

# Phase differences between SCN neurons and their role in photoperiodic encoding; a simulation of ensemble patterns using recorded single unit electrical activity patterns

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## Abstract

In mammals, a major circadian pacemaker is located in the suprachiasmatic nuclei (SCN), at the base of the anterior hypothalamus. The pacemaker controls daily rhythms in behavioral, physiological and endocrine functions and is synchronized to the external light–dark cycle via the retinohypothalamic tract. The SCN are also involved in photoperiodic processes. Changes in day-length are perceived by the SCN, and result in a compression or decompression of the SCN ensemble pattern, which appears to be effectuated by changes in phase relationship among oscillating neurons. By simulation experiments, we have previously shown that the duration of the single unit activity pattern is of minor importance for the broadness of the population activity peak. Instead, the phase distribution among neurons is leading to substantial differences in the broadness of the population pattern. We now show that the combination of (i) changes in the single unit activity pattern and (ii) changes in the phase distribution among oscillating neurons is also effective to encode photoperiodic information. Moreover, we simulated the ensemble waveform of the SCN with recently recorded single unit electrical activity patterns of mice under long and short photoperiods. We show that these single unit activity patterns cannot account for changes in the population waveform of the SCN unless their phase distribution is changed. A narrow distribution encodes for short photoperiods, while a wider distribution is required to encode long photoperiods. The present studies show that recorded patterns in single unit activity rhythms, measured under long and short day conditions, can be used in simulation experiments and are informative in showing which attributes of the neuronal discharge patterns leads to the capacity of the SCN to encode photoperiod.

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## 1. Introduction

Virtually all living systems have developed ways to anticipate daily changes in their environment. This allows animals and plants to anticipate to changes that are bound to come, rather than to follow them passively. Daily changes caused by the earth's rotation around its axis, result in 24 h rhythms in environmental conditions, such

as temperature, water and food availability, and light. To anticipate 24 h rhythms in the environment, organisms have innate circadian systems, or clocks, that have a genetic basis for rhythm generation (Takahashi et al., 2001; Reppert and Weaver, 2002). For the proper functioning of these circadian systems, they have to be synchronized, or entrained, to the daily external cycle. The most important synchronizing stimulus in the environment is light, rather than the change of temperature or other environmental stimuli (Meijer and Rietveld, 1989; Morin and Allen, 2005). Photic information is either directly perceived by circadian clocks, or it is transmitted via specialized

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pathways between light recipient elements, and the clock (Groos, 1982). In mammals, light is perceived exclusively by the eyes (Meijer et al., 1999; Yamazaki et al., 1999), and the retinohypothalamic tract mediates light transmission to the site of the mammalian pacemaker: the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Moore and Lenn, 1972; Ralph et al., 1990; Nelson and Takahashi, 1991; Meijer et al., 1998; Yamazaki et al., 2000).

Seasonal changes in the environment are caused by the earth's rotation around the sun, resulting in changes in day-length in the course of the year. Changes in day-length are perceived by animals, and are used to determine the time of the year. Adaptations to the changing seasons can be observed in many different organisms, and are commonly referred to as 'photoperiodicity'. In mammals, information on day-length is transmitted to and processed by the SCN. As a result, the SCN plays a crucial role in controlling both daily and seasonal rhythms (Mrugala et al., 2000; Sumova et al., 1995, 2003). The rhythm generating capacity of SCN neurons is explained by a molecular feedback loop, in which protein products inhibit the expression of specific clock genes (Kume et al., 1999; Reppert and Weaver, 2002; Hastings and Herzog, 2004). Rhythms in clock gene expression or in their protein products can be recorded within the SCN (Abe et al., 2002; Reddy et al., 2002; Hastings et al., 2003; Hastings and Herzog, 2004; Hamada et al., 2004; Nakamura et al., 2005; Nagano et al., 2003; Maywood et al., 2006). The rhythms show sinusoidal patterns, and for most (but not all) clock genes, expression is high during the day and low during the night. Likewise, circadian rhythms can be recorded in electrical impulse frequency in the SCN (Gillette et al., 1993; Groos and Hendriks, 1982). Electrical impulse frequency of neuronal populations of the SCN is high during the day and

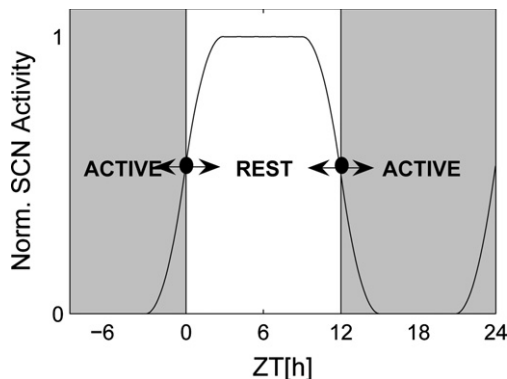


Fig. 1. SCN electrical activity in nocturnal rodents. Rats and mice are active during the night, when the electrical activity of the SCN is low, and rest during the day, when the electrical activity of the SCN is high. The peak width, or duration of electrical activity, was defined as the difference between the half-maximum amplitude of the rising and the declining phase. For short days, the resting phase becomes smaller and the activity phase becomes longer, while for long days, the resting phase increases and the activity interval decreases. In the figure, the darker background denotes nighttime, while the white background denotes daytime.

low during the night. The electrical impulses are thought to be a major output of the SCN (Schwartz et al., 1987) and carry information on the time of day to other parts of the brain, including the pineal gland. Under long or short photoperiods, the waveform changes that are generated by the SCN show remarkable changes. In long days, gene expression profiles show long durations of elevated activity, and electrical activity patterns are broad, while in short days, the expression profiles and electrical activity patterns show narrow activity peaks (Mrugala et al., 2000; Schaap et al., 2003; Sumova et al., 1995, 2003). Recordings of single cell electrical activity and of *Per1* gene expression profiles have shown that neurons show phase differences (Brown et al., 2005; Schaap et al., 2003; Yamaguchi et al., 2003; Quintero et al., 2003). Moreover, it has been

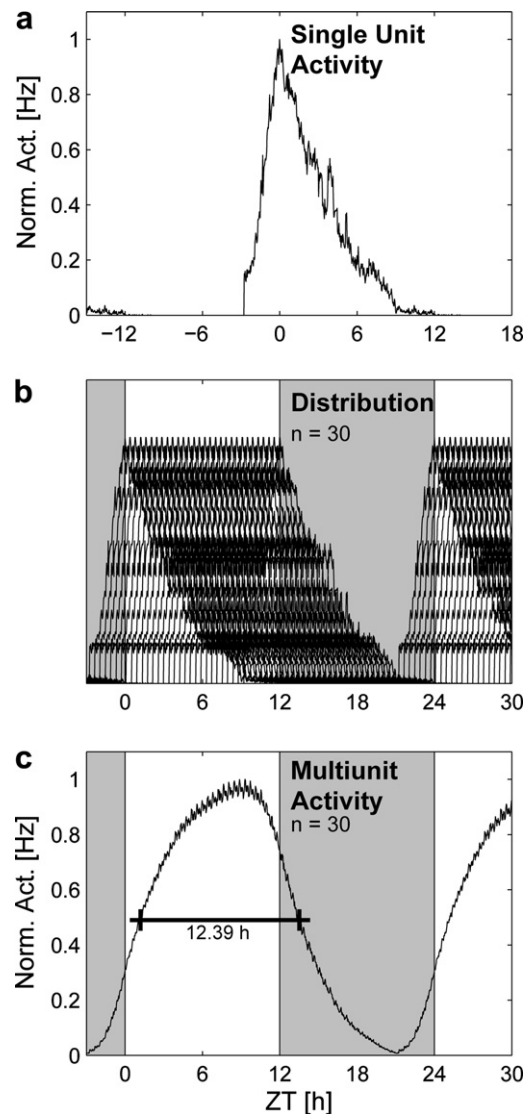


Fig. 2. Multiunit activity pattern obtained by linearly distributing a normalized experimentally obtained single unit activity pattern of a rat. (a) Average normalized single unit activity pattern of a rat measured in a 12 h:12 h light–dark regime. (b) 30 single units are linearly distributed over the day. (c) Summed activity of 30 units. The resulting multiunit activity pattern is normalized and shows a width of 12.39 h.

show that individual neurons of the SCN exhibit electrical activity patterns that are remarkably short as compared to the population waveform pattern.

Simulation studies have indicated that these phase differences may play an important role in the ability of the SCN to encode for day-length (Schaap et al., 2003; Rohling et al., 2006). It has been proposed that in short days, phase differences between neurons decrease, while in long days they increase. Recordings of mouse SCN neurons under short and long day-length have recently confirmed these predictions. Long term recordings of the electrical activity patterns of single SCN cells have shown an increment in phase distribution among oscillating neurons in long days and a decrease in phase distribution in short days (VanderLeest et al., 2007). While the precise phase distribution between the neurons is significantly different between long

and short days, the available data do not allow quantifying the distribution. In the present paper, we combine the results from rat and mouse SCN recordings with simulation experiments, and investigate the influence of different phase distributions between the neurons on the population activity patterns of the rat and mouse SCN.

## 2. Methods

A simulation environment has been created using Matlab to evaluate how single neurons can influence the ensemble electrical activity pattern of the SCN, and first described in Rohling et al. (2006). In the simulation environment, a number of single unit activity patterns could be distributed over the circadian cycle. We used different waveforms for the single unit activity patterns, or used measured single unit patterns (rat: Schaap et al., 2003; mouse: VanderLeest et al., 2007). The single unit activity patterns were established by calculating the mean single unit activity

