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NMDA receