

Research report

Serotonin antagonists do not attenuate activity-induced phase shifts of circadian rhythms in the Syrian hamster

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Abstract

A variety of observations from several rodent species suggest that a serotonin (5-HT) input to the suprachiasmatic nucleus (SCN) circadian pacemaker may play a role in resetting or entrainment of circadian rhythms by non-photic stimuli such as scheduled wheel running. If 5-HT activity within the SCN is necessary for activity-induced phase shifting, then it should be possible to block or attenuate these phase shifts by reducing 5-HT release or by blocking post-synaptic 5-HT receptors. Animals received one of four serotonergic drugs and were then locked in a novel wheel for 3 h during the mid-rest phase, when novelty-induced activity produces maximal phase advance shifts. Drugs tested at several doses were metergoline (5-HT_{1/2} antagonist; i.p.), (+)-WAY100135 (5-HT_{1A} postsynaptic antagonist, which may also reduce 5-HT release by an agonist effect at 5-HT_{1A} raphe autoreceptors; i.p.), NAN-190 (5-HT_{1A} postsynaptic antagonist, which also reduces 5-HT release via an agonist effect at 5-HT_{1A} raphe autoreceptors; i.p.) and ritanserin (5-HT_{2/7} antagonist; i.p. and i.c.v.). Mean and maximal phase shifts to running in novel wheels were not significantly affected by any drug at any dose. These results do not support a hypothesis that 5-HT release or activity at 5HT₁, 2 and 7 receptors in the SCN is necessary for the production of activity-induced phase shifts in hamsters. © 1998 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Circadian rhythms of rodents can be synchronized to daily cycles of light–dark (LD), and several non-photic stimuli, including food access [28] and locomotor activity [10,19,26,38]. Activity has proven to be a powerful zeitgeber, capable of producing very large phase shifts and period changes of free-running rhythms [14,38,45,46] and capable of altering the phase angle of entrainment to LD cycles [27,31,42] and subsequent free-running period [27,39].

There is substantial evidence that phase resetting effects of activity on circadian rhythms are mediated by a neuropeptide Y (NPY) containing pathway from the thalamic intergeniculate leaflet (IGL) to the dominant circadian pacemaker located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus. Three hour bouts of activity

stimulated by confinement to a novel wheel during the middle of the subjective day (the rest phase in nocturnal species) can induce phase advance shifts of 2–3 h in hamsters. These shifts can be mimicked by microinjection of NPY into the SCN area, both in vivo and in SCN slices in vitro [1,16,22]. In addition, SCN microinjections of antiserum raised against NPY prevent activity-induced phase shifts without affecting the level of activity in response to wheel confinement (WC) [3]. Lesions of the IGL that eliminate NPY immunoreactivity in the SCN prevent phase shifting to WC or saline injections in hamsters [17,21] and prevent entrainment to forced treadmill running schedules in mice [20].

Another neurotransmitter that has been implicated in activity-induced resetting of circadian rhythms is serotonin (5-HT). The SCN in hamsters receives a large serotonergic input from the median raphe [24] and 5-HT release is increased in the SCN in response to increased activity during the mid-subjective day [8,9]. Serotonin agonists (8-OH-DPAT) have been shown to induce phase advances when administered systemically to hamsters [7,44] as well as to rats [11] in the mid-subjective day. These phase shifts

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are not dependent on the expression of activity, which is consistent with a role for 5-HT as a mediator of activity-induced shifts, rather than as merely an inducer of activity [4]. In addition, direct application of 5-HT agonists to the rat SCN *in vitro* induces phase shifts of electrical firing rates that are similar to phase shifts of behavioral rhythms induced by locomotor activity *in vivo* [35,36]. These *in vitro* shifts appear to be mediated by a 5-HT₇ receptor [18]. Finally, neurotoxic lesions of the serotonergic terminals in the mouse SCN prevent entrainment to daily scheduled activity [12,20]. Other studies have also reported attenuation of activity-induced phase shifts following 5-HT lesions in hamsters, but these may have been confounded by a decreased running response to the non-photic stimulus [7,32].

Despite the convergence of evidence implicating 5-HT in the induction of phase shifts by activity, some studies fail to support this hypothesis. In two studies, neurotoxic lesions of 5-HT input to the SCN did not prevent activity-induced phase shifts in hamsters [6,23]. Also, at least one study has reported that 8-OH-DPAT does not induce phase shifts in the hamster when microinjected directly to the SCN, although it does produce shifts when delivered systemically or into the raphe nuclei, where it presumably inhibits activity of 5-HT neurons [25].

The present study was designed as an additional test of the 5-HT hypothesis of non-photic phase shifting. If 5-HT is necessary for non-photic phase shifting, then it should be possible to block or attenuate activity-induced phase shifts pharmacologically with drugs that block or attenuate 5-HT activity. One recent study has shown that injections of ritanserin, a 5-HT_{2/7} receptor antagonist, can attenuate, but not prevent, phase shifts to arousal in response to handling and a saline injection late in the subjective day in hamsters [43]. However, it is conceivable that 5-HT antagonists might affect shifting by attenuating the arousal response to the injection procedure, and thereby reducing the salience of the non-photic stimulus. In the present study, we used systemic and central injections of 5-HT antagonists (ritanserin, metergoline, (+)-WAY100135) and mixed agonists (NAN-190) to evaluate whether 5-HT release and binding at 5-HT₁, 5-HT₂ or 5-HT₇ receptors is necessary for activity-induced phase shifting in hamsters.

2. Method

2.1. Subjects

Male adult Syrian Hamsters were obtained from Charles River (St. Constant, PQ, Canada). At the onset of the experiments, hamsters receiving systemic injections were 80–100 g, and hamsters receiving intra-SCN cannulae were 100 to 120 g. All animals were housed in ventilated isolation cabinets with a 14:10 LD cycle. Hamsters had *ad libitum* access to food and water.

2.2. Apparatus

Hamsters were housed in polypropylene cages (45 × 25 × 20 cm) with wire floors and 17 cm running wheels. Rotation of the wheel was detected by a microswitch connected to an interface and 486 PC (Activity Counting System, Simon Fraser University). Data were collected in 10 min time bins and periodically transferred to a Macintosh computer for display and analyses.

Wheel confinement was performed in either a Wahmann Wheel (33 cm) or in a plastic cage similar to the home cages, but with wire mesh surrounding the 17 cm wheel to keep the hamster locked inside. The percentage of animals that run continuously tends to be greater in the larger Wahmann wheels, although activity counts are sometimes less given the larger circumference. An unpublished analysis of one set of data in this lab determined that the amount of activity during WC and the magnitude of resulting phase shifts did not differ significantly between the two types of activity cages ($n = 27$ per group).

2.3. Drugs

The four drugs used in the present study were metergoline (Research Biochemicals International, Natick, MD), (+)-WAY100135 (also known as WAY100478 and a gift of Wyeth Ayerst, USA), NAN-190 (RBI) and ritanserin (RBI). Metergoline is a non-specific 5-HT_{1/2} antagonist which has been demonstrated to block the phase shifting effects of 8-OH-DPAT in the rat hypothalamic slice preparation [35]. A dose of 2 mg/kg s.c. was used in DMSO vehicle.

(+)-WAY100135 is a 5-HT_{1A} antagonist that has been reported to have agonist properties at the somatodendritic autoreceptor in the rat [2] and the cat [13] and thus decreases 5-HT release. 8-OH-DPAT-induced phase shifts of hamster circadian rhythms have been blocked with the 5-HT_{1A} antagonist pindolol [44], which has little affinity for the 5-HT₇ receptor [18], suggesting that 5-HT_{1A} receptors may play a role in non-photic phase shifting in hamsters. Thus, (+)-WAY100135 allowed a test of the 5-HT hypothesis in two ways, by blocking post-synaptic 5-HT_{1A} receptors and by activating raphe somatodendritic autoreceptors, thereby decreasing 5-HT release. Doses of 10 and 15 mg/kg s.c. were used in a saline and polyethylene glycol vehicle (0.75 mg/ml).

The mixed agonist NAN-190 binds with high affinity to 5-HT_{1A} receptors, and with fairly high affinity to 5-HT₇ receptors [18]. It decreases 5-HT release, presumably by activating 5-HT somatodendritic receptors in the raphe [41] and blocks postsynaptic 5-HT_{1A} receptors [15]. Like (+)-WAY100135, NAN-190 allowed a test of the 5-HT hypothesis in two ways, by decreasing 5-HT release and by blocking post synaptic 5-HT_{1A/7} receptors. Doses of 5 and 10 mg/kg i.p. in 100% DMSO vehicle were used.

The final drug used was ritanserin, a 5-HT_{2/7} receptor antagonist. This drug prevents 8-OH-DPAT-induced phase shifts in the rat hypothalamic slice [18]. It also prevents 5-HT-induced attenuation of photic activation of SCN cells [47] and attenuates arousal-induced phase shifts in Syrian hamsters [43]. Systemic doses were 2 and 4 mg/kg i.p. in DMSO vehicle, and intra-SCN doses were 1, 2, 8, 16 and 32 nmol in 5 μ l DMSO. DMSO neither induces phase shifts nor modulates phase shifts in response to light or activity (unpublished observations and present study).

2.4. Surgery

Hamsters used for central injections were anesthetized with 80 mg/kg i.p. of sodium pentobarbital. Coordinates for the placement of the 9 mm, 22 gauge guide cannula (Plastics One, Roanoke, VA) were 0.3 mm lateral to the midline, 7.0 mm ventral to the surface of the skull, with the anterior/posterior position set at bregma. The incisor bar was set at 2 mm below the inter-aural level. The cannula was fixed to the skull with four jeweler's screws and dental cement. A dust cap/dummy cannula was placed in the guide cannula and removed only for injections, after which it was replaced. The length of the injection cannula and dummy cannula were such that they extended 1 mm beyond the tip of the guide cannula.

2.5. Histology

At the conclusion of the study, animals which had been implanted with cannulas were injected with an overdose of pentobarbital. The animals were perfused transcardially with saline, followed by 10% formalin. Brains were removed, and fixed for at least 24 h in formalin, followed by a 24 h post-fix in sucrose-formalin for cryoprotection. Coronal sections (50 μ m) through the hypothalamus were collected with a freezing stage microtome, mounted on slides, and stained for Nissl substance with cresyl violet. Sections were examined under a microscope to determine placement of the tip of the cannula. Data from animals with placements more than 500 μ m from the border of the SCN were excluded from analysis.

2.6. Procedure

Phase shifts in response to WC were measured using the Aschoff type II design [30]. Animals were entrained to a 14:10 LD cycle and received an injection of the test drug 7 h prior to dark onset (zeitgeber time 5, ZT5, where ZT12 is dark onset, by convention). For central injections, animals were gently restrained while the drug was administered slowly over 30 s, and the cannula left in place for another 30 s to minimize backflow. At ZT6, animals were locked in a novel activity wheel. Fresh sawdust bedding was placed under the wheels, as this appears to enhance running activity. DD was started when the animals were

placed into the activity cages. Animals were returned to their home cages after 3 h (i.e., at ZT9) under dim red light, or with the aid of an infrared camera (Find-R-Scope™). The animals were left in dim red light (RR, $\sim$ 1 lux), or constant darkness (DD, $<$ 0.1 lux) for 3 to 5 days, after which they were re-entrained to the LD cycle for at least 10 days before the next test.

2.7. Data analysis

The onset of nocturnal activity was used to measure phase shifts. Onsets were defined as the first bin containing 50 counts after an interval of 240 min in which activity did not exceed this level in any 10 min bin. The count requirement was relaxed for a few animals that on some days did not reach an activity level of 50 counts in one bin, but in these cases the criteria were consistent between pre and post manipulation onsets. Onset times were determined for the 5 days prior to the manipulation and an average taken. Onset times were determined for 3 days post manipulation, the second of which was used for analysis of the phase shifts induced by the manipulation. In a few cases, onset on day 3 was used due to equipment failure or hypoactivity on day 2. Our previous studies have shown that phase shifts measured on day 2 and 3 following a running stimulus in the middle of the light period are not significantly different using the Aschoff type II method in hamsters.

Repeated measures ANOVAs with Student–Newman–Keuls post-hoc tests, or paired *t*-tests were used to detect significant effects of WC and drug treatments on circadian

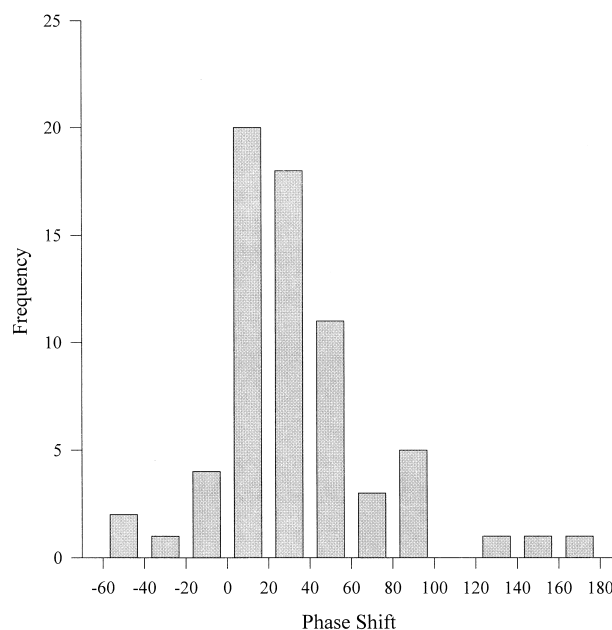


Fig. 1. Frequency histogram of phase shifts to a control DD test ($n = 67$). DD was started at ZT6, and phase shifts were assessed on the second full day of DD.

phase and activity levels. Means are reported with their standard deviations.

3. Results

3.1. Control tests

Animals released into DD at ZT6 without drug injections or WC exhibited a mean phase advance of 35 ± 39 min ($n = 67$) on day 2 of DD. Fig. 1 presents a frequency

histogram of the phase shifts produced by the type II design. The modal phase shift was 0–20 min in size.

3.2. Metergoline

Four conditions were tested; DD alone, WC alone, DMSO + WC, and metergoline (2 mg/kg) + WC. Preliminary studies showed that metergoline alone does not produce phase shifts in the subjective day or night (unpublished observations; and Ref. [29]) Fig. 2A presents the mean and maximum phase shifts for all conditions, and a

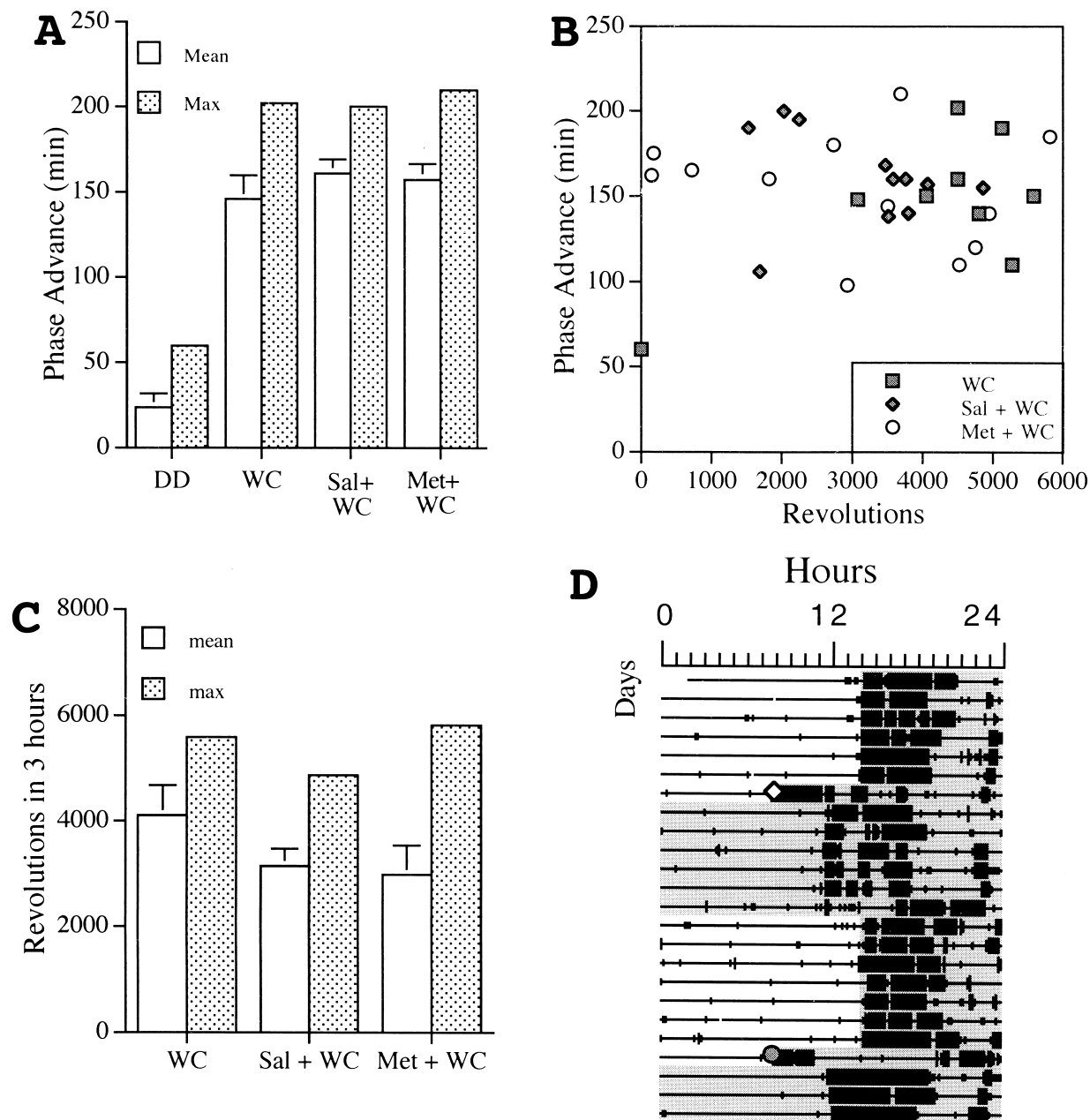


Fig. 2. Metergoline (2 mg/kg s.c.). (A) Mean (+S.E.) and maximum phase shift of each treatment conditions. (B) Scatterplot of raw data (activity level vs. phase shift) for each WC treatment condition. (C) Mean (+S.E.) and maximum activity level produced in each WC condition. (D) Sample actogram of one animal which received both (a) novel WC and (b) novel WC with metergoline.

scatterplot of the raw data is provided in Fig. 2B. A repeated measures ANOVA detected a significant effect of treatment ($F_{3,30} = 47.504$, $p < 0.05$). Post-hoc tests determined that phase advances produced in the DD control condition were significantly smaller than those found in all other conditions. However, there was no significant difference between the metergoline + WC and the DMSO + WC conditions (paired $t_{11} = 0.833$, $p > 0.05$; e.g., Fig. 2D). Mean levels of activity-induced by WC also did not differ between the groups ($F_{2,17} = 1.348$, $p > 0.05$; Fig. 2C).

3.3. (+)-WAY100135

Six conditions were tested; DD alone, (+)-WAY100135 (15 mg/kg) alone, WC alone, saline + WC, (+)-WAY100135 (15 mg/kg) + WC, and (+)-WAY100135 (10 mg/kg) + WC. Means and raw data are plotted in Fig. 3A, B, respectively. A repeated measures ANOVA (excluding the (+)-WAY100135 alone condition which came from a separate set of subjects, and which did not produce a phase shift different from DD alone) detected a signifi-

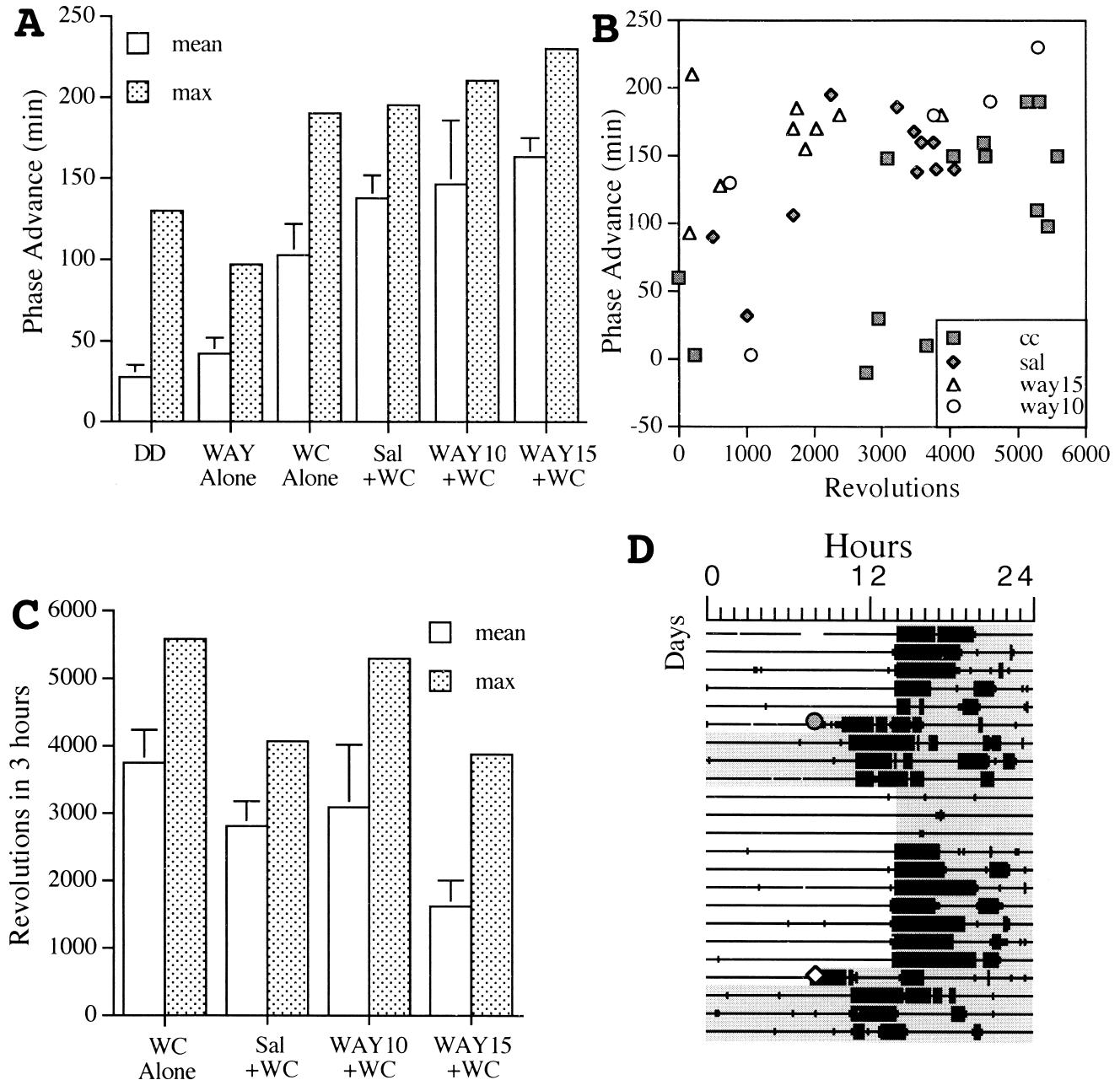


Fig. 3. (+)-WAY100135 (10 and 15 mg/kg s.c.). (A) Mean (+S.E.) and maximum phase shift of each treatment conditions. (B) Scatterplot of raw data (activity level vs. phase shift) for each WC treatment condition. (C) Mean (+S.E.) and maximum activity level produced in each WC condition. (D) Sample actogram of one animal which received both (a) (+)-WAY100135 (15 mg/kg) and novel WC and (b) saline and novel WC.

cant effect of treatment ($F_{4,33} = 15.57$, $p < 0.05$). Post-hoc tests determined that the DD alone condition yielded significantly smaller phase shifts than those observed in all other conditions, and that (+)-WAY100135 (10 or 15 mg/kg) did not significantly attenuate the phase shifts induced by WC (e.g., Fig. 3D). An injection of (+)-WAY100135 alone did not produce a significant shift compared to DD alone (paired $t_9 = -1.34$, $p > 0.05$; Fig. 3A).