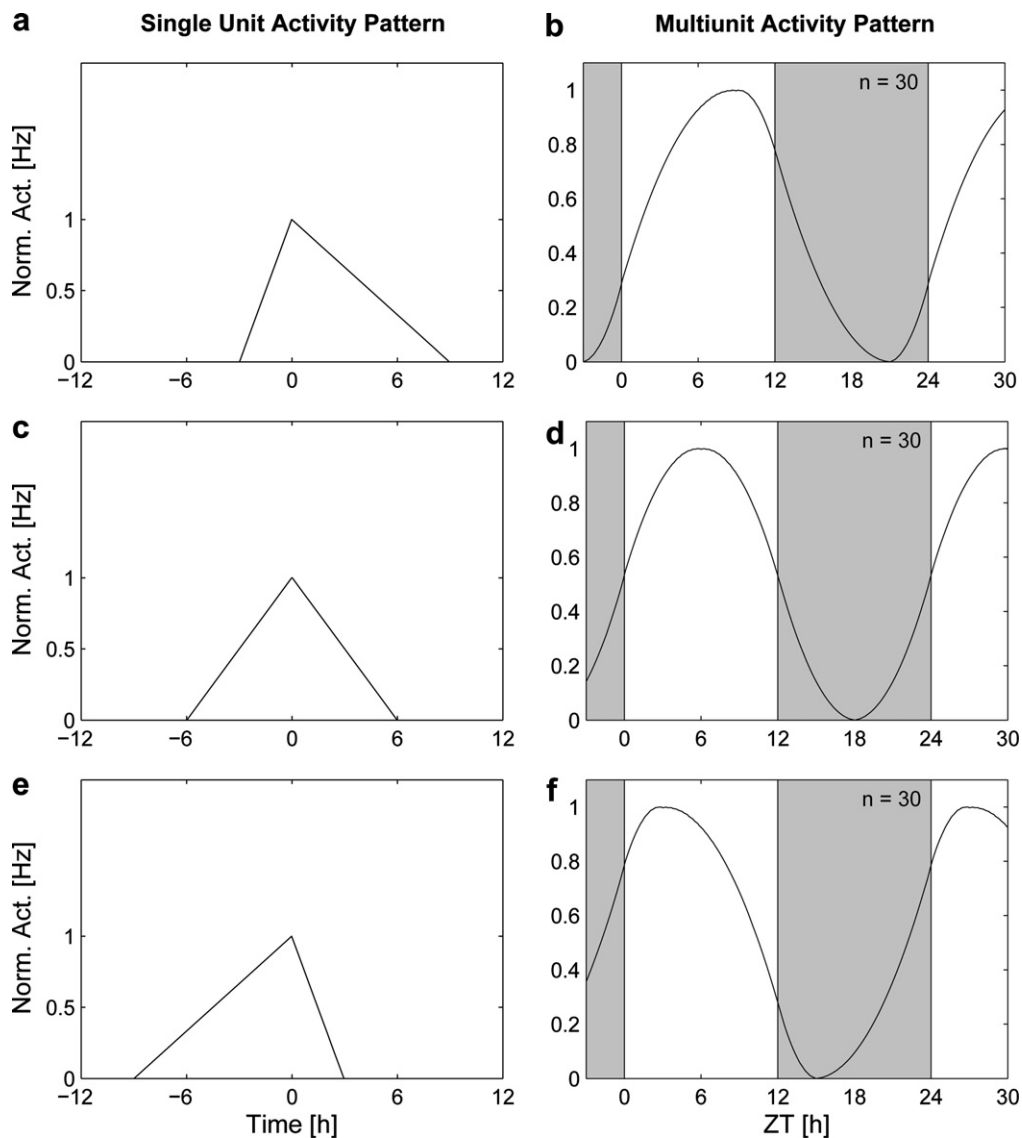


Fig. 3. Single unit activity pattern shape can affect the shape of the normalized multiunit activity pattern. Three different artificial single unit activity shapes (a, c, e) are used to obtain different multiunit activity patterns (b, d, f). To obtain the multiunit activity pattern, 30 single unit activity patterns were distributed according to a linear distribution in a 12 h:12 h light–dark schedule. The multiunit activity patterns on the right result from the corresponding single unit waveform on the left.

pattern from the different recorded units. For this purpose, the peaks of all normalized single unit activity patterns were aligned. The effects of different single unit activity patterns and of different distributions between these neurons on the multiunit activity pattern were evaluated.

We used linear and normal phase distributions, as described in Rohling et al. (2006). In the linear distribution, peak time of the neurons is distributed evenly over the day, while in the normal distribution, a Gaussian function was applied ( $e^{-(x-\mu)^2/2\sigma^2}/(\sigma * \sqrt{2\pi})$ ). In all figures, we show the results from linear distributions.

Multiunit activity patterns were quantified by their peak width. The peak width, or the duration of electrical activity, was defined as the time difference between the half-maximum amplitude of the rising and declining phase of the rhythm (Fig. 1). To enable comparisons between simulations, we plotted the data as normalized values (maximum activity = 1). Mice and rats are nocturnal and therefore active during the night. The SCN electrical activity patterns of rodents show high activity during the day and low activity during the night. Thus, the half-maximum amplitude of the rising phase of the rhythm coincides with activity offset, and the half maximum of the declining phase with activity onset. To gain insight in the adjustment of the SCN rhythm to long and short photoperiods, or in the compression or decompression of the SCN ensemble activity pattern, we determined the influence of single unit discharge patterns and phase distributions on the width of the electrical activity pattern of the SCN.

### 3. Results

Single unit activity patterns that have been measured in the rat and mouse are relatively narrow as compared to the population activity pattern. In rats, kept in 12 h light–

12 h dark schedules, the mean width of a single unit activity pattern is  $4.4 \pm 0.6$  h (Fig. 2a, Schaap et al., 2003). It has been shown that neurons show differences in phase (Brown et al., 2005; Schaap et al., 2003), and that their summed activity pattern accounts for the ensemble behavior of the population. A linear (Fig. 2b) and a normal distribution (data not shown) can both result in broad multiunit activity patterns (Fig. 2c), that resemble the recorded SCN waveforms in the rat (Brown et al., 2005; Schaap et al., 2003; Gillette et al., 1993; Prosser, 1998; Yannielli et al., 2004; Groos and Hendriks, 1982). Multiunit activity patterns are not only determined by the distribution of neurons but also by the circadian pattern of individual cells. Simulated discharge patterns show that the shape of single unit activity patterns affects not only the multiunit activity pattern, but also the peak time of the multiunit pattern. If the single unit activity pattern is characterized by a steep activity onset, and a slow activity offset, the multiunit activity pattern shows the opposite waveform and displays a slower onset and a faster offset (Fig. 3a and b). A symmetrical single unit pattern leads to symmetrical population patterns (Fig. 3c and d). When, vice versa, a single unit pattern has a slow onset and a fast offset, the resulting multiunit pattern has a steep onset and a shallow offset (Fig. 3e and f).

