 and Dbp amplitudes are attenuated in peripheral tissues including kidney (Mohri et al., 2003). In experimental models of aortic constriction, Per2 and Bmal1 expression were altered in the SCN, as well as in the aorta and heart (Martino et al., 2007). In addition a BMAL1 polymorphism was identified in SHR, and BMAL1 haplotypes were associated with type 2 diabetes and hypertension in human subjects (Woon et al., 2007). Deletion of Bmal1 from smooth muscle decreased blood pressure levels without affecting SCN controlled locomotor activity (Xie et al., 2015) and contributed to disruption of blood pressure circadian rhythms in db/db mice (Su et al., 2012).

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Moreover, in a recent study, the canonical clock genes NPAS2, PER2, ROR $\beta\beta$, BMAL1 and ROR α were associated with non-dipper phenotype of young onset hypertension in humans (Leu et al., 2014).

In spite of all the studies indicating the disturbance of rhythms or the involvement of clock genes, we still do not know whether these hypertension associated changes are mediated by the SCN, or not, since in most of these studies, SCN changes were not evaluated (Shimba et al., 2011; Xie et al., 2015). **However other studies show that in transgenic hypertensive animals with an additional renin gene, in spite of their reversed rhythm indicating the involvement of the SCN, an SCN lesion does not remove the already developed hypertension (Witte et al 1998). All these studies indicating the involvement of the SCN in the etiology of hypertension stimulated us to investigate whether the observed circadian disturbances and changes in the SCN could be the cause or consequence of hypertension.** To examine this question we conducted experiments with both SHR and the 2 kidney, one clamp (2K1C) reno-vascular hypertensive rats (Okamura et al., 1986). Here, the SHR represents a centrally mediated, top-down model of hypertension and the 2K1C-induced hypertensive rats; a peripherally induced, bottom-up model of hypertension. As functional output of the SCN, we evaluated circadian phase, **total amount** and amplitude of locomotor activity and running wheel rhythms of animals before, and after the establishment of hypertension, under both DD (0 lux) and dim light (LL) 5 lux conditions. **At the end of, both the pre-hypertensive and the hypertensive stage, experiments blood pressure and heart rate levels were measured.**

2. Materials and Methods

Animals

Experiments were conducted with a total of 36 male SHR and WKY rats (bred from parents purchased from Harlan, UK). Additionally, 8 Wistar Albino rats (Harlan, Zeist, The Netherlands) were used for the induction of hypertension experiments. All SHR and WKY rats participating in the experiments were bred in our own animal facility in order to avoid possible interference of stressors (i.e., transportation, changes in housing conditions, etc.) in the development of hypertension (Tucker & Johnson, 1981). Before the experiments, animals were housed in groups, under a 12:12h light: dark (LD) cycle (± 100 and 5 lux, respectively; lights on 07:00 h), in a temperature controlled environment ($21 \pm 2^\circ\text{C}$) with *ad libitum* access to tap water and food (Rat Chow). Animals were separated from their mothers after weaning at the age of 4 weeks. During the experiments the animals were housed individually in sound and light isolated so-called “circadian cages” (38x38x39 cm) where all behavioral rhythm measurements were done. In these “circadian cages”, rats had free access to a running wheel, food and water and were handled twice a week. **Locomotor activity was monitored by counting the interruptions of two overlapping infrared beams in the ceiling of the cage. Running wheel activity was monitored in a 34.5 cm running wheel (eight pulses per rotation) (Techniplast, Buguggiate, Italy). The “circadian cages” were designed and made in Netherlands Institute for Neuroscience, and are connected with an on-line computer system collecting automatically all behaviors at a frequency of 1 registration per 6 minutes (CIRMIC developed by Wil Witting) (Palm, 2001).**

At the end of the experiments blood pressure and heart rate levels of animals were measured by the intra-carotid catheterization method and animals were sacrificed. All

1 experiments were conducted under the approval of the ethical committee of the Royal
2 Netherlands Academy of Art and Sciences (KNAW) and the Netherlands Institute for
3 Neuroscience (NIN). Two sets of experiments were conducted to test the functionality of the
4 SCN.

5 *First set of experiments:*

6 First, 4 weeks old male SHRs (n=9) and their normotensive controls, male WKYs (n=9), were
7 used, in order to evaluate endogenous rhythmicity of the SCN during the pre-hypertensive
8 stage (4-10 weeks). Secondly 9 male SHR and 9 male WKY rats at the age of 7-13 weeks
9 (hypertensive stage) were kept in the “circadian cages”, under the same conditions as the
10 pre-hypertensive group, to test the circadian rhythmicity when animals were developing and
11 establishing hypertension. Locomotor and running wheel activity rhythms of animals were
12 registered during the 10-day periods of constant darkness (DD; 0 lux) and subsequent 10-
13 days of constant very low dim light conditions (dim light LL; 5 lux) in two repeating sessions
14 (Fig. 1a and 1b).

15 *Second set of experiments:*

16 In the second set of experiments, 8 male Wistar albino rats (7 weeks of age) were placed in
17 the “circadian cages” seven days before the surgery to induce hypertension with the 2
18 Kidney 1 Clamp method (2K1C) (see *Surgery* below) and housed in similar conditions as
19 during the first set of experiments. After surgery, animals were placed in constant darkness
20 (DD; 0 lux) for 15 days (this includes one week recovery from surgery and another 7 days for
21 circadian rhythm analyses), subsequently in constant very dim light (LLdim; 5 lux) for 10 days
22 and finally in constant darkness (DD2) again for 10 days (Fig. 1c).

