Research paper

Chronic social defeat stress suppresses locomotor activity but does not affect the free-running circadian period of the activity rhythm in mice

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A B S T R A C T

In mammals, daily rhythms in behavior and physiology are under control of an endogenous clock or pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN assures an optimal temporal organization of internal physiological process and also synchronizes rhythms in physiology and behavior to the cyclic environment. The SCN receives direct light input from the retina, which is capable of resetting the master clock and thereby synchronizes internally driven rhythms to the external light-dark cycle. In keeping with its function as a clock and pacemaker, the SCN appears to be well buffered against influences by other stimuli and conditions that contain no relevant timing information, such as acute stressors. On the other hand, it has been suggested that chronic forms of stress may have gradually accumulating effects that can disturb normal clock function and thereby contribute to stress-related disorders. Therefore, in the present study we investigated whether chronic intermittent social stress affects the endogenous period and phase of the free-running activity rhythm in mice. Adult male mice were maintained in constant dim red light conditions and exposed to a daily 20 min social defeat stress session for 10 consecutive days, either during the first half of their activity phase or the first half of their resting phase. The overall amount of running wheel activity was strongly suppressed during the 10 days of social defeat, to about 50% of the activity in non-defeated control mice. Activity levels gradually normalized during post-defeat recovery days. Despite the strong suppression of activity in defeated animals, the endogenous free-running circadian period of the activity rhythm and the phase of activity onset were not affected. These findings are thus in agreement with earlier studies suggesting that the circadian pacemaker in the SCN that is driving the rhythmicity in activity is well-protected against stress. Even severe social defeat stress for 10 consecutive days, which has a major effect on the levels of activity, does not affect the pace of the endogenous clock.

1. Introduction

In mammals, daily rhythms in physiology and behavior are under control of an endogenous clock that is located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Dibner et al., 2010; Saper, 2013). The SCN serves as a pacemaker that directly drives the rhythms or coordinates rhythms that reside in other tissues and organs. The SCN receives a direct neuronal input from the eyes and light is the main time cue used by the SCN to synchronize internal rhythms to the environmental cycles in the external world.

A disturbance in the fine-tuned temporal organization of physiological processes and behavior may have serious consequences for health and well-being. Indeed, desynchrony of internal rhythms has been implicated in a variety of maladies and diseases, including psychiatric disorders, neurological disorders, metabolic syndrome, and

inflammation (Jones and Benca, 2015; Maury et al., 2014; Videnovic and Zee, 2015; Wright et al., 2015). Likewise, desynchrony between internal rhythms and the external environment can also cause health problems, as is the case with jet lag and shift work. Shiftwork for example, is associated with sleep-wake problems, fatigue, and poor attention (Caruso, 2015; Herichova, 2013), which can also be observed after long-distance flights across time zones (Samuels, 2012; Weingarten and Collop, 2013).

In this context, it is an important question whether the circadian timing system is sensitive to disturbance by stressors and whether such circadian disturbance might then contribute to the development of stress-related disorders. Many earlier studies have suggested that the SCN, in keeping with its function as a clock and pacemaker, appears to be well buffered against the influence of acute stressors (Meerlo et al., 2002; Richter, 1967). For example, studies in rodents have shown that

acute social defeat stress may lead to severe disturbances in the daily rhythms of activity, body temperature and heart rate, but it does not affect the endogenous phase and period of these rhythms under constant conditions (Meerlo et al., 1997b, 2002; Meerlo and Daan, 1998). In other words, the endogenous pacemaker driving the rhythms appears to be unaffected, but its output can be masked by disturbances elsewhere in the body. On the other hand, it has been suggested that chronic stress may have more severe effects that accumulate over time and perhaps can disturb normal clock function, if not directly, perhaps indirectly by affecting other systems that communicate with the circadian system (Koch et al., 2017). For this reason, in the present study we assessed the effects of repeated social defeat stress for 10 successive days on free running activity rhythms in mice.

2. Material and methods

2.1. Animals and housing

A total of 45 male C57BL/6J mice (Janvier Labs, Le Genest-Saint-Isle, France) and 15 male CD-1 mice (Charles River, Sulzfeld, Germany) were used for the experiments. The C57BL/6J mice were between 2 and 3 months-old at the beginning of the experiment. They were used as experimental animals and were assigned to either a control group or a social defeated group. The male CD-1 mice were 3 to 5 months old and were trained to be used as aggressors for the social defeats. All animals were individually housed in cages with running wheels. The mice had free access to food and water throughout the study and the rooms were temperature controlled (21 ± 1 °C). All efforts were made to minimize animal suffering. The experiments were conducted in accordance with the Dutch rules and regulations and approved by the Central Authority for Scientific Procedures on Animals (CCD).

2.2. Experimental design

Fig. 1 shows the timeline of the experiment. After an initial phase of habituation under a standard 12-12 h light-dark (LD) cycle, animals were kept under constant dim red light from the start of the baseline period onwards and throughout the remainder of the experiment. Running wheel activity was recorded and compared among the three blocks: baseline, social defeat and recovery; each block consisted of 10 days. In the first experiment, half of the mice were subjected to a social defeat stress during their circadian activity phase for 10 consecutive days. In the second experiment, half of the mice were subjected to a social defeat stress during their resting phase, also, for 10 consecutive days. These time frames for stress exposure were chosen because these are the phases at which the circadian clock in the SCN and the free-running activity rhythm rodents have been shown to be sensitivity to the phase shifting effects of various non-photic stimuli (Mrosovsky, 1996). These phases therefore seemed most relevant in the context of our study on stress. In both experiments, the social defeat stress took place at a fixed external time of day. Because mice were free-running with their own endogenous period that slightly deviated from 24 h, the defeats at fixed external times took place at a slightly different internal time every day.
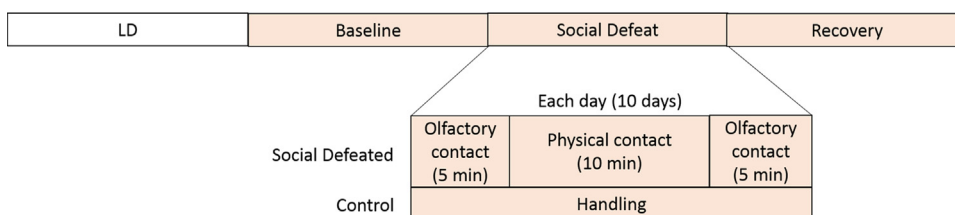
2.3. Social defeat

Social defeat sessions took place under dim red light, similar to that in the home room of the experimental mice, and care was taken to not expose them to any other light. Each social stress session lasted 20 min and was divided as follows: Phase 1 (5 min) was the initiation phase, during which the experimental animal was placed in the aggressor's cage, separated by a transparent and perforated acrylic partition, allowing olfactory and visual contact. Phase 2 (10 min) was the actual defeat time, which started by removing the partition, after which the aggressor threatened and attacked the experimental animal. If during the interaction phase, the intruder received more than 10 attacks before 10 minutes, the animals were separated and the remaining time was added to Phase 3 (5 min). In phase 3, the mice were separated by the partition again. At the end of the procedure, the intruders returned to their home cage. Social defeated animals were exposed to a new aggressor each day, to avoid habituation. Control mice were placed in an empty cage for the same duration as the defeat procedure.

2.4. Activity recordings and data processing

Running wheel rotations were recorded and stored in 2 min bins throughout the study. The free-running circadian period of the activity rhythm was calculated for each of the 10-day time blocks (baseline, social defeat, recovery) by means of a periodogram analysis based on the Sokolove and Bushell algorithm (ChronoShop 1.04; Spoelstra, 2015). Based on the individual free-running period, the total activity per circadian hour and circadian day was calculated. The phase of activity onset was calculated by a procedure previously described (Meerlo et al., 1997b). Briefly, the time of activity onset was calculated by determining the crossings between a 1 h running mean and a 24 h running mean of the original raw data. The time of activity onset for the last day of each 10-day block (baseline, defeat, recovery) was then transformed to circadian time, based on the free-running period for each individual mouse.

2.5. Statistics

To assess the effects of social defeat stress on free-running circadian period and phase, repeated measures ANOVA was used with between-subjects factor GROUP (control and social defeat) and within-subjects factor TIME (10-day time blocks for baseline, social defeat, and recovery). To determine differences between the two groups in the overall amount of daily activity, repeated measures ANOVA was applied separately for the three 10-day time blocks with between-subjects factor GROUP (control and social defeat) and within-subjects factor DAYS (10 successive days within a time block). Finally, to assess differences between the two groups in the daily distribution of activity, a repeated measures ANOVA was applied separately for the average daily activity profile in each 10-day time blocks with between-subjects factor GROUP (control and social defeat) and within-subjects factor HOURS (24 circadian hours). Newman–Keuls test was used as a post-hoc when appropriate. Results were considered statistical significant when $p < 0.05$.

Fig. 1. Timeline of the experiment. After entrainment to a 12:12 light-dark cycle, animals were exposed to constant dim red light throughout the rest of the experiment. The timeline of the experiment consisted in three blocks of 10 days each: Baseline, Social defeat and Recovery. During the Social Defeat block, mice from the social defeated group were plac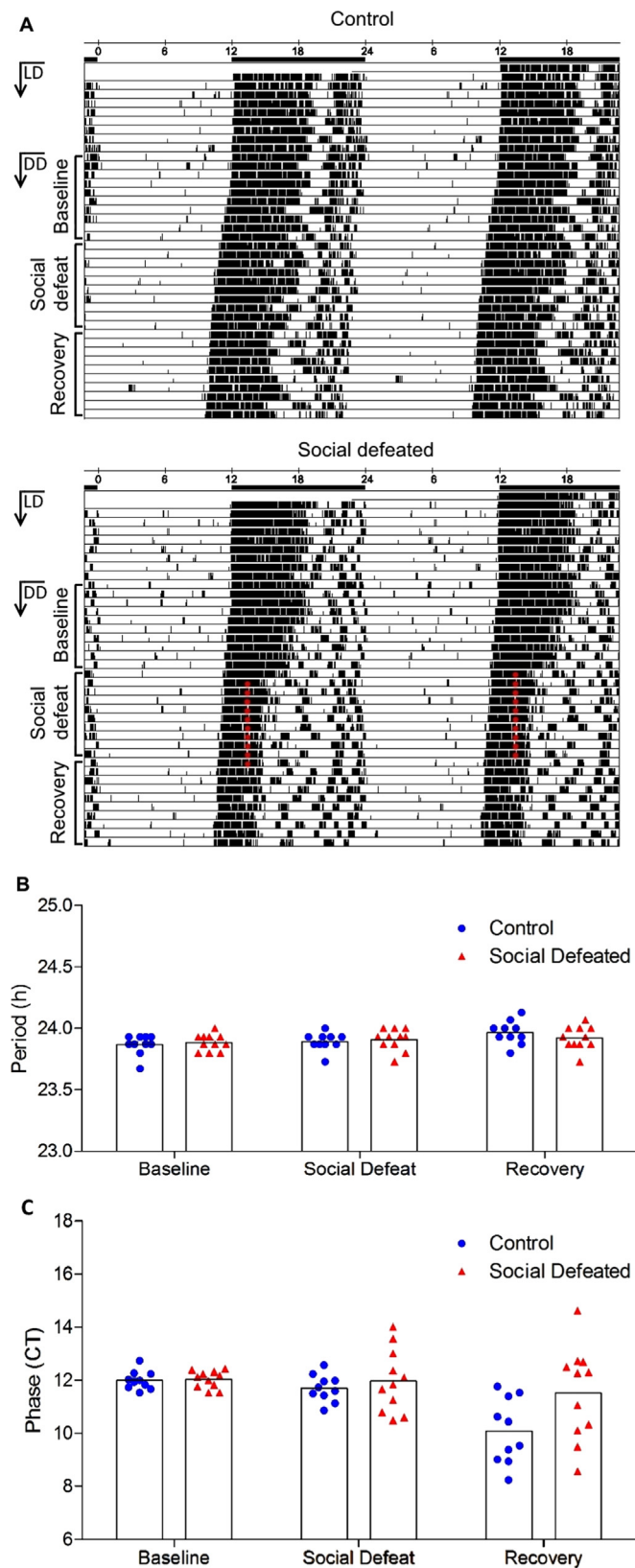ed in a cage with an aggressive animal for a total of 20 min each day. Control animals were handled and placed in a different cage. During Baseline and Recovery blocks, animals were left undisturbed in their home cages.

Fig. 2. Effects of repeated social defeat stress during the active phase on free-running activity rhythms. Panel (A) Representative actograms of an individual control animal and an animal subjected to social defeat stress on 10 consecutive days during the active phase (indicated by the red line). Observe the suppression of activity that occurred as a consequence of the social defeat stress. Panel (B) The intrinsic circadian period of the activity rhythm during the 3 different phases of the experiment (Baseline, Social Defeat and Recovery). No differences in period between groups were observed. Panel (C) Activity onset phase in the last day of each block of the experiment. No differences between groups were observed. Bars in panel B and C represent group means and symbols represent the individual animals. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

because of technical issues with their running wheels and incomplete activity recordings, resulting in a total of 10 and 11 animals in the control and social defeated group, respectively. Fig. 2A shows actograms from an individual control animal and an animal exposed to social defeat stress in the active phase. Fig. 2B displays the average circadian period for the control group and defeat group during the three successive 10-day-blocks. There was no difference in free-running period between control and socially defeated animals in any of the 3 time blocks. In the control group, the free-running circadian period for the three successive 10-day blocks was 23.86 ± 0.02 h (baseline), 23.89 ± 0.02 h (experiment), and 23.97 ± 0.03 h (recovery). For the defeated mice, the free-running period was 23.88 ± 0.02 h (baseline), 23.91 ± 0.02 h (social defeat), and 23.92 ± 0.03 h (recovery). Fig. 2C shows the average circadian time of the activity onset on the last day of each 10-day block. Repeated measures ANOVA indicated a trend for a GROUP difference ($F_{(1,19)}$ = 11.87, p = 0.06) and a trend for interaction between GROUP x DAYS ($F_{(2,38)}$ = 2.93, p = 0.07).

Fig. 3A depicts the amount of activity per circadian day and changes herein across the three 10-day time blocks. The amount of daily activity during the 10-day baseline block was not different between the two groups. For daily activity during the 10-day experimental block, ANOVA revealed an overall effect of GROUP ($F_{(1,19)}$ = 11.87, p < 0.01). Daily activity was strongly suppressed in the socially defeated mice as compared to the control mice. Activity levels in the defeated animals gradually normalized during the first couple of post-defeat days and, overall, ANOVA did not indicate a significant difference between control and defeated mice in the 10-day recovery block ($F_{(1,19)}$ = 0.94, p = 0.34), although there was a trend for a GROUP x DAYS interaction ($F_{(9,171)}$ = 1.76, p = 0.08).

Fig. 3B shows the average daily activity profiles of the two groups for the three successive 10-day blocks. As expected, the two groups of mice had similar activity profiles during the 10-day baseline block. However, for the 10-day experimental block, repeated measures ANOVA revealed an effect of GROUP ($F_{(1,19)}$ = 11.88, p < 0.01) and a GROUP X HOURS interaction ($F_{(23,437)}$ = 3.15 p < 0.01). Post-hoc tests indicated that the socially defeated mice were significantly less active than the controls from CT13 to CT17 (Newman-Keuls, p < 0.05 for each time). The average activity profile during the 10-day recovery block did no longer significantly differ between the groups.

3.2. Social defeat during the resting phase

In the second experiment, data from two animals were excluded due to problems with their running wheels, giving a total of 11 and 9 animals in the control and social defeated group. Fig. 4A shows actograms from an individual control animal and an animal exposed to social defeat stress in the resting phase. The free-running periods for the 3 successive 10-day time blocks are shown in Fig. 4B. ANOVA did not indicate any difference between control and socially defeated mice for any of these time blocks. In the control group, the free-running circadian period for the three successive 10-day blocks was 23.96 ± 0.03 h (baseline), 23.99 ± 0.05 h (experiment), and 24.01 ± 0.03 h (recovery). For the defeated mice, the free-running period was

3. Results

3.1. Social defeat during the active phase

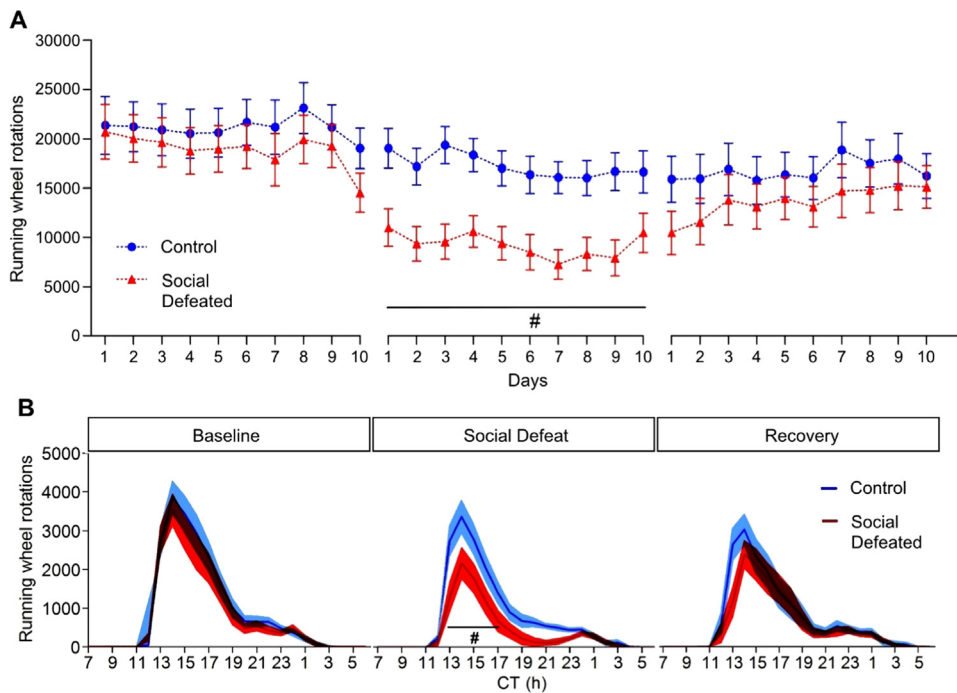Data from two animals in the first experiment had to be excluded

Fig. 3. Effects of repeated social defeat stress during the active phase on total activity and distribution of activity. Panel (A) Total activity per day during the three phases of the experiment (Baseline, Social Defeat and Recovery) in control animals (blue lines and symbols) and animals subjected to social defeat stress on 10 consecutive days (red lines and symbols). Animals that were exposed to social defeat showed suppressed running wheel activity compared to control animals. In the Recovery block, there was no longer difference between defeated and control group activity. Data shown are group mean ± SEM. Panel (B) Mean daily activity profile for each 10-day block of the experiment. Defeated mice displayed reduced activity particularly from CT13 to CT17 during Social Defeat days. Lines represent mean and colored area the SEM. For both panel A and B, # indicates a significant difference between groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

23.90 ± 0.03 h (baseline), 23.89 ± 0.06 h (social defeat), and 24.00 ± 0.03 h (recovery). Fig. 4C shows the average circadian time of activity onset on the 10th day of each 10-day block. ANOVA did not indicate any difference between control and socially defeated mice.

Fig. 5A illustrates the amount of activity per circadian day and changes herein across the three- 10-day time blocks. The amount of daily activity during the baseline was not significantly different between the two groups. For daily activity during the 10-day experimental block, ANOVA indicated that activity in the socially defeated animals was strongly suppressed, relative to the activity level of the control mice (overall effect of GROUP: $F_{(1,18)} = 31.94$ $p < 0.001$). For the 10-day recovery block, ANOVA revealed a significant overall effect of GROUP ($F_{(1,18)} = 7.44$, $p = 0.01$) and a significant GROUP x DAY interaction ($F_{(9,162)} = 4.22$, $p < 0.001$). Activity levels in the defeated animals gradually returned to those seen in control mice but were still significantly lower on the first 2 days of the recovery phase (Newman-Keuls, $p < 0.05$).

Fig. 5B shows the average daily activity profiles of the control and defeated mice for the three successive 10-day blocks. The average activity profiles during the baseline period were slightly but significantly different between the groups, as ANOVA indicated a significant GROUP x HOURS interaction ($F_{(23,414)} = 1.67$, $p < 0.05$). Post-hoc analysis indicated that the would-be defeated group ran less than the control mice at CT15 (Newman-Keuls: $p < 0.05$). For the 10-day experimental block, repeated measures ANOVA revealed an overall effect of GROUP ($F_{(1,18)} = 30.61$, $p < 0.001$) and a significant GROUP x HOURS interaction ($F_{(23,414)} = 11.50$, $p < 0.001$). Post-hoc tests indicated that the socially defeated mice were significantly less active than the controls for a large part of the active phase from CT13 to CT19 (Newman-Keuls: $p < 0.05$ in each case). Even during the 10-day recovery period, the average activity profile of the defeated animals was still significantly different from that of the control mice: ANOVA showed an overall effect of GROUP ($F_{(1,18)} = 7.57$, $p = 0.01$) and a GROUP x HOURS interaction ($F_{(23,414)} = 4.38$, $p < 0.001$). Post-hoc tests demonstrated that defeated animals ran less than controls from CT13 to CT17 (Newman-Keuls, $p < 0.05$ for each time point).

4. Discussion

The results of the present experiments in mice show that exposure to uncontrollable social defeat stress for 10 successive days causes a major suppression of activity levels but does not affect the clock responsible for the rhythmicity in activity: neither repeated defeat in the circadian active phase nor repeated defeat in the resting phase had a significant effect on the endogenous free-running period of the activity rhythm or the circadian time of activity onset.

The suppression of locomotor activity in socially defeated animals is in line with previous studies in rats and mice exposed to acute or chronic stressors (Meerlo et al., 2002; Richter, 1967). Particularly, social defeat stress has been found to lead to pronounced reductions in activity that in some cases persist for several days after the last defeat experience (Bartlang et al., 2015; Meerlo et al., 1996a, 1997b, 1999). In the present study, the suppression of activity seemed to be slightly stronger and more persistent in mice exposed to defeat in the resting phase as compared to mice exposed to defeat in the active phase. However, since the two experiments were independently carried out in different cohorts of mice, this extrapolation of the findings needs to be considered with care. The social stress-induced suppression of activity may be partly due to a motivation deficit and viewed as a depressive-like behavior, as already observed in social defeated rats (Meerlo et al., 1996b; Rygula et al., 2005) and mice (Krishnan et al., 2007; Kudryavtseva et al., 1991). Another possibility is that the activity reduction could be caused by pain in the defeated mice, since they were bitten by the aggressors. However, besides the caution we took so that the animals would not be injured, another study with social stress showed that avoidance behavior observed in defeated mice was not caused by difference in locomotor activity, since it was not different from control animals (Krishnan et al., 2007, supplemental data). Another hypothesis is that defeated mice present a higher sleep debt and might be asleep instead of running. To our knowledge, no study has investigated the effects of chronic social stress on the sleep-wake cycle, but studies with one or two defeats showed that sleep debt seemed higher in defeated animals, as they present more slow-wave activity
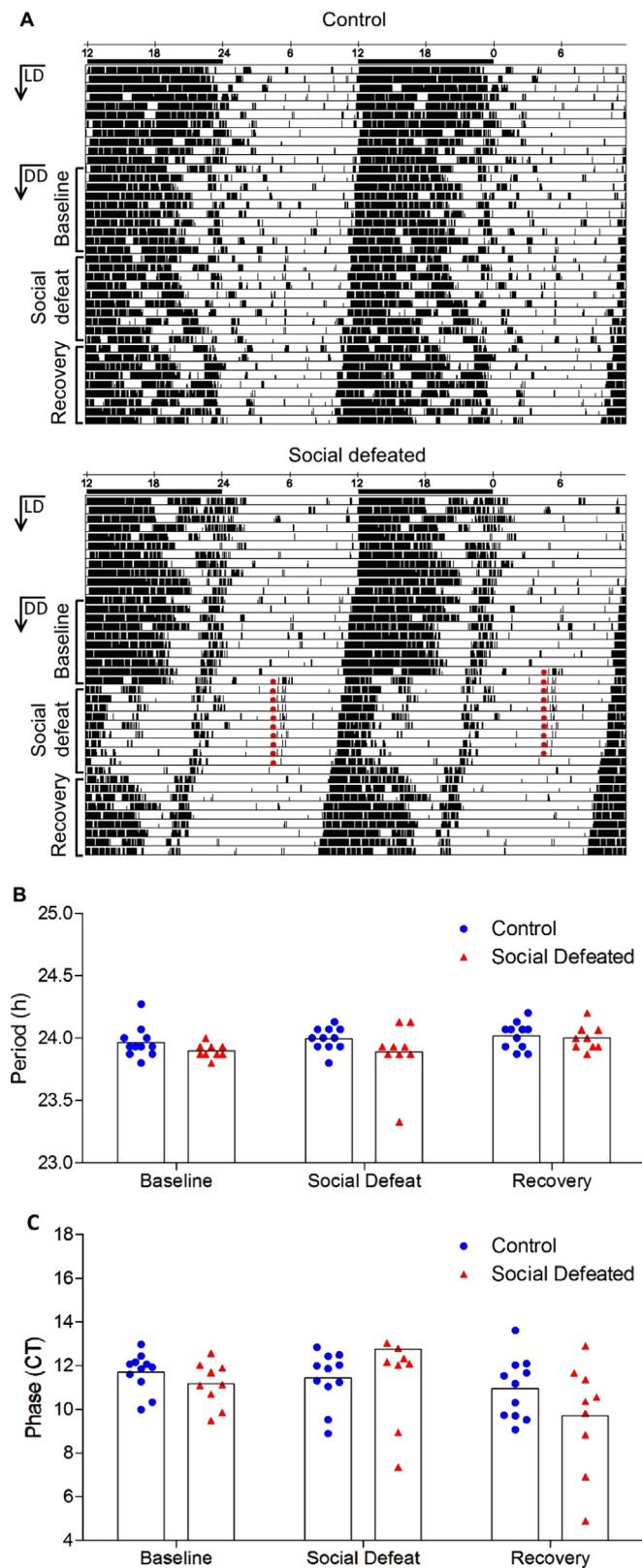
temperature during the resting phase that can last for several days after the end of the stressor (Meerlo et al., 1996a, 1997b, 1999; Tornatzky and Miczek, 1993). In one study in rats, the reduction in activity after social defeat stress strongly correlated with the increase in resting temperature: the more activity was suppressed during the active phase, the more body temperature was elevated during the resting phase (Meerlo et al., 1996a). This finding indicates that the change in activity *per se* is unlikely to be responsible for the change in body temperature, but the correlated change suggests that the two may share a common mechanism.