We also explored changes in the width of the single unit activity pattern and their effect on the broadness of the

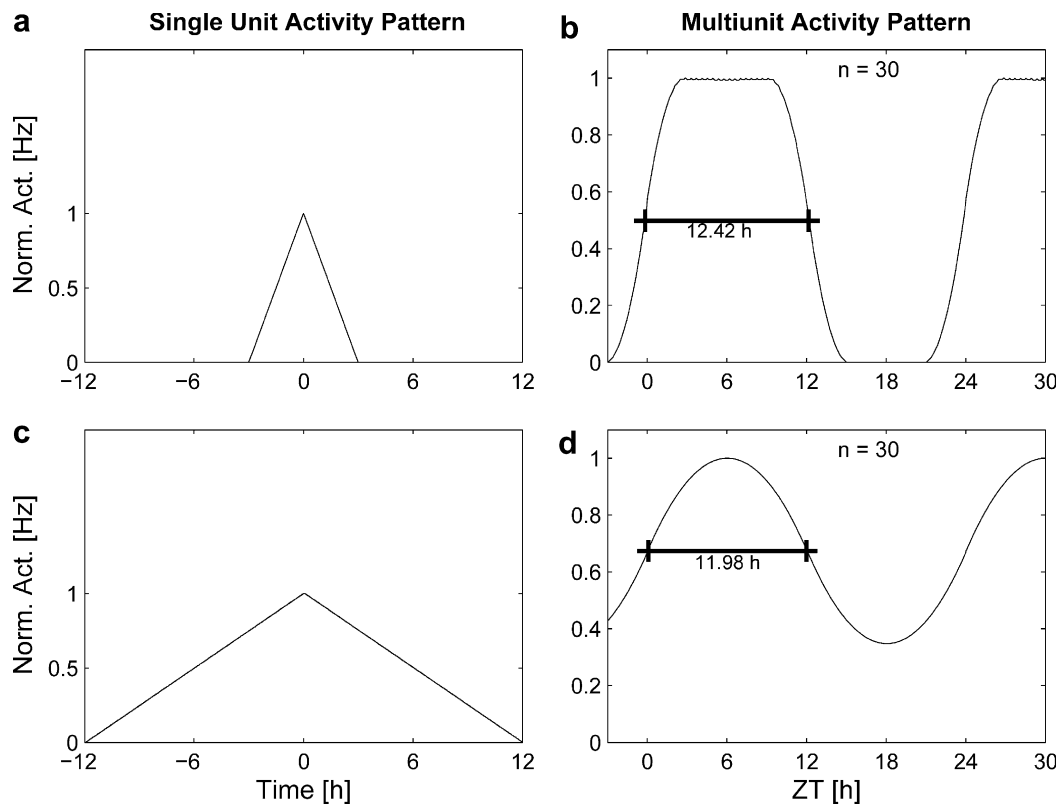


Fig. 4. The influence of single unit activity width on the width of the multiunit activity pattern. One narrow artificial single unit activity pattern (a) and one broad pattern (c) are used to obtain multiunit activity patterns by distributing the single unit patterns according to identical linear distributions over 12 h. The upper right panel (b) shows the normalized multiunit activity pattern resulting from the distribution of 30 single unit patterns as shown in (a). The lower right panel (d) shows the normalized multiunit activity pattern resulting from distributing 30 single unit activity patterns as shown in (c).

multiunit activity pattern. To this purpose, the single unit patterns were narrowed (by 50%) or broadened (doubled) while their phase distribution was kept constant. The width of the multiunit activity pattern was not changed significantly by this manipulation (Fig. 4). For narrow single units, a peak width of 12.42 h was obtained, while for broad single unit patterns, the multiunit peak width was 11.98 h.

When the phase distribution between neurons was changed, the width of the multiunit pattern altered significantly. For narrow distributions, a mean population peak width of 8.88 h was found, while for broad distributions, a peak width of 15.62 h was obtained (Fig. 5). A change in single unit activity pattern, in combination with a change in phase distribution, appeared to result in substantial effects on the population waveform as well. For narrow single unit activity patterns, in combination with narrow distributions of the neurons, we observed that the multiunit peak width was strongly compressed to 8.25 h, while the combination of a broad single unit pattern with a broad distribution resulted in a broader multiunit peak of 11.98 h (Fig. 6).

Fig. 7 shows measured single unit activity patterns in mice for short days and for long days. There were no significant differences between the average peak width of the mean neuronal discharge patterns under long and short days (Fig. 7a and b). However, the patterns were broader

during daytime than during the night, both for short and for long day-length (Fig. 7c–f; VanderLeest et al., 2007). In the present paper, we used these measured patterns to simulate encoding for day-length in the mouse SCN. When the same linear distribution was applied to the single unit discharge patterns, measured under short and long days, the resulting multiunit activity pattern was not significantly different (Fig. 8a and b). When the distribution was altered, on the other hand, significant changes in multiunit patterns were observed. A more narrow distribution was required to mimic narrow multiunit activity patterns, such as those recorded under short days, while a broadening of the distribution was required to mimic long day-length patterns (Fig. 8c and d).