1 *Surgery (2K1C- Goldblatt Hypertension)*

2 A total of eight animals were operated under Hypnorm (Janssen, High Wycombe,
3 Buckinghamshire, UK; 0.05ml/100g BW) and Dormicum (Roche, Almere, The Netherlands;
4 0.04ml/100g BW) anaesthesia in order to induce hypertension (n=5) with the modified
5 version of 2K1C hypertension (Okamura et al., 1986) and for sham surgery (n=3). Laparotomy
6 was performed to expose the abdominal aorta by a longitudinal incision on the abdominal
7 midline, the aorta between the renal arteries was lifted to put a surgical thread (4-0 Perma-
8 Hand Said Ethicon) underneath, after separating visible nerve fibers travelling with the aorta.
9 The aorta was tied together with a blunted injection needle (25 gauge, 1^{1/4} inch needle) on
10 top of it by the aid of surgical thread such that after removing the needle the aorta was
11 narrowed to the diameter of the needle. The abdominal wall muscles and the skin were
12 sutured separately. Sham operated animals underwent the same procedure without
13 narrowing the aorta. Animals recovered overnight and were given Temgesic (Schering-
14 Plough, Utrecht, The Netherlands, 0.01ml/100g BW, sc) to reduce post-operative pain.

15 *Blood Pressure Measurements and Data Analyses*

16 Blood pressure measurements were performed using an intra-carotid catheter in the first
17 half of the animals' inactive period (between circadian time (CT) 2 and 6) at the age of 10
18 weeks for the pre-hypertensive group and at the age of 13 weeks for the hypertensive group
19 and 13.5 weeks for 2K1C animals and their sham-operated controls. For this procedure
20 animals were anesthetized with isoflurane (Isoflurane; Pharmachemie BV, Haarlem, The
21 Netherlands, 3.5% oxygen) and consequently by sodium pentobarbital (Nembutal, Ceva
22 Sante Animal B.V, Maassluis, The Netherlands, 0.06 ml/100g BW) in order to stabilize blood
23 pressure and heart rate levels within its circadian variations. Blood pressure and heart rate

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1 were measured and recorded for ten minutes via intra-carotid catheter connected to a
2 computer using HDAS (Hemodynamic Data Analyses Software- Maastricht University).
3 Consequently, after the blood pressure and heart rate measurements, animals were
4 sacrificed by overdose of Nembutal.

5 All behavioral data were depicted in double-plotted actograms . Four sessions of 10 days,
6 from DD1 to LL2, were analyzed with computer based systems for precise period estimates
7 (TAU, Minimitter Co, Inc. Oregon, USA) and measuring the amount of behavior (CIRMIC
8 ANALYSE, Wil Witting) with Chi-squared method. In every session, only the last seven days
9 of measurements were taken into account for evaluation in order to avoid the effect of the
10 transition period.

11 Total amount of activity was calculated by adding all activity bins over last 7 circadian
12 cycles of a total period of 10 days that the animals kept under alternating constant
13 conditions. Total amount of locomotor activity is expressed as total amount of infrared
14 interruptions, and total amount of running wheel is expressed as total amount of wheel
15 rotations.

16 The amplitude of the locomotor and running-wheel activities was analysed by Actiwatch
17 and Sleep 5 software (Cambridge Neurotechnology, Cambrdige, UK) using cosinor method
18 (Van Someren et al., 1999; Van Someren, 2007) and logged at 6-min intervals.

19 *Statistics*

20 Statistical analyses were done using *one way ANOVA* when two groups were compared
21 and a *repeated measures ANOVA* was performed with DD1, LL1, DD2 and LL2 as the within
22 subject factor (light), and with groups as SHR vs WKY or IndH vs shm as between subjects
23 factor (group). In this way, effects of light and group were examined in an overall test.

If the p value detected less than 0.05, changes were considered significant. All data presented as mean $\pm$ S.E.M.

Results

Free-running circadian locomotor and wheel running activities were measured in four groups of rats by means of period (τ) and magnitude as well as the amplitude changes. In the “circadian cages”, SHR and WKY animals were exposed to two alternating sessions of constant darkness (0 lux; DD1 and DD2) and constant (dim-5 lux) light (LLdim1 and LLdim2) (Fig.1a and 1b). In the group of animals with induced hypertension (2K1C) and sham operations after the pre-operative DD (DD0), only DD1, LL1 and DD2 sessions were included in the analysis (Figure 1c).

Figure 2 and supplementary Table 1 indicate the actual values of period (τ) changes of locomotor and wheel running rhythms throughout the experiments. In the first alternating sessions of constant darkness (DD1) and constant dim light (LL1), there was no significant period difference between pre-hypertensive SHRs and WKYs for either locomotor or running wheel activities ($p=1.000$; $n=9$). However, differences in periods became apparent in DD2, where the SHRs had significantly shorter periods than WKYs for both type of activities (locomotor activity; 23.68 ± 0.04 h vs 24.02 ± 0.06 h; $p<0.001$; $n=9$ and wheel running activity; 23.71 ± 0.05 h vs 24.07 ± 0.15 h; $p=0.04$; $n=9$). Although periods became longer, no difference was observed between groups during LL2 ($p>0.05$). On the other hand, a repeated measures of ANOVA showed that periods differed significantly between constant conditions ($F(3,48)=112.010$, $P<0.001$). Post hoc tests using the Bonferroni correction revealed a significant shortening in periods for DD2 compared to DD1 sessions of locomotor activity only in SHRs (DD1; 24.1 ± 0.078 vs DD2; 23.68 ± 0.056 , $p=0.015$).

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Representative actograms of SHR and WKY animals during the pre- and hypertensive period are shown in Figure 4a and 4b.

During the hypertensive state, also no significant differences in periods for locomotor and running wheel activities were observed between SHRs and WKYs at DD1 and LL1 ($p > 0.05$). However, the periods became significantly shorter in DD2 (for locomotor activity, $P=0.002$, $n=9$; for running wheel activity, $p<0.001$, $n=9$) and subsequently longer in LL2 (for locomotor activity, $p=0.002$; for running wheel activity, $p=0.004$) sessions in SHRs compared to WKYs (Figure 2; for the actual values see supplementary Table 1). **A repeated measures of ANOVA revealed a significant light and group effect on period length for locomotor activity ($F(3,48)= 9.936$, $p<0.001$) and running wheel activity ($F(3,48)= 9.162$, $p<0.001$). With post hoc tests using the Bonferroni correction significant differences between DD1 and DD2 in SHRs for locomotor (DD1; 24.033 ± 0.074 vs DD2; 23.678 ± 0.062 $P=0.005$) and running wheel activity periods (DD1; 24.022 ± 0.098 vs DD2; 23.667 ± 0.054 $P=0.043$) was detected, with periods in DD2 being shorter. However, no significant differences on periods were observed in hypertensive SHRs between the LL1 and LL2 sessions for both types of activities (locomotor activity; $P=0.540$. running wheel activity; $P=0.128$). In WKYs, no period differences was observed between DD1 and DD2 (locomotor activity; $P=0.568$. running wheel activity; $P=0.171$) as well as between LL1 and LL2 for both types of activities (locomotor activity; $P=1.000$, running wheel activity; $P=1.000$).**