One often proposed mechanism for stress-induced changes in activity patterns and physiological rhythms is a disturbance of the circadian system. However, the current study, performed under constant conditions, showed that the circadian pacemaker driving the rhythmicity in activity continued to run at the same pace: despite the suppression of overall activity levels, the free-running circadian period of the activity rhythm and the timing of activity onset was not affected.

The finding that severe stress does not affect the free-running period of the activity rhythm is in agreement with previously published work in rodents exposed to social defeat or other stressors (Meerlo et al., 2002, Richter, 1967). A number of studies specifically addressed the question of whether the changes in activity and body temperature rhythm that result from uncontrollable social stress are a consequence of changes in the endogenous circadian timing system. In one study, rats were subjected to social defeat stress in the first half of the activity phase (Meerlo et al., 1997b), and in another study social defeat occurred in the middle of the resting phase (Meerlo and Daan, 1998). In neither one of these studies social stress had an effect on the phase or the period of the free running rhythms under constant conditions (Meerlo et al., 1997b; Meerlo and Daan, 1998). The present study in mice showed that even repeated defeat stress on 10 consecutive days did not have the proposed long-lasting effects that would culminate in an altered period. And overall, there was no significant difference between defeated and control groups in the phase of activity onset, although there was a trend for a difference when animals were defeated in the active phase. In this case however, it seemed that control mice, rather than defeated animals, had a slightly earlier time of activity onset during the last 10-day time block of the experiment compared to baseline.

Bartlang and colleagues recently reported on the effects of chronic intermittent social stress on activity rhythmicity in mice. The animals were exposed to a social conflict for 19 consecutive days, either in the light or in the dark phase, after which they were kept in constant darkness to study their free-running rhythms. In contrast to our findings, they reported a phase delay in peaks of activity in both C57BL/6N mice and C57BL/6J mice, especially in animals defeated in the dark phase. They also found a small but significant shortening of the free-running period of about 10 min in C57BL/6N mice but not in C57BL/6J mice (Bartlang et al., 2015). The change in phase, as the authors discussed, could be explained by a change in the shape of the rhythm, caused by a conditioned fear suppression of the activity, which was also observed in our mice, even when the social defeats occurred during the

during non-REM sleep (Meerlo et al., 1997a, 1997c; Meerlo and Turek, 2001).

In previous studies, social defeat stress was found to not only affect the activity rhythm but also rhythms in physiology, including body temperature. Social defeat stress is often associated with elevated body
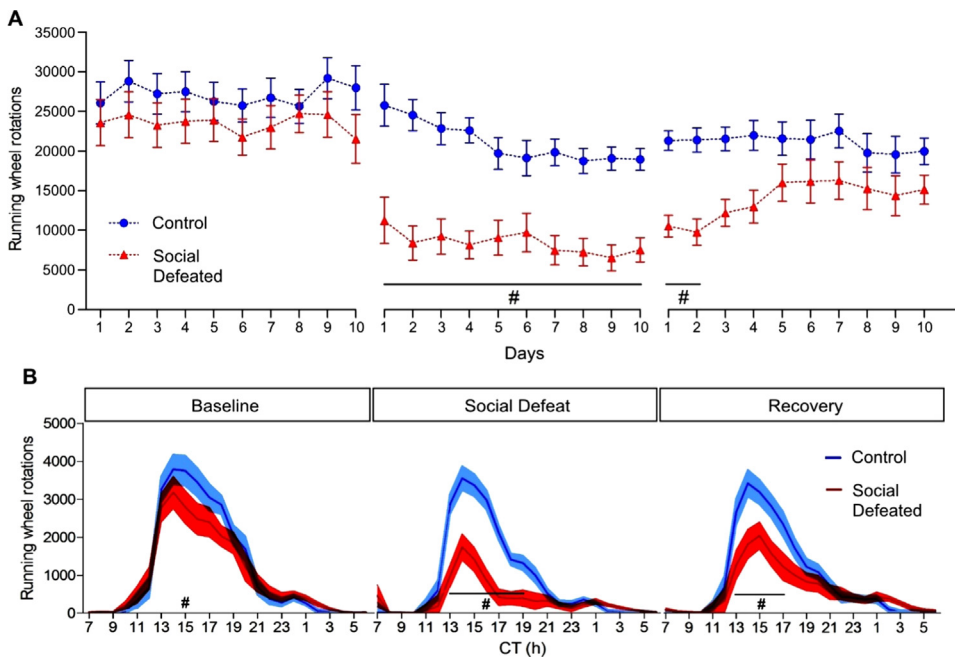
Fig. 5. Effects of repeated social defeat stress during the resting phase on total activity and distribution of activity. Panel (A) Total activity per day during the three phases of the experiment (Baseline, Social Defeat and Recovery) in control animals (blue lines and symbols) and animals subjected to social defeat stress on 10 consecutive days (red lines and symbols). Animals that were exposed to social defeat showed suppressed running wheel activity compared to control animals. This suppression gradually disappeared in the course of the recovery phase but was still significantly different during the first 2 days of recovery. Data shown are group mean ± SEM. Panel (B) Mean daily activity profile for each 10-day block of the experiment. During Baseline, the would-be defeated group showed less running wheel activity at CT 15. During the 10-day Social Defeat phase, defeated animals displayed strongly suppressed activity particularly from CT 13 to CT 19. And during the Recovery phase, defeated animals on average still ran less than controls from CT 13 to CT 17. Lines represent mean and colored area the SEM. For both panel A and B, # indicates a significant difference between groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