#### 4. Discussion

Evidence strongly supports the conclusion that the photoperiodic measurement of time and the circadian rhythms in mammals are both controlled by the SCN (Sumova et al., 1995). Single unit electrical activity recordings in the SCN of rats, kept under 12 h light–12 h dark cycles have shown that single units are active for relatively short (up to 7 h) intervals of time (Brown et al., 2005; Schaap et al., 2003). In the rat, Schaap et al. (2003) showed a mean single unit activity pattern that is a-symmetrical, with a

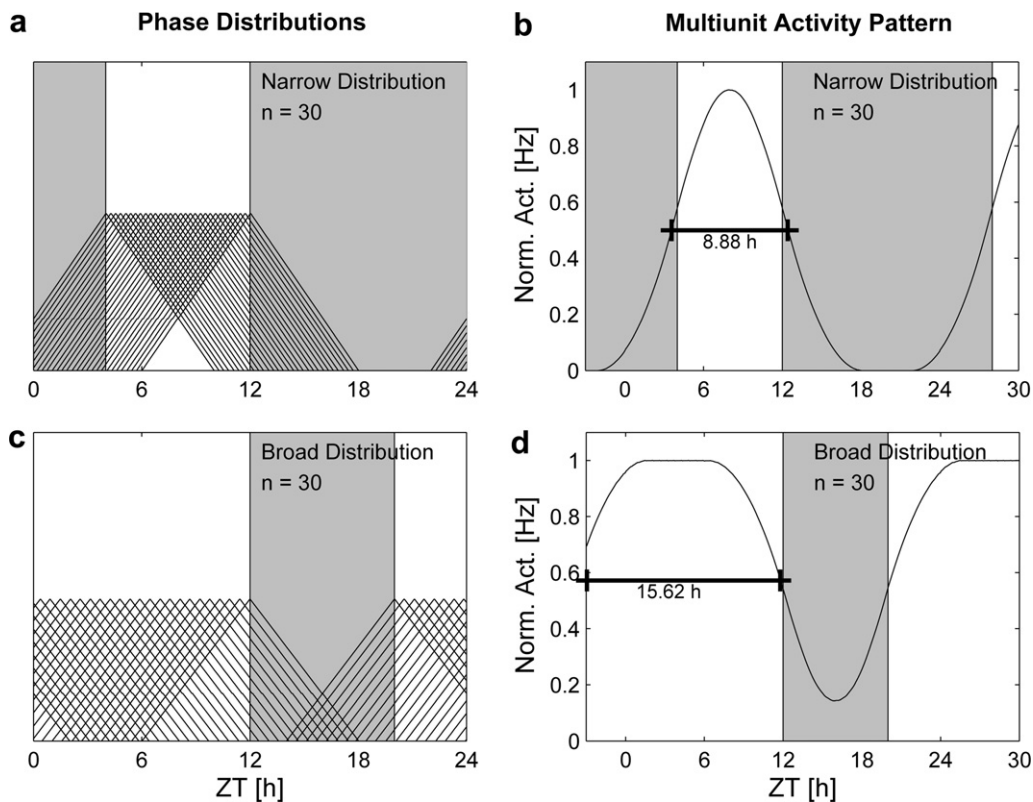


Fig. 5. The influence of the phase distribution of single unit patterns on the width of the multiunit activity pattern. One narrow linear phase distribution (8 h) (a) and one broad linear phase distribution (16 h) (c) are used to obtain multiunit activity patterns. The single unit patterns in (a) and (c) are identical. The upper panel (b) shows the multiunit activity pattern resulting from the distribution of 30 single unit patterns shown in (a). The lower panel (d) shows the multiunit activity pattern resulting from the distribution of 30 single unit patterns shown in (c).

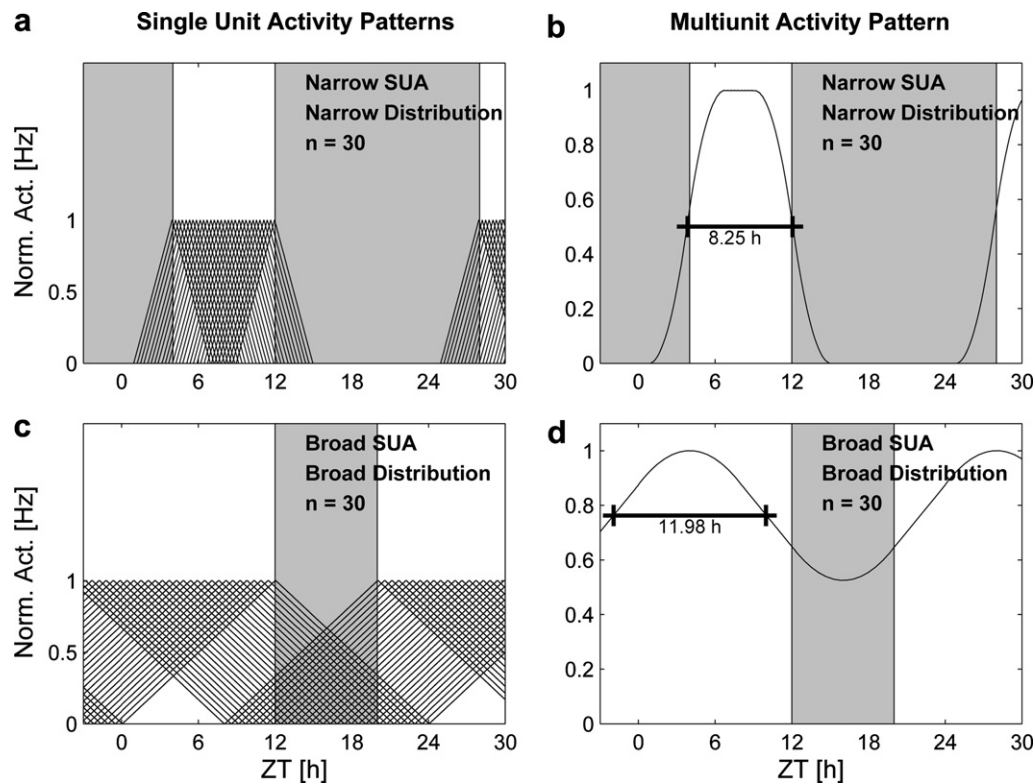


Fig. 6. The influence of the combination of phase distribution of single unit patterns together with single unit width on the width of the multiunit activity pattern. A narrow linear phase distribution, distributing 30 narrow artificial single unit activity patterns over 8 h (a) is used to obtain a multiunit activity pattern (b). This corresponds to a short day-length. A broad linear phase distribution which distributes 30 broad single unit activity patterns over 16 h (c) results in the multiunit activity pattern of (d). This corresponds to a long day-length.

step rising phase and a slower declining phase. This pattern results at the population level in a pattern that is gradually increasing and rapidly decreasing. This summed activity pattern may contrast the primary expectation, but is in fact consistent with multiunit activity patterns that have been described for the rat (Meijer et al., 1997; Schaap et al., 2003).

The observed phase differences between individual discharge patterns prompted us to investigate the role of phase differences in photoperiodic encoding processes within the SCN. A priori, one may expect that the waveform changes of the SCN under long and short photoperiod reflect a change in individual discharge patterns. As an alternative, one may propose that population waveform changes are caused by differences in phase distribution between oscillating neurons, while individual patterns do not change. We investigated these most extreme alternatives in a series of simulation studies.

Changes in the broadness of single unit discharge patterns were realized by decreasing the peak width to 50% of its initial value, or by doubling the width of the peak. The results indicated that these substantial changes in individual discharge patterns have little effect on the half-maximum electrical activity level. For linear distributions of neurons, this manipulation resulted, in fact, in a counterproductive effect on the population waveform, and the half-maximum discharge pattern narrowed as a conse-

quence of the broadening of the individual discharge pattern. For normal distributions, this manipulation resulted in a minor increment in peak width (Rohling et al., 2006). This counterintuitive result is explained, in part, by the increment in activity during the trough of the electrical activity pattern. This issue raises the question how the output signal of the SCN is actually read by downstream brain areas that receive the information. In other words, are downstream areas sensitive to changes in electrical activity pattern, and is the half maximum an indicator of the functional output signal, or alternatively, are these areas sensitive to the absolute discharge rate that is produced by the SCN. While changes in the electrical activity pattern were not effective in changing the broadness of the population signal at half-maximum discharge levels, they did increase the broadness of the peak at a fixed discharge rate. It will be of great importance to investigate, in vivo, by simultaneous SCN and behavioral recordings, how SCN electrical activity relates to behavioral activity levels.

While single unit activity waveform changes were not effective to change the broadness of the electrical activity pattern at half-maximum levels, changes in phase relation were most effective. Widening the phase relation in a linear phase distribution resulted in an increase in population peak width at half-maximum discharge levels (i.e. from 12.35 h to 15.62 h), while a decrease in phase relation resulted in a decrease in peak width (to 8.88 h). When