During the hypertensive stage, animals started experiments at the age of 7 weeks (DD1) and the first light session (LL1) was ended at the age of 10 weeks, this time period coincided exactly with the second alternating sessions of constant darkness and constant light (DD2 and LL2) in the pre-hypertensive animals (7 to 10 weeks, Figure 1b). Although (as expected)

no significant differences were observed in period length between LL2 of pre-hypertensive and LL1 of hypertensive stages of SHR, period length of locomotor activity in DD2 (23.69 ± 0.04 h) in the pre-hypertensive group was significantly shorter than period length in DD1 (24.03 ± 0.06 h) in the SHR hypertensive group ($p < 0.001$). This shortening of period length of running wheel activity in DD2 (23.71 ± 0.05 h) during the pre-hypertensive stage was also evident compared to DD1 (24.02 ± 0.10 h) in the hypertensive SHR ($p = 0.022$, $n = 9$). In WKYs such changes were not observed between age of 7 and 10 weeks.

Using similar analysis as above, no significant differences in period length during DD1, LL1 and DD2 sessions ($p > 0.05$ for all three) could be detected between the animals where hypertension was induced (2K1C) and their sham operated controls (Figure 2, for the actual values see the supplementary material, Table 1).

Similar to period length no significant changes were observed in level (or total amount) of locomotor activity rhythms of SHR in comparison to WKYs during the pre- hypertensive stage, with the exception of a prominent increase in the total amount of the running wheel activity rhythm in SHR during the DD2 period (134.64 ± 9.7 vs 72.72 ± 19.29 ; $p = 0.011$, $n = 9$). In the hypertensive stage, significant changes in total amount of activity were observed in locomotor activity rhythms in both LL1 (16.53 ± 1.15 vs 12.56 ± 1.36 ; $P = 0.045$, $n = 9$) and LL2 (16.20 ± 1.95 vs 10.95 ± 0.97 ; $P = 0.025$, $n = 9$) sessions. The running wheel activity rhythm during the hypertensive state showed a higher level of activity during DD2 (175.26 ± 16.05 vs 96.82 ± 28.36 ; $P = 0.029$) and LL2 (89.19 ± 20.58 vs 30.99 ± 10.85 ; $P = 0.024$) (Figure 3a-3d and supplementary material Table 2) in SHR. Interestingly, in the SHR, the increase of activity coincided with shorter circadian periods in DD while conversely, when the periods got longer in the LL sessions, the activity levels were relatively decreased, in agreement with literature

1 (Aschoff & Pohl, 1978; Yamada et al., 1988). **Repeated Measures of ANOVA revealed a**
2 **significant light and group effect on period ($F(3,48)= 3.519$, $P=0.022$) and total amount**
3 **($F(3,48)=5.587$ $P=0.002$) of Running Wheel activity. The inverse relation between period**
4 **and total amount of activity was evident after Post hoc tests using the Bonferroni**
5 **correction and significantly different in SHR_s especially between DD2 and LL2 (periods,**
6 **DD2 vs LL2; 23.71 ± 0.05 vs 25.56 ± 0.16 , $P<0.001$; activity level, DD2 vs LL2; 134.6 ± 9.72 vs**
7 **80.41 ± 15.65 ; $P=0.008$) of wheel-running activity in the pre-hypertensive stage.** None of
8 these differences concerning the response to light were present in WKYs, neither in
9 locomotor nor in running wheel activities (in each case; $P>0.05$) at the same age period with
10 pre-hypertensive SHR_s. **Repeated Measures of ANOVA revealed a no significant light and**
11 **group (light*group) effect on total amount of ($F(3,48)= 1.209$, $P=0.317$) of locomotor and**
12 **running wheel activity ($F(3,48)= 0.660$, $P=0.581$). Therefore a similar pattern, meaning an**
13 **inverse relation between activity levels and period changes, was visible at DD2 and LL2**
14 **sessions of locomotor (periods, DD2 vs LL2; 23.678 ± 0.062 vs 25.367 ± 0.078 , $P<0.001$;**
15 **activity levels, DD2 vs LL2; 20.819 ± 1.985 vs 16.204 ± 1.533 ; $P=0.005$) and wheel-running**
16 **activities (periods, DD2 vs LL2; 23.667 ± 0.054 vs 25.467 ± 0.094 , $P<0.001$; activity levels, DD2**
17 **vs LL2; 175.267 ± 23.063 vs 89.198 ± 16.454 ; $P=0.001$) for each group, but highly significant in**
18 **SHR_s (values above) during the hypertensive stage.** The SHR_s' total amount of running
19 wheel activities in DD2 at pre-hypertensive stage (134.64 ± 9.72) was not significantly
20 different ($p=0.073$) from the total amount of activity in DD1 during the hypertensive stage
21 (91.03 ± 20.54), nor between LL2 and LL1 ($p=0.960$). Additionally, there wasn't any significant
22 differences for activity levels for running wheel activity in any session in the WKYs. On the
23 other hand, in SHR_s activity levels were higher during the hypertensive stage as compared
24 to activity levels during the pre-hypertensive stages for running wheel activity in DD1 ($91,04$

1 $\pm 20,54$ vs $46,39 \pm 3,699$, $p=0.048$), LL1 ($81,77 \pm 20,39$ vs $29,06 \pm 6,19$, $p=0.025$), DD2 ($175,3 \pm$
 2 $16,05$ vs $134,6 \pm 9,72$, $p=0.046$) and LL2 ($89,20 \pm 20,58$ vs $80,46 \pm 15,65$, $p=0.740$) sessions.
 3 Interestingly no significant changes were observed in the levels of these rhythms in animals
 4 with induced hypertension (2K1C) or their sham operated controls (Figure 3e and 3f, for
 5 detailed information see supplementary material, Table 2).

6 **In the case of amplitude changes significant differences were observed between SHR and**
 7 **WKYs at LL1 ($P=0.032$, $n=3$), DD2 ($P=0.034$, $n=3$) and LL2 ($P=0.010$, $n=3$) sessions for**
 8 **locomotor activity and at DD2 session for running wheel activity ($P=0.044$, $n=3$) at pre-**
 9 **hypertensive stage (Figure 4a and 4b). In a similar manner with changes in the total**
 10 **amount of activity at the hypertensive stage, amplitude of running wheel activity was**
 11 **significantly different in SHR compared to WKY at DD2 ($P=0.002$, $n=5$) and LL2 ($P=0.008$,**
 12 **$n=5$) sessions at hypertensive stage (Figure 4d). In addition to this, repeated measures of**
 13 **ANOVA revealed a significant light and group effect on period ($F(3,48)= 3.519$, $P=0.022$)**
 14 **and amplitude ($F(3,12)=5.172$ $P=0.016$) of running wheel activity. Post hoc tests using**
 15 **Bonferroni correction indicated a light responsivity in amplitude and this was significantly**
 16 **evident in running wheel activity between LL1 and DD2 sessions at pre-hypertensive stage**
 17 **(periods, LL1 vs DD2; 23.71 ± 0.116 vs 25.55 ± 0.161 , $P<0.001$; amplitude levels, LL1 vs DD2;**
 18 **603.667 ± 247.640 vs 2641.333 ± 432.986 ; $p=0.011$). Similarly, there was light and group**
 19 **effect on period ($F(3,48)= 9.162$, $P<0.001$) and amplitude ($F(3,21)= 9.599$, $P<0.001$) of**
 20 **running wheel activity at hypertensive stage. After post hoc tests using Bonferroni**
 21 **correction, there was a difference between LL1 and DD2 (periods, LL1 vs DD2;**
 22 **25.233 ± 0.099 vs 23.667 ± 0.054 , $P<0.001$; amplitude levels, DD2 vs LL2; 2054.000 ± 468.954 vs**
 23 **4935.000 ± 486.212 ; $P<0.001$) as well as DD2 and LL2 (periods, DD2 vs LL2; 23.667 ± 0.054 vs**
 24 **25.467 ± 0.094 , $P<0.001$; amplitude levels, DD2 vs LL2; 4935.000 ± 486.212 vs**

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3 1 2653.200±357.375; P=0.002) sessions at hypertensive stage. A within-group analyses on
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5 2 amplitude revealed significant differences between DD1 and DD2 in SHRs for running
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7 3 wheel activity both at pre-hypertensive (DD1 vs DD2; 751.667±270.397 vs
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10 4 2641.333±432.986; P=0.042) and hypertensive stages (DD1 vs DD2; 1700.800±257.627 vs
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12 5 4935.000±486.212; P=0.001). However, no significant difference (p>0.05) was observed for
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14 6 any type of activity in hypertensive SHRs between the LL1 and LL2 sessions throughout the
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16 7 experiments. In WKYs, there was no significant difference (p>0.05) between DD1 and DD2
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18 8 as well as LL1 and LL2 session neither for locomotor nor for the running wheel activity in
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20 9 any stage of the experiments.

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24 10 Blood pressure and heart rate levels of the animals were measured at the end of the
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26 11 experiments. At the end of the pre-hypertensive stage (at 10 weeks of age) no significant
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28 12 changes in any of the cardiovascular parameters were observed in SHRs compared to WKYs.
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31 13 However, at the beginning of the hypertensive stage (at 13 weeks of age) except heart rate
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33 14 all three parameters namely, systolic, diastolic and mean arterial blood pressure levels in
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35 15 SHRs were significantly higher (p< 0.05, individual examples represented in Figure 4c and
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37 16 overall results in Table 1) compared to WKYs. Measurements in a separate group of 4
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39 17 animals at the age of 11.5 weeks revealed that the SHRs establish the hypertension around
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41 18 that age with a MAP >140 mmHg for all 4 animals (data not shown). In the animals with
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43 19 induced hypertension the systolic blood pressure level was 167 mmHg and significantly
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45 20 higher (p= 0.037) than in sham operated controls (122 mmHg, Table 1).

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50 21 **DISCUSSION**

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53 22 The present study demonstrates that changes in the functionality of the circadian time
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55 23 keeping system, as reflected by changes in locomotor and wheel running activity precede the

1 development of spontaneous hypertension. In addition since SHR show changes in the
2 period length of their free-running locomotor and running wheel activity rhythms before and
3 after the development of hypertension the present study also suggests changes in the SCN
4 before animals develop hypertension. Moreover since animals with induced hypertension do
5 not show such circadian alterations, the present data strongly support a change in the
6 circadian system already before the animals develop spontaneous hypertension.

7 **Circadian system changes in SHRs but not in 2K1C hypertension rats**

8 In the current study, we used circadian locomotor and wheel running activities as measures
9 of changes in the global functionality of the SCN (Acosta-Galvan et al., 2011; Buijs et al.,
10 2017; Buijs, 1996). **In man and in SHRs, in addition to neural (Mann et al., 1983) and**
11 **humoral (Imai et al., 1993) mechanisms, locomotor activity is one of the main factors**
12 **influencing or associated with variations in blood pressure (Rowlands et al., 1980; Zaretsky**
13 **et al., 2016). The exception seems to the TGR animals, here adult animals have a motor**
14 **activity peaking in the dark period while blood pressure peaked in the light (inactive**
15 **phase) (Lemmer et al., 1993). Moreover, in TGR in the pre-hypertensive period blood**
16 **pressure was in phase with the rhythm in locomotor activity (Witte & Lemmer, 1999) why**
17 **the expression of an additional renin gene gives this disturbance is not clear yet.** Although
18 several studies on changes in blood pressure rhythms in the course of development of
19 hypertension have been published (Minami et al., 1988; Morris & Keller, 1982; Munakata et
20 al., 1990; Wijnen et al., 1980), none of them explored rhythmic changes of (locomotor)
21 activity patterns during development of hypertension. Until now, the only available data
22 show alterations in circadian rhythms, obtained from SHRs which had already established
23 hypertension (Peters et al., 1994; Rosenwasser et al., 1996). The present study provides

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3 1 several indications for functional changes of the SCN in SHR in the course of development of
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5 2 hypertension. Using alternating DD and LLdim sessions we revealed changes in circadian
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7 3 behavior before and after the animals established hypertension. In DD conditions SCN's
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9 4 overt rhythmicity was expressed, while the LLdim conditions revealed the effects of the
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11 5 response of the circadian system to dim light, which in general is an inhibition of locomotor
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13 6 activity. By exposing animals to alternating constant conditions in the course of the
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15 7 development of hypertension from the age of 4 weeks to 10 weeks in the pre-hypertensive
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17 8 stage and from 7 weeks to 13 weeks in the hypertensive stage, we revealed functional
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19 9 changes of the circadian system preceding and after the development of hypertension. We
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21 10 used a comparable experimental setup covering the ages of 7-13 weeks in 2K1C reno-
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23 11 vascular hypertensive rats, to test whether they exhibit similar changes as the SHR during
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25 12 the second dark sessions (DD2). Interestingly, changes in **the total amount and amplitude of**
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27 13 **activity rhythms** in SHR, reached a critical level already in DD2 at the age of 7 weeks and
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29 14 became more clear at the age of 10-13 weeks when the hypertension is established (SHR's
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31 15 MAP was above 140 mmHg at the age of 11,5 weeks, current study). The **total amount and**
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33 16 **amplitude of the activity rhythm** in DD2 during the pre-hypertensive stage tended to be
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35 17 higher than that in DD1 during the hypertensive stage. Although this difference was not
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37 18 significant it does suggest that the increase in amplitudes of activity rhythms between these
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39 19 ages in hypertensive rats could be an indicator of the future changes in blood pressure
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41 20 (Hashimoto, 1994; Minami et al., 1988; Munakata et al., 1990a).
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49 21 **There is also a difference in the activity levels and amplitude changes in response to light**
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51 22 **between the SHR and WKY animals. When in SHR the circadian periods of activity were**
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53 23 **shortened in DD, activity levels and amplitudes especially for running wheel activity**
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55 24 **increased and when periods got longer in LLdim the activity levels and the amplitudes**
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1 decreased (Aschoff & Pohl, 1978; Yamada et al., 1988). No such light response in any activity
2 rhythm was observed in WKYs at the early age except in DD2 for wheel running. At the age
3 of 10-13 weeks the difference in the light responsive pattern was evident and the activity
4 levels and amplitudes of running wheel rhythms were significantly higher in DD2 and LLdim2
5 sessions in SHR compared to WKY at this age. This observation agrees with previous studies
6 whereby shortening of the free-running period in SHRs was associated with an increased
7 activity level (Peters et al., 1994; Rosenwasser & Plante, 1993), although it is not known how
8 exactly the level of activity and the amplitude of the activity rhythm are related (Berger et
9 al., 2007; Edgar et al., 1991). Similarly, in TGR transgenic hypertensive rats, when blood
10 pressure levels were increased, a decrease in the period length of the blood pressure rhythm
11 was observed, preceding the overall increase in amplitudes in circadian rhythms including
12 activity (Canal-Corretger et al., 2001b). That hypertension per se does not increase the levels
13 of the locomotor activity rhythm can be concluded from our results with the induced
14 hypertension, in these animals in contrast to the SHRs a decrease in the total amount of
15 running wheel activity rhythm was observed as compared to the sham animals in DD
16 conditions. Therefore our experimental results, might have clinical significance by indicating
17 that changes in locomotor activity rhythms preceding the development of hypertension
18 indicate changes in the SCN.

20 Altered pre-hypertensive period length, indicates early SCN changes in SHRs

21 SHRs entrain to LD cycle normally, although they start activity earlier than WKYs and
22 before the dark onset (Yilmaz et al., unpublished observations; Peters et al., 1994). In
23 current study, activity rhythms became significantly longer during LL2 in SHRs during the

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3 1 hypertensive stage, this together with the shortening of the rhythms during DD2 in the pre-
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5 2 hypertensive stage, could indicate early changes in the SCN possibly related with the
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7 3 development of hypertension. Accordingly, shorter tau in DD but longer tau in LL for activity
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9 4 rhythms are the characteristics of the hypertensive rather than hyperactive trait of rats
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11 5 derived from SHR_s (Rosenwasser et al., 1996). A change in blood pressure cannot be the
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13 6 reason for this change in activity since the animals only start to develop a higher blood
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15 7 pressure after 11 weeks as we observed in the current study. Thus overt locomotor activity is
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17 8 altered in SHR_s before the development of hypertension.
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22 9 As we have seen, the period length is already changed in the SHR_s during the pre-
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24 10 hypertensive stage in DD2 indicating a possible change in the SCN, interestingly this
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26 11 coincides with the moment that also in the SHR running wheel activity increases. At the
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28 12 neuroanatomical level, SHR_s have more VIP in their SCN which is the neurotransmitter
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30 13 responsible for light entrainment of the rhythms (Avidor et al., 1989; Peters et al., 1994). The
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32 14 abundance of VIP might make the SHR_s more responsive to changes in the environmental
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34 15 light than WKY_s as observed in our experiments to very dim light at 5 lux. Mutant mice over
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36 16 expressing the VIP VPAC2 receptor have a significant shortening in their activity rhythms and
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38 17 accelerated circadian entrainment (Shen et al., 2000). Moreover, VPAC2 deficient mice have
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40 18 abnormal blood pressure rhythms due to their abnormal activity patterns (Sheward et al.,
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42 19 2010). Together these data suggest that the SCN is changed in SHR_s possibly via other
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44 20 mechanisms interacting with the SCN, regulating period length and locomotor activity
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46 21 (Canal-Corretger et al., 2001a; Harper et al., 2008; Wang et al., 2015). Interestingly in
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48 22 humans, short sleep duration is associated with the development of hypertension, although
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50 23 whether this is the result of the simultaneous development of the metabolic syndrome is not
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52 24 clear yet (Gangwisch et al., 2006). As for now evidence for the involvement of the SCN in the
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development of hypertension in humans can only be inferred from morphological changes observed previously (Goncharuk et al., 2001) and the fact that melatonin taken at night significantly reduces blood pressure levels (Scheer et al., 2004).

In conclusion: The early functional changes of the circadian timing system in SHRs strongly suggest that early changes in the SCN and circadian system may precede the development of hypertension. **Possibly this is the reason that the environmental mutation of neglecting the light dark signal, that mankind has experienced in the last hundred years may have contributed significantly to the development of hypertensive problems in the population.**

At the other hand the absence of changes in rhythms after the induction of hypertension indicates that, overall an increase in blood pressure is not sufficient to change the rhythmicity of SCN, at least not in the short term.

Declaration of interest

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Figure Legends

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Figure 1. Light scheduling during the experiments. Alternating constant dark (DD1 and DD2-0 lux) and constant dim light (LL1 and LL2- 5 lux) regimes during experimental scheduling displayed. At the pre-hypertensive stage animals started experiments at the age of 4 weeks and experiments were ended at the age of 10 weeks (a). The experiments both in the hypertensive stage (b) and in the induction of hypertension group (c) started at the age of 7 weeks and ended at the age of 13 and 13.5 weeks, respectively. At the end of the experiments blood pressure and heart rate were measured.

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Figure 2. Period changes in locomotor and wheel running activity during consecutive

constant dark (DD-0 lux) and constant dim light (LL-5 lux) conditions. Periods (hours, mean $\pm$ S.E.M.) of locomotor (a, c, e) and wheel running activity (b, d, f) rhythms of SHR (n=9 for each stage), WKY (n=9 for each stage), and IndH (n=4) and sham animals (n=3) in DD1, LL1, DD2 and LL2 sessions shown, respectively. * $p < 0.05$, indicates statistically significant difference with a t-test between age-matched SHRs and WKYs.

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Figure 3. Changes in total amount of locomotor and wheel running activity during consecutive constant dark (DD) and constant dim light (LLdim) conditions. Total amount of (a.u., mean $\pm$ S.E.M.) of general activity (a, c, e) and running wheel activity (b, d, f) rhythms of SHR_s (n=9 for each stage), WKY_s (n=9 for each stage), and IndH (n=4) and sham animals (n=3) in DD1, LL1, DD2 and LL2 shown, respectively. * p<0.05, indicates statistically significant difference with a t-test between age-matched SHR_s and WKY_s.

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Figure 4. Amplitude changes in locomotor and wheel running activity during consecutive constant dark (DD) and constant dim light (LLdim) conditions. Amplitudes (a.u., mean $\pm$ S.E.M.) of general activity (a, c, e) and running wheel activity (b, d, f) rhythms of SHR_s (n=3), WKY_s (n=3) at pre-hypertensive stage, and SHR (n=5) and WKY (n=4) at hypertensive stage, respectively. * p<0.05, indicates statistically significant difference with a t-test between age-matched SHR_s and WKY_s.

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Figure 5. Representative actograms of SHR_s and WKY_s. Representative actograms of SHR and WKY_s at the pre-hypertensive (a) and hypertensive (b) stages (black bars- DD, 0 lux; dark grey bars- LL, 5 lux) displayed. Representative blood pressure and heart rate recordings of an individual SHR and a WKY animal shown (c).

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Ajda Yilmaz, Andries Kalsbeek, and Ruud M. Buijs

Table 1 of 1

Table 1. Blood pressures and heart rate variables of SHR, WKY, IndH and Sham animals during different stages of the experimental protocol.

	Pre-hypertensive Stage (10 wks*)		Hypertensive Stage (13 wks)		Induction of hypertension (13.5 wks)	
	SHR (n=8)	WKY (n=9)	SHR (n=6)	WKY (n=7)	IndH (n=4)	Sham (n=3)
SBP [†] (mmHg)	129.75±15.78	102.11±5.56	167.00±4.58*	98.28±8.87	167.00±8.71*	122.66±11.39
DBP [‡] (mmHg)	92.75±13.46	76.00±5.30	137.16±4.54*	68.42±8.20	113.33±6.35	95.66±8.83
MAP [§] (mmHg)	105.12±14.03	84.88±5.45	147.00±3.66*	77.57±8.34	131.66±6.76	104.00±9.53
HR [¶] (bpm)	273.37±18.16	283.44±9.71	314.66±11.39	289.57±10.17	302.33±16.16	336.66±5.48

Data presented as mean ± S.E.M. * indicates statistically significant difference between age-matched SHR and WKY as well as IndH and Sham animals (p< 0.05, t- test). •wks: weeks, †SBP: Systolic Blood Pressure, ‡DBP: Diastolic Blood Pressure, §MAP: Mean Arterial Pressure, ¶HR: Heart Rate.

Supplementary data to:

Circadian behavioral changes in the etiology of spontaneous hypertension but not after the induction of hypertension

Ajda Yilmaz, Andries Kalsbeek, and Ruud M. Buijs

Supplementary Table 1 of 2

Supplementary Table 1. Periods (hours, mean $\pm$ S.E.M.) of locomotor and wheel running activity rhythms of SHR (n=9), WKY (n=9), IndH (n=4) and sham animals (n=3) in DD1, LL1, DD2 and LL2 sessions during pre- and hypertensive stages, respectively.