resting phase. It is unclear why the stress effect on period only occurred in one strain, but it might partly explain the difference from our current study, which was done in the C57BL/6J strain. Interestingly, another study showed that expression of the Period2 clock gene in the SCN was not affected by their protocol of chronic social stress (Bartlang et al., 2014). The latter might suggest that the small change in the period of the activity rhythm observed in one mouse line may be unrelated to the central pacemaker, which is in line with the general picture that the circadian pacemaker is resistant to stress.

A number of earlier studies performed in the golden hamster showed phase shifts in response to a wide variety of stimuli, some of which might be considered stressors, including aggressive social interactions (Mrosovsky, 1988, Mistlberger et al., 2003) and immobilization or restraint (Van Reeth et al., 1991). However, subsequent studies revealed that these phases shifts were not a direct consequence of the stressor but were the result of high intensity wheel running that occurred afterwards (for review, see Meerlo et al., 2002). For example, aggressive interactions between male hamsters induced phase shifts in some studies (Mrosovsky, 1988; Mistlberger et al., 2003), but not in all (Refinetti et al., 1992). In the first studies mentioned, the fighting was consistently followed by a period of running wheel activity, whereas in the latter experiment it was not. It thus seems that the aggressive and presumably stressful interaction only resulted in phase shifts when it induced an increase in locomotor activity. Stress per se did not appear to be the critical aspect of the stimulus. Similarly, immobilization or restraint was sometimes found to be associated with phase shifts (Van Reeth et al., 1991) but also in this case, rather than being the result from restraint stress per se, shifts only occurred when animals displayed wheel running after being released from restraint (Mistlberger et al., 2003; Mistlberger and Antle, 2006). Taken together, these studies in the golden hamster, provide indirect evidence supporting our conclusion that stress by itself does not perturb the central circadian oscillator."

One potential limitation of our study is that we only assessed the effects of repeated social stress at roughly two circadian phases, i.e., the middle of the resting phase and the first half of the active phase. One might argue that stress perhaps could affect the circadian clock and the free-running activity rhythm at other phases. While this needs to be tested, we specifically chose these circadian phases for stress exposure because these are the phases at which the circadian clock in the SCN and the free-running activity rhythm appears to be most sensitivity to

the phase shifting effects of non-photic stimuli discussed in the previous paragraph (Mrosovsky, 1996). These phases thus seemed most relevant in the context of our study on stress.

While the central circadian pacemaker in the SCN may be well-protected against the effects of stress, the possibility that stress can perturb peripheral oscillators that reside in other tissues and organs throughout the body, which are normally under regulatory control of the SCN cannot be excluded. In fact, although chronic social stress in mice did not change Period2 expression in the SCN, it produced a phase advance in the expression of this clock gene in the adrenal glands (Bartlang et al., 2014). Also, treatment with dexamethasone, a synthetic analogue of the glucocorticoid stress hormone, does not affect the SCN, but does shift the rhythms in clock gene expression in liver, kidney, and heart tissue (Balsalobre et al., 2000). These findings are consistent with the fact that glucocorticoid receptors, which are abundantly present in most tissues, are no longer expressed in the adult SCN (Rosenfeld et al., 1988). Hence, through glucocorticoids mechanism, stress might affect oscillatory processes in many tissues while leaving the central oscillator in the SCN untouched. It thus remains possible that changes in the phase relations among multiple clocks in the brain and body underlie some aspects of stress pathology. Therefore, more studies are necessary to assess the effects of different stressors on peripheral oscillators and the mechanisms involved.

In conclusion, while effects of stress on peripheral oscillators need to be investigated, the current study supports our earlier studies showing that acute social defeat stress does not affect the central pacemaker in the SCN. The current experiments extend our earlier findings by showing that even chronic intermittent social defeat stress for 10 days does not affect the free-running period of the activity rhythm that is driven by the master clock.

Declarations of interest

None.

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