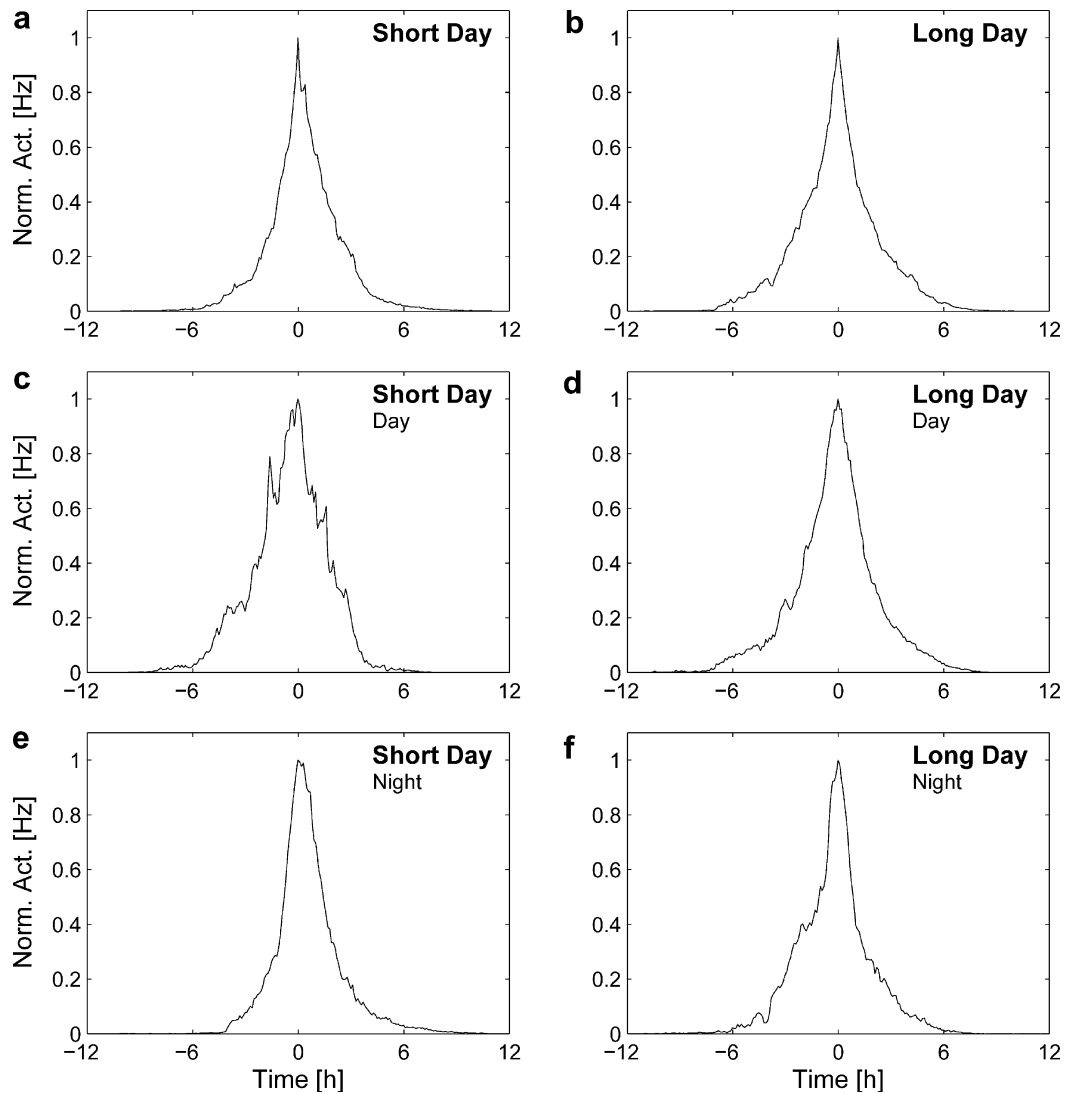


Fig. 7. Six average single unit patterns from mice measured in short and long days (taken from VanderLeest et al., 2007). The normalized average single unit activity patterns of mice are shown, for short and for long day-lengths. The width of the single unit patterns averaged over 24 h for short days is 3.24 h (a) and for long days 3.47 h (b). The widths of the average single unit patterns that were measured exclusively during daytime were, for short days 4.01 h (c) and for long days 3.44 h (d). The widths of the average single unit patterns measured during the night were 2.80 h (e) and 3.14 h (f) for short and long days respectively.

absolute discharge levels were investigated, this manipulation was equally effective and broadness of the peaks under short, normal and long photoperiod were 8.9 h, 12.4 h and 16.5 h respectively.

Although our simulations indicate that changes in phase distribution are an effective way to code for photoperiod, they do not exclude the possibility that coding for photoperiod involves a combination of the two processes, i.e. a change in individual waveform patterns, and a change in phase distribution among the neurons. Simulation studies show that a combination of these manipulations, also result in waveform changes at the population level. A decrement in phase relation, together with a decrement in single unit peak width results in a narrow population electrical activity peak. An increment in phase relation together with an increment in single unit peak width causes an increased

peak width, and a considerable decrease in the amplitude of the rhythm, due to a substantial rise in the trough. As the compression and decompression of the population discharge pattern was already observed by a change in phase distribution alone, we conclude that the change in single unit activity width is of minor importance for the system to code for photoperiod, but that large changes in single unit activity patterns may also occur. While changes in broadness of individual discharge patterns play a minor role for the waveform of the population signal, changes in phase distribution appear to be essential in coding for day-length.

Experimental recordings of single SCN neurons of the mouse have been performed after the animals were entrained to long (LD 16:8) and short (LD 8:16) light dark cycles. This procedure resulted in changes in multiunit