	General Activity				Running Wheel Activity			
	DD1	LL1	DD2	LL2	DD1	LL1	DD2	LL2
Pre-Hypertensive stage								
SHR	24.1 $\pm$ 0.04	25.35 $\pm$ 0.12	23.68 $\pm$ 0.04*	25.33 $\pm$ 0.12	24.18 $\pm$ 0.09	25.50 $\pm$ 0.19	23.71 $\pm$ 0.05*	25.55 $\pm$ 0.16
WKY	24.1 $\pm$ 0.1	25.1 $\pm$ 0.10	24.02 $\pm$ 0.06	25.1 $\pm$ 0.13	24.32 $\pm$ 0.1	25.12 $\pm$ 0.09	24.07 $\pm$ 0.15	25.15 $\pm$ 0.16
Hypertensive stage								
SHR	24.03 $\pm$ 0.06	25.20 $\pm$ 0.10	23.67 $\pm$ 0.07*	25.36 $\pm$ 0.08*	24.02 $\pm$ 0.10	25.23 $\pm$ 0.11	23.66 $\pm$ 0.06*	25.46 $\pm$ 0.08*
WKY	24.25 $\pm$ 0.07	24.9 $\pm$ 0.07	24.00 $\pm$ 0.05	24.94 $\pm$ 0.07	24.25 $\pm$ 0.08	24.13 $\pm$ 0.07	23.97 $\pm$ 0.04	25.02 $\pm$ 0.10
Induction of hypertension								
IndH	24.45 $\pm$ 0.15	25.32 $\pm$ 0.025	24.12 $\pm$ 0.048	–	24.32 $\pm$ 0.19	25.27 $\pm$ 0.075	24.2 $\pm$ 0.14	–
Sham	24.3 $\pm$ 0.005	25.4 $\pm$ 0.002	24.3 $\pm$ 0.1	–	24.4 $\pm$ 0.5	25.6 $\pm$ 0.208	24.4 $\pm$ 0.1	–

* p<0.05, states statistically significant difference with a t-test between age-matched SHRs and WKYs

Ajda Yilmaz, Andries Kalsbeek, and Ruud M. Buijs

Supplementary Table 2 of 2

Supplementary Table 2. Total amount of (a.u., mean $\pm$ S.E.M.) of locomotor and running wheel activity rhythms of SHR_s (n=9), WKY_s (n=9), IndH (n=4) and sham animals (n=3) in DD1, LL1, DD2 and LL2 sessions during pre- and hypertensive stages, respectively.

	General Activity				Running Wheel Activity			
	DD1	LL1	DD2	LL2	DD1	LL1	DD2	LL2
Pre-Hypertensive stage								
<i>SHR</i>	14.88 $\pm$ 1.10	12.21 $\pm$ 0.45	15.59 $\pm$ 1.65	14.54 $\pm$ 0.83	46.88 $\pm$ 3.69	29.06 $\pm$ 2.19	134.64 $\pm$ 9.72*	80.46 $\pm$ 15.64
<i>WKY</i>	14.51 $\pm$ 1.77	12.40 $\pm$ 1.08	13.68 $\pm$ 1.40	14.54 $\pm$ 0.83	55.22 $\pm$ 14.95	36.00 $\pm$ 13.78	72.72 $\pm$ 19.29	73.44 $\pm$ 21.07
Hypertensive stage								
<i>SHR</i>	16.12 $\pm$ 1.24	16.53 $\pm$ 1.15*	20.81 $\pm$ 2.28	16.20 $\pm$ 1.95*	91.03 $\pm$ 20.54	81.76 $\pm$ 20.39	175.26 $\pm$ 16.05*	89.19 $\pm$ 20.58*
<i>WKY</i>	14.25 $\pm$ 0.94	12.56 $\pm$ 1.36	15.87 $\pm$ 1.57	10.95 $\pm$ 0.97	45.08 $\pm$ 9.67	43.52 $\pm$ 19.69	96.82 $\pm$ 28.39	30.99 $\pm$ 10.85
Induction of hypertension								
<i>IndH</i>	10.64 $\pm$ 1.22	9.66 $\pm$ 1.00	9.76 $\pm$ 0.86	–	9.76 $\pm$ 1.02	8.60 $\pm$ 0.60	11.10 $\pm$ 1.03	–
<i>Sham</i>	11.34 $\pm$ 2.24	10.22 $\pm$ 1.29	11.08 $\pm$ 3.02	–	26.47 $\pm$ 12.05	9.66 $\pm$ 2.04	17.77 $\pm$ 4.69	–

* p<0.05, states statistically significant difference with a t-test between age-matched SHR_s and WKY_s.

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Table 1 of 1

Table 1. Blood pressures and heart rate variables of SHR, WKY, IndH and Sham animals during different stages of the experimental protocol.

wks)	Pre-hypertensive Stage (10 wks•)		Hypertensive Stage (13 wks)		Induction of hypertension (13.5	
	SHR (n=8)	WKY (n=9)	SHR (n=6)	WKY (n=7)	IndH (n=4)	Sham (n=3)
SBP [†] (mmHg) 122.66±11.39	129.75±15.78	102.11±5.56	167.00±4.58*	98.28±8.87	167.00±8.71*	
DBP [‡] (mmHg)	92.75±13.46	76.00±5.30	137.16±4.54*	68.42±8.20	113.33±6.35	95.66±8.83
MAP [§] (mmHg) 104.00±9.53	105.12±14.03	84.88±5.45	147.00±3.66*	77.57±8.34	131.66±6.76	
HR [¶] (bpm) 336.66±5.48	273.37±18.16	283.44±9.71	314.66±11.39	289.57±10.17	302.33±16.16	

Data presented as mean ± S.E.M. * indicates statistically significant difference between age-matched SHR and WKY as well as IndH and Sham animals (p< 0.05, t- test). •wks: weeks, †SBP: Systolic Blood Pressure, ‡DBP: Diastolic Blood Pressure, §MAP: Mean Arterial Pressure, ¶HR: Heart Rate.

A- Pre-hypertensive Stage **B- Hypertensive Stage** **C- Induction of Hypertension (2K1C)**

Figure 1. Light scheduling during the experiments. Alternating constant dark (DD1 and DD2- 0 lux) and constant dim light (LL1 and LL2- 5 lux) regimes during experimental scheduling displayed. At the pre-hypertensive stage animals started experiments at the age of 4 weeks and experiments were ended at the age of 10 weeks (a). The experiments both in the hypertensive stage (b) and in the induction of hypertension group (c) started at the age of 7 weeks and ended at the age of 13 and 13.5 weeks, respectively. At the end of the experiments blood pressure and heart rate were measured.

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Figure 2. Period changes in locomotor and wheel running activity during consecutive constant dark (DD-0 lux) and constant dim light (LL-5 lux) conditions. Periods (hours, mean $\pm$ S.E.M.) of locomotor (a, c, e) and wheel running activity (b, d, f) rhythms of SHR (n=9 for each stage), WKY (n=9 for each stage), and IndH (n=4) and sham animals (n=3) in DD1, LL1, DD2 and LL2 sessions shown, respectively. * p<0.05, indicates statistically significant difference with a t-test between age-matched SHRs and WKYs.

Figure 3. Changes in total amount of locomotor and wheel running activity during consecutive constant dark (DD) and constant dim light (LLdim) conditions. Total amount of (a.u., mean $\pm$ S.E.M.) of general activity (a, c, e) and running wheel activity (b, d, f) rhythms of SHRs (n=9 for each stage), WKYs (n=9 for each stage), and IndH (n=4) and sham animals (n=3) in DD1, LL1, DD2 and LL2 shown, respectively. * $p < 0.05$, indicates statistically significant difference with a t-test between age-matched SHRs and WKYs.

Figure 4. Amplitude changes in locomotor and wheel running activity during consecutive constant dark (DD) and constant dim light (LLdim) conditions. Amplitudes (a.u., mean $\pm$ S.E.M.) of general activity (a, c, e) and running wheel activity (b, d, f) rhythms of SHRs (n=3), WKYs (n=3) at pre-hypertensive stage, and SHR (n=5) and WKY (n=4) at hypertensive stage, respectively. * $p < 0.05$, indicates statistically significant difference with a t-test between age-matched SHRs and WKYs.

Figure 5. Representative actograms of SHRs and WKYs. Representative actograms of SHR and WKYs at the pre-hypertensive (a) and hypertensive (b) stages (black bars- DD, 0 lux; dark grey bars- LL, 5 lux) displayed. Representative blood pressure and heart rate recordings of an individual SHR and a WKY animal shown (c).

Supplementary data to:

Functional changes of the Suprachiasmatic nucleus in spontaneous hypertension but not after the induction of hypertension

Ajda Yilmaz, Andries Kalsbeek, and Ruud M. Buijs

Supplementary Table 1 of 2

Supplementary Table 1. Periods (hours, mean $\pm$ S.E.M.) of locomotor and wheel running activity rhythms of SHR (n=9), WKY (n=9), IndH (n=4) and sham animals (n=3) in DD1, LL1, DD2 and LL2 sessions during pre- and hypertensive stages, respectively.

	General Activity				Running Wheel Activity			
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Pre-Hypertensive stage								
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Hypertensive stage								
<i>SHR</i>	24.03 $\pm$ 0.06	25.20 $\pm$ 0.10	23.67 $\pm$ 0.07*	25.36 $\pm$ 0.08*	24.02 $\pm$ 0.10	25.23 $\pm$ 0.11	23.66 $\pm$ 0.06*	25.46 $\pm$ 0.08*
<i>WKY</i>	24.25 $\pm$ 0.07	24.9 $\pm$ 0.07	24.00 $\pm$ 0.05	24.94 $\pm$ 0.07	24.25 $\pm$ 0.08	24.13 $\pm$ 0.07	23.97 $\pm$ 0.04	25.02 $\pm$ 0.10
Induction of hypertension								
<i>IndH</i>	24.45 $\pm$ 0.15	25.32 $\pm$ 0.025	24.12 $\pm$ 0.048	–	24.32 $\pm$ 0.19	25.27 $\pm$ 0.075	24.2 $\pm$ 0.14	–
<i>Sham</i>	24.3 $\pm$ 0.005	25.4 $\pm$ 0.002	24.3 $\pm$ 0.1	–	24.4 $\pm$ 0.5	25.6 $\pm$ 0.208	24.4 $\pm$ 0.1	–

* p<0.05, states statistically significant difference with a t-test between age-matched SHRs and WKYs

Ajda Yilmaz, Andries Kalsbeek, and Ruud M. Buijs

Supplementary Table 2 of 2

Supplementary Table 2. Total amount of (a.u., mean $\pm$ S.E.M.) of locomotor and running wheel activity rhythms of SHR_s (n=9), WKY_s (n=9), IndH (n=4) and sham animals (n=3) in DD1, LL1, DD2 and LL2 sessions during pre- and hypertensive stages, respectively.

	General Activity				Running Wheel Activity			
	DD1	LL1	DD2	LL2	DD1	LL1	DD2	LL2
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WKY	14.51 $\pm$ 1.77	12.40 $\pm$ 1.08	13.68 $\pm$ 1.40	14.54 $\pm$ 0.83	55.22 $\pm$ 14.95	36.00 $\pm$ 13.78	72.72 $\pm$ 19.29	73.44 $\pm$ 21.07
Hypertensive stage								
SHR	16.12 $\pm$ 1.24	16.53 $\pm$ 1.15*	20.81 $\pm$ 2.28	16.20 $\pm$ 1.95*	91.03 $\pm$ 20.54	81.76 $\pm$ 20.39	175.26 $\pm$ 16.05*	89.19 $\pm$ 20.58*
WKY	14.25 $\pm$ 0.94	12.56 $\pm$ 1.36	15.87 $\pm$ 1.57	10.95 $\pm$ 0.97	45.08 $\pm$ 9.67	43.52 $\pm$ 19.69	96.82 $\pm$ 28.39	30.99 $\pm$ 10.85
Induction of hypertension								
IndH	10.64 $\pm$ 1.22	9.66 $\pm$ 1.00	9.76 $\pm$ 0.86	–	9.76 $\pm$ 1.02	8.60 $\pm$ 0.60	11.10 $\pm$ 1.03	–
Sham	11.34 $\pm$ 2.24	10.22 $\pm$ 1.29	11.08 $\pm$ 3.02	–	26.47 $\pm$ 12.05	9.66 $\pm$ 2.04	17.77 $\pm$ 4.69	–

* p<0.05, states statistically significant difference with a t-test between age-matched SHR_s and WKY_s.