A repeated measures ANOVA revealed a significant effect of treatment on the amount of wheel running during WC ($F_{2,11} = 21.144$, $p < 0.05$; Fig. 3C). Post-hoc tests determined that (+)-WAY100135 (10 mg/kg) + WC and

saline + WC did not differ, but both were significantly greater than (+)-WAY100135 (15 mg/kg) + WC.

3.4. NAN-190

Two doses (5 and 10 mg/kg) of NAN-190 were tested, using separate groups of hamsters. Each group received DMSO + WC, as well as NAN-190 + WC. Mean phase shifts in the two dose conditions were not significantly different (independent $t_{20} = 0.222$, $p = 0.83$), thus the data were combined. There was no significant difference in phase shifts between the NAN-190 + WC and DMSO + WC conditions ($t_{21} = -0.899$, $p > 0.05$; Fig. 4A, D).

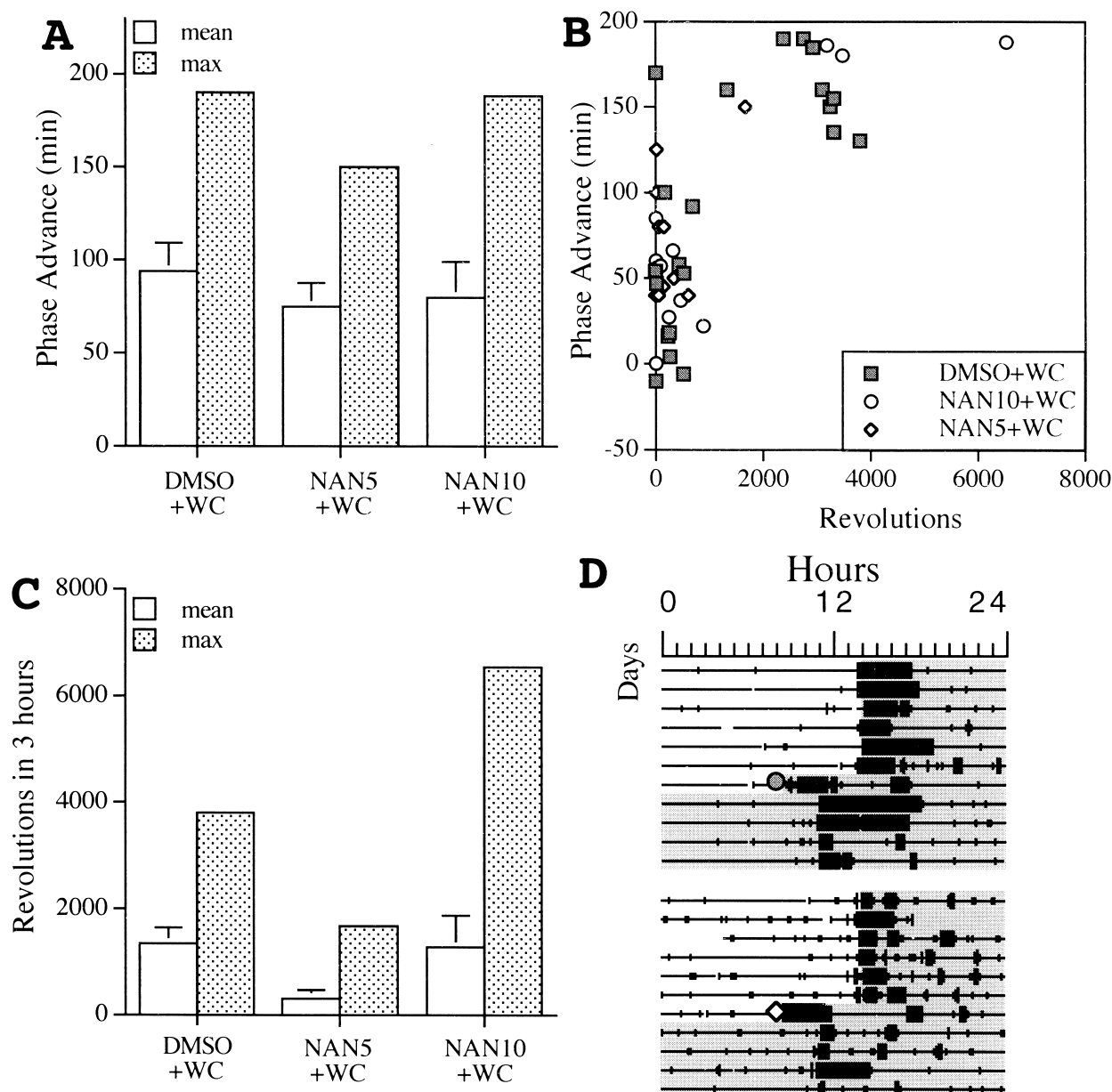


Fig. 4. NAN-190 (5 and 10 mg/kg i.p.). (A) Mean (+S.E.) and maximum phase shift of each treatment conditions. (B) Scatterplot of raw data (activity level vs. phase shift) for each WC treatment condition. (C) Mean (+S.E.) and maximum activity level produced in each WC condition. (D) Sample actogram of one animal which received both (a) NAN-190 (10 mg/kg, i.p.) with novel WC and (b) DMSO with novel WC.

A scatterplot reveals that a substantial number of animals did not run during WC in both drug and control conditions (Fig. 4B). As activity-induced phase shifts are not expected without activity, the data were reanalyzed using only those animals exhibiting activity greater than 1000 revolutions in 3 h. One animal from the NAN-190 (5 mg/kg) + WC group met this criterion (1672 revolutions, 150 min phase advance) and three animals from the NAN-190 (10 mg/kg) + WC group met the criterion (mean activity, 4399; mean phase advance 184 ± 4 min), as did 9

DMSO + WC animals (mean activity, 2905; mean phase advance 162 ± 23 min). A *t*-test determined that phase shifts of the NAN-190 treated animals (pooled for analysis) did not differ from the phase shifts observed in the DMSO treated animals ($t_{11} = 1.12$, $p > 0.05$).

Mean activity levels for the whole data set are presented in Fig. 4C. Despite the low activity during WC, neither the NAN-190 (10 mg/kg) + WC group nor the NAN-190 (5 mg/kg) + WC group differed from their respective DMSO + WC control conditions in induced activity ($t_{11} =$

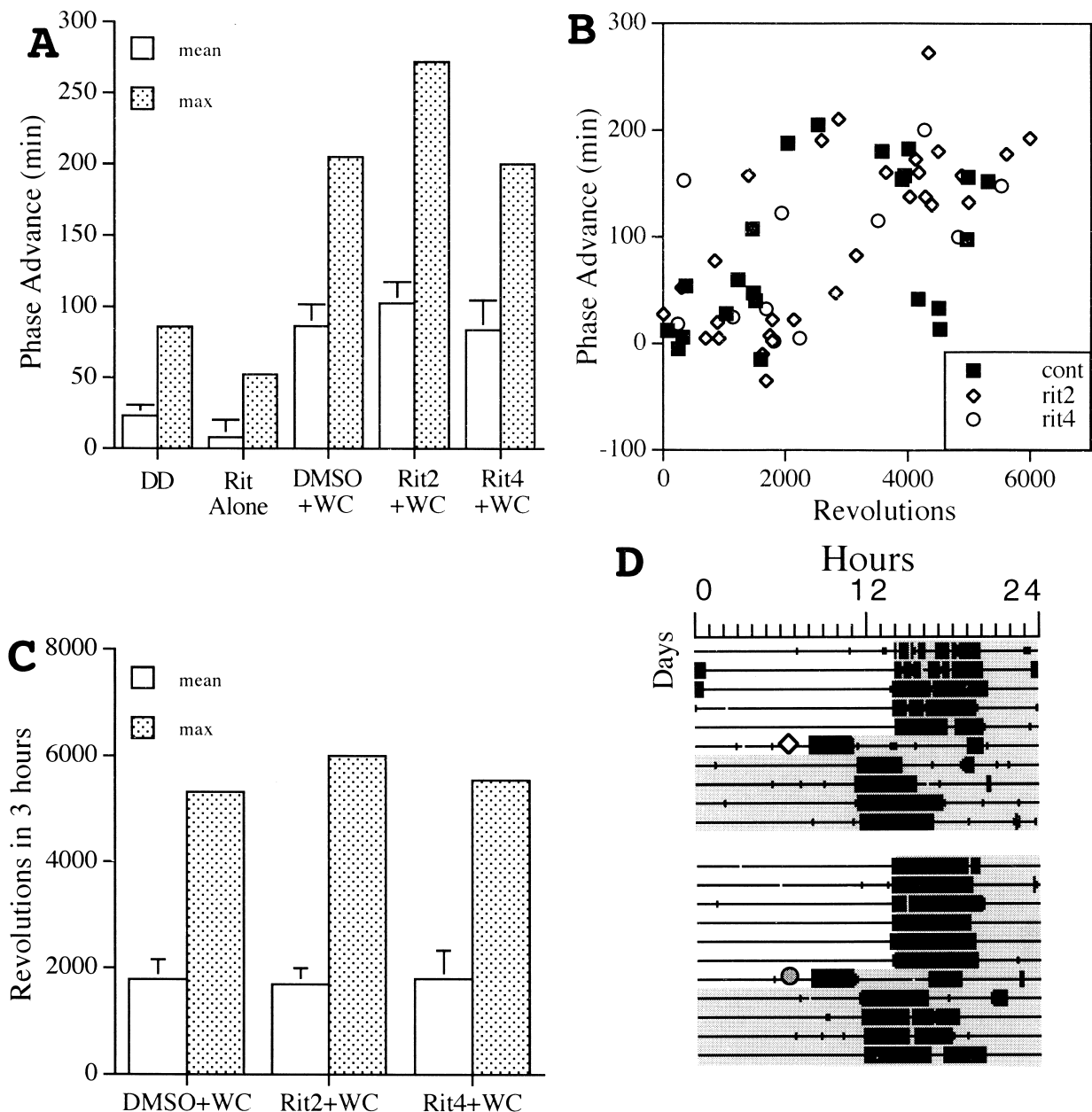


Fig. 5. Systemic ritanserin (2 and 4 mg/kg, i.p.). (A) Mean (+S.E.) and maximum phase shift of each treatment conditions. (B) Scatterplot of raw data (activity level vs. phase shift) for each WC treatment condition. (C) Mean (+S.E.) and maximum activity level produced in each WC condition. (D) Sample actogram of an animal which received both (a) DMSO (i.p.) with novel WC and (b) ritanserin (2 mg/kg, i.p.) with novel WC.

-0.768 , $p > 0.05$; and $t_9 = -0.740$, $p > 0.05$, respectively).

3.5. Ritanserin

Ritanserin was tested systemically at two doses (2 mg/kg and 4 mg/kg). Ritanserin alone (both doses combined) did not produce a significant phase shift compared to DD (Fig. 5A). A repeated measures ANOVA failed to detect a significant effect of ritanserin at either dose on phase shifts induced by running during WC ($F_{2,26} = 1.89$, $p > 0.05$; Fig. 5A, B, D). There was also no significant difference among treatment conditions in the amount of

wheel running during the WC ($F_{2,26} = 2.13$, $p > 0.05$; Fig. 5C).

In the central injection group, 20 of 24 cannulated animals were determined to have acceptable placements (i.e., within 400 μm of the border of the SCN). Two separate repeated measures ANOVAs were used to examine the data, as two groups of 10 animals were used, one group for doses of 0, 1, 2, and 8 nmol of ritanserin, and the other for doses of 0, 16, and 32 nmol of ritanserin. The first repeated measures ANOVA did not detect any significant effects of treatment with ritanserin + WC at doses of 0, 1, 2, and 8 nmol ($F_{3,16} = 0.58$, $p > 0.05$; Fig. 6A, D). Similarly, the second repeated measures ANOVA failed to

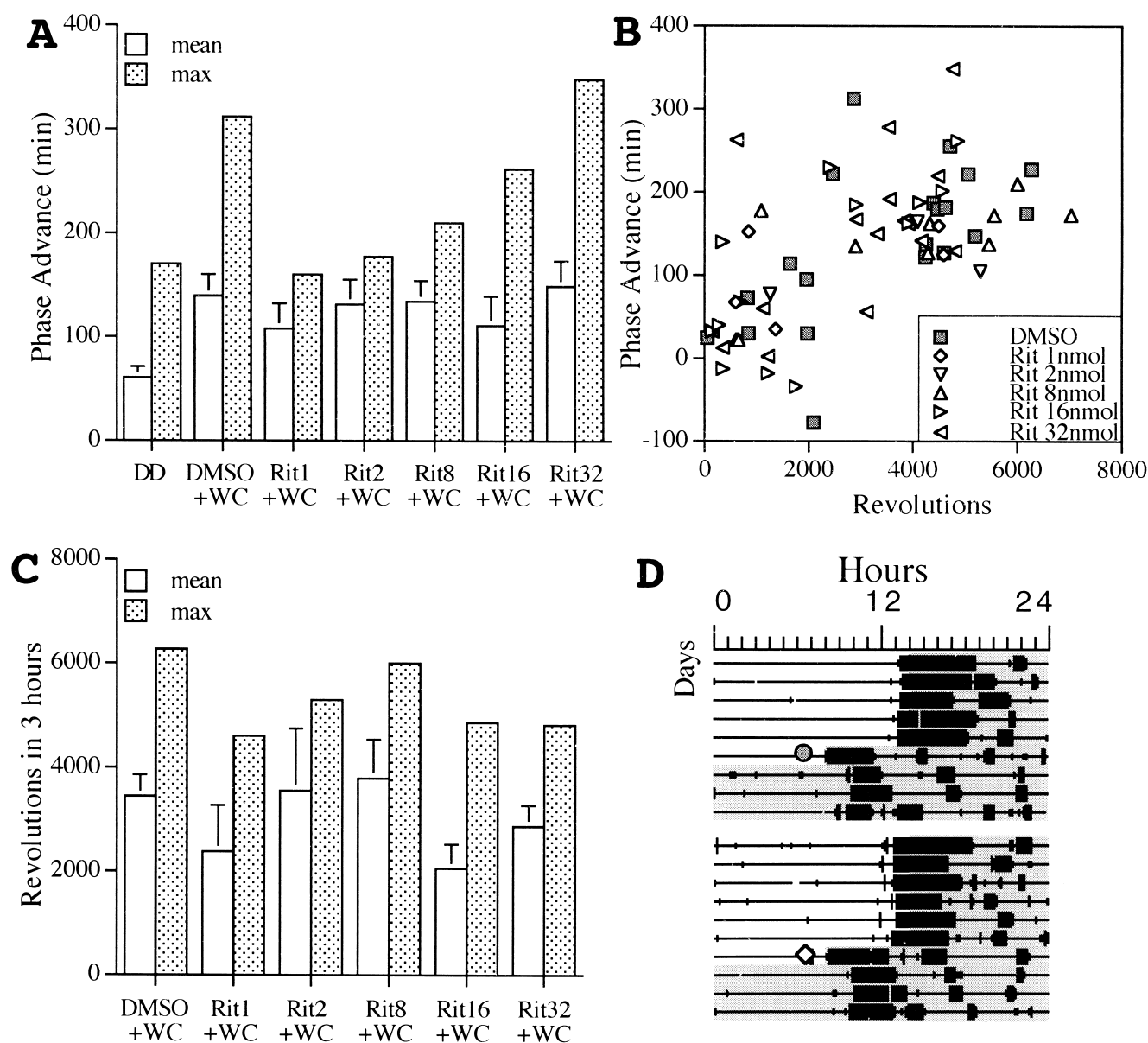


Fig. 6. Intra-SCN ritanserin (0 to 32 nmol). (A) Mean (+S.E.) and maximum phase shift of each treatment conditions. (B) Scatterplot of raw data (activity level vs. phase shift) for each WC treatment condition. (C) Mean (+S.E.) and maximum activity level produced in each WC condition. (D) Sample actogram of an animal which received both (a) ritanserin (8 nmol in 0.5 μl DMSO) to the SCN and novel WC and (b) DMSO (0.5 μl to the SCN) and novel WC.

detect an significant effect of treatments with ritanserin + WC at doses of 0, 16, and 32 nmol ($F_{2,18} = 1.14$, $p > 0.05$; Fig. 6A).

A strong relationship between activity and resulting phase shift is apparent in a scatterplot of the raw data (Fig. 6B). The mean activity levels (presented in Fig. 6C) in the treatment groups did not differ for either set of animals ($F_{3,15} = 1.41$, $p > 0.05$ for 0, 1, 2, and 8 nmol dosage group; $F_{2,18} = 2.49$, $p > 0.05$ for the 0, 16, and 32 nmol dosage group).

4. Discussion

Several studies have demonstrated that 5-HT agonists administered systemically in hamsters induce phase shifts with some similarities to those produced by running activity [4,7,25,44]. In addition, 5-HT release in the hamster SCN increases during wheel running activity [8,9]. Despite this circumstantial evidence suggesting that 5-HT release in the SCN might mediate activity-induced phase shifts, the present study failed to attenuate activity-induced phase shifts with the 5-HT_{1/2} antagonist metergoline, the selective 5-HT_{2/7} antagonist ritanserin, and the mixed 5-HT_{1A} agonists (+)-WAY100135 and NAN-190.

One interpretation of these results is that serotonergic transmission is not necessary for activity-induced resetting of the circadian clock in hamsters. Close comparison of the PRCs for activity and 8-OH-DPAT provides some evidence in support of this idea. The peak of the PRC for 8-OH-DPAT-induced phase advances is around CT7 [25] or CT8 [4,7], with mean shifts near 90 min. The peak of activity-induced phase shifts is between CT4-6 [5] with mean shifts of greater than 180 min. The PRC for NPY, the other neurotransmitter thought to be involved with activity-induced resetting of the circadian clock, has a shape more similar to that of the activity PRC. The discrepancies in the details of the PRCs to activity and 8-OH-DPAT may be taken to suggest that the two stimuli affect the clock through different means, although other explanations are possible. Alternatively, activity might activate both NPY and 5-HT afferents to the SCN, and both inputs may be capable of inducing phase shifts, but the latter may not be necessary for a full phase shifting response to activity, at least under the conditions utilized in this study.

This explanation does not rule out the possibility that there may be a species difference in the role of 5-HT within the circadian system. In vitro studies clearly show that rat SCN can be phase shifted by 5-HT agonists, including 8-OH-DPAT [18,34,36]. In vivo studies have also demonstrated phase shifting effects of 8-OH-DPAT or quipazine administered to the SCN of rats through an intracerebral catheter placed in the 3rd ventricle above the dorsal border of the SCN [11]. In mice, lesions of 5-HT inputs to the SCN prevent entrainment to a daily activity

schedule [12,20]. Similar lesions in hamsters fail to block activity-induced phase shifts in those studies that adequately addressed potential confounds with activity levels [6,23]. Conceivably, 5-HT transmission associated with non-photic stimuli functions to modulate the hamster SCN pacemaker's response to light [29] but may not be necessary for phase resetting effects of activity in this species. This would be consistent with the previously reported behavioral evidence showing that intra-SCN injections of 5-HT agonists do not produce phase shifts in hamsters (Ref. [25]; M. Antle and R. Mistlberger, unpublished observations). Studies of the phase shifting effects of 5-HT in the hamster SCN in vitro are needed to further evaluate this hypothesis.

Given that activity-induced phase shifts were not attenuated using drugs that reduce 5-HT output (i.e., NAN-190, and (+)-WAY100135), it is unlikely that lack of attenuation in the other drug conditions was due to the involvement of a novel 5-HT receptor that is not adequately blocked by 5-HT_{1,2}, and 7 receptor antagonists. It is possible that the hyperpolarization produced by these autoreceptor agonists was insufficient to counteract activation of raphe 5-HT cells during locomotor activity. Microdialysis could be used to assess the degree to which SCN 5-HT release associated with wheel running is reduced by pretreatment with either (+)-WAY100135 or NAN-190.

Another possible explanation for the failure of 5-HT antagonists to block phase shifts induced by wheel running is that the doses used were insufficient. Each dose was selected based on previously reported findings demonstrating a strong behavioral or pharmacological effect. Metergoline at 2 mg/kg blocks activity-induced attenuation of phase shifts to light pulses in the late subjective night in hamsters [29] and induces salt intake in rats [40]. (+)-WAY100135 significantly decreases hippocampal 5-HT at a dose of 10 mg/kg [2] and depresses activity in the raphe at all doses tested up to 1 mg/kg [13]. We used a maximum dose of 15 mg/kg due to indications that higher doses might inhibit induction of activity by WC. NAN-190 has been reported to decrease hippocampal 5-HT release in rats at doses as low as 0.03 mg/kg [15,41]. Also, NAN-190 at a dose of 5 mg/kg potentiates phase advances induced by light pulses in hamsters [37]. Ritanserin at systemic doses of 1, 2, and 5 mg/kg significantly attenuates phase advance shifts induced by stress/arousal in hamsters [43]. Ritanserin (5 mM) also blocks serotonergic suppression of light-induced increases in firing rate in the SCN of hamsters [47]. The concentrations used in the present experiment were between 2 mM and 64 mM. Given that all of the drugs were used at doses that are considered to be high and that have demonstrable neural or behavioral effects, it is unlikely that lack of attenuation is attributable to insufficient amount of drug.

It has recently been proposed that systemically administered 8-OH-DPAT may act at the dorsal raphe to reduce 5-HT output to the IGL and thus disinhibit the IGL [25].

Once disinhibited, the IGL would release NPY and GABA, which would induce phase shifts by their actions at the SCN. According to this model, activation of postsynaptic 5-HT_{1A} and 5-HT₇ receptors in the SCN is not sufficient to induce phase shifts, and therefore is not likely to mediate behaviorally-induced phase shifts. This proposal is consistent with our demonstration here that blockade of SCN 5-HT₇ receptors by intra-SCN injections of ritanserin fails to attenuate activity-induced phase shifts. However, the model would also predict that blockade of 5-HT receptors at the IGL by systemically administered antagonists like metergoline, ritanserin and WAY100135 would induce phase shifts. We did not observe significant phase shifting effects to these drugs by comparison with vehicle control procedures, suggesting that the model needs revision, or that additional antagonists need to be tested. The scatter-plots of the (+)-WAY100135 and metergoline data do illustrate a few cases in which there were large phase shifts in drug-treated animals despite levels of running activity that are typically inadequate to induce shifts. However, a few animals in the control conditions were also observed to exhibit large phase shifts despite low activity. It has recently been shown that the size of phase shifts to activity are to some extent phase dependent, and that less activity may be needed for maximal shifting at the phase used in the present study [5].

An alternative explanation for these cases of large shifts despite low activity levels is that 5-HT antagonists may sensitize the circadian system to activity at the level of the SCN. Consistent with this idea, global 5-HT depletion did not prevent activity-induced phase shifts despite significantly decreasing activity levels [23]. In addition, another study has shown that 5-HT and NPY are mutually inhibitory in their effects on phase of the rat SCN in vitro, which implies that blockade of 5-HT transmission might enhance non-photic shifting if this is mediated by NPY transmission [34]. Failure to see potentiation of activity-induced shifting in our drug conditions may reflect a ceiling effect for non-photic shifting in response to the particular stimulus conditions utilized. Further investigation of the dose response curves for activity at various circadian times in hamsters pretreated with 5-HT antagonists may help determine if acute attenuation of 5-HT transmission within the SCN sensitizes or disinhibits the circadian pacemaker's response to activity.

One issue raised in the review of this manuscript was that in the Aschoff Type II design used in this study the hamsters were subjected to a compound stimulus of dark onset and wheel running. If both stimuli induce shifts, and if 5-HT mediates only one type of shift, then 5-HT antagonists might not attenuate shifting to the compound stimulus. However, our own control data indicates that dark onset induces minimal shifting, and previous studies show that larger phase shifts to dark pulses in hamsters maintained in constant light are mediated by dark-induced running activity [45]. Therefore, in the present design,

hamsters were exposed to only a single true non-photic zeitgeber. Although wheel running can be induced with the lights on, this would likely result in less activity, and the light might attenuate the size of non-photic shifts, as has been recently shown for phase shifts induced by 5-HT [33].

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