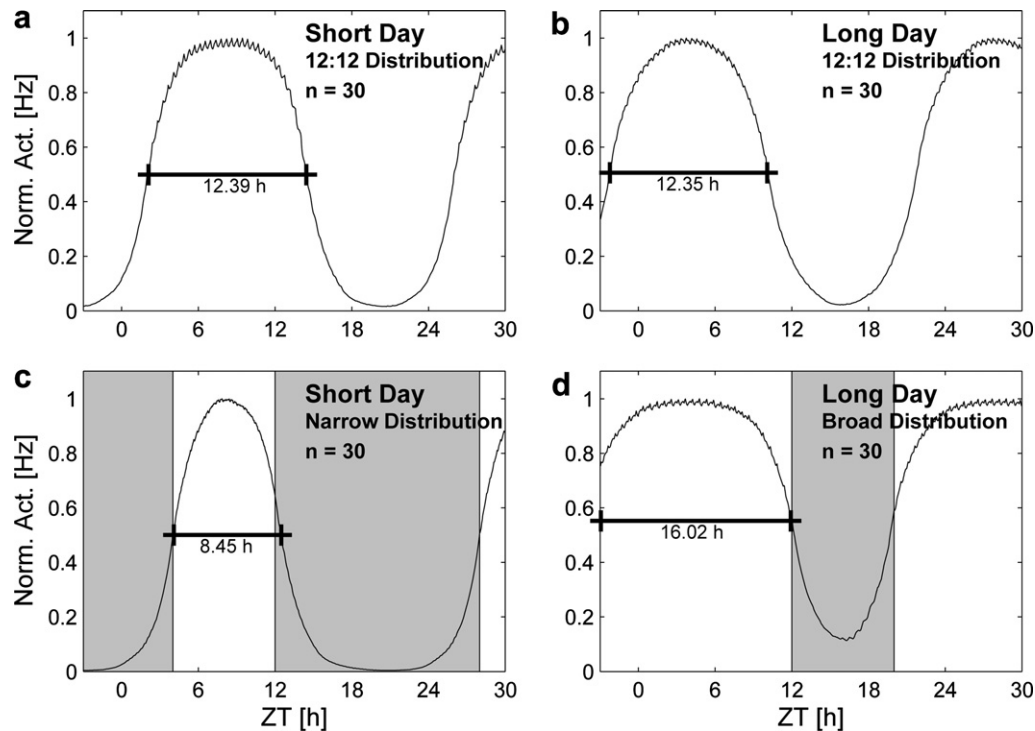


Fig. 8. The influence of averaged single unit activity patterns of mice for short and long day-length on the multiunit activity pattern. (a) Multiunit activity pattern with a width of 12.39 h is obtained by distributing 30 average single unit patterns for a short day-length (see Fig. 7a) according to a linear distribution over 12 h. (b) A multiunit activity pattern of comparable width (12.35 h) is obtained by distributing 30 average single unit activity patterns for a long day-length (see Fig. 7b) according to the same linear distribution as used in (a). These simulations did not result in multiunit patterns that carry day-length information. (c) A narrow distribution of 30 average single unit patterns for a short day-length (see Fig. 7a) distributed over 8 h results in a narrow multiunit activity pattern (8.45 h). (d) A broad linear distribution of 30 average single unit activity patterns for a long day-length (see Fig. 7b) distributed over 16 h results in a broad multiunit activity pattern (16.02 h). All multiunit patterns are normalized.

waveform patterns in slices containing the SCN, and in vivo recordings showed that these photoperiod-induced changes remained consistent for at least 4 days after release in constant darkness (VanderLeest et al., 2007). Single unit activity recordings revealed little difference between the duration of electrical activity patterns of single neurons under long and short days (3.47 h and 3.24 h respectively). While we cannot exclude that an increase in the number of recorded neurons would reveal differences in individual waveform changes, we stress that small changes are not sufficient to result in the substantial population discharge patterns that are recorded in rats, hamsters and mice (recording studies: Mrugala et al., 2000; VanderLeest et al., 2007; Schaap et al., 2003; simulation: Rohling et al., 2006; this study). We can also not exclude that specific subsets of neurons exist within the SCN that do follow the photoperiod, and that we have missed in our recordings. Finally, we cannot exclude that other parameters, such as a gene expression profiles, do change with photoperiod. For instance, some genes may reflect the electrical activity pattern of the SCN as a whole, and may therefore follow the population discharge pattern. While all these uncertainties exist, the recording studies in mice revealed unequivocally that electrical activity patterns in mouse from long and short days show clear differences in phase relation. In long days, a large distribution of phases was

observed, with many neurons that peaked also in the 'silent' phase of the cycle (i.e. the subjective night), while in short days the neurons showed a much tighter synchrony in terms of their phase differences. The small phase differences in short days result not only in narrow population activity patterns, but also in an increment in circadian amplitude. Vice versa, an increase in phase difference results not only in a broadening of the multiunit activity pattern. These effects of long and short day on circadian amplitude have been described for different clock genes, and for rat electrical activity rhythms (Schaap et al., 2003; Sumova et al., 1995, 2003).

The simulations of the present paper show how the measured single unit activity patterns may contribute to the population signal under short and long day-lengths. In these simulations, we incorporated the finding that in long day-length, the distribution is significantly larger (VanderLeest et al., 2007). We applied a linear distribution and a normal distribution to the measured neuronal discharge patterns, and investigated the outcome for the population discharge pattern. We choose for these distributions as insufficient single units have been recorded to characterize and quantify the distribution of neurons within the SCN ( $n = 26$  under both photoperiods), and we believe that a multitude of these numbers would be required to describe this distribution. In fact, our finding may still be consistent



with unimodal (Yamaguchi et al., 2003), bimodal (Jagota et al., 2000; Pittendrigh and Daan, 1976) or trimodal (Quintero et al., 2003; Meijer et al., 1997) distributions, and for all of these distributions there is evidence in the literature.

The pineal gland is considered to play an important role in photoperiodic time measurement. The circadian oscillator in the SCN is connected to the pineal gland via a multisynaptic neural pathway (Moore, 1996). The hormone melatonin is secreted by the pineal and its synthesis is stimulated by the SCN (Goldman, 2001). The melatonin production is low during the day and high during the night. This inverse relation between the length of the day and the duration of melatonin secretion is found in many mammals and during nighttime, the melatonin production can be suppressed by light (Nelson and Takahashi, 1991). The duration of the melatonin production serves as a photoperiodic message, as the length of the day is encoded in the melatonin signal and decoded in the target tissues of the hormone. The signal is compressed during long summer days and decompressed during short winter days. Our present simulations were based on recordings in C57 mice. These mice have no melatonin which raises the question whether C57 mice are a good model to study photoperiodicity. In experiments where the pineal gland is removed, a loss of melatonin leads to the inability of the reproductive system and body fat regulatory systems to discriminate between long and short days. However, entrainment of circadian rhythms to cycles of light and darkness proceeds in the absence of melatonin (Goldman, 2001). We observed that C57 mice responded to the photoperiod with a change in their behavioral activity pattern, as well as with a change in their SCN electrical activity rhythms. We believe therefore that photoperiodic changes in behavioral activity are independent from melatonin, but are correlated with the waveform of the SCN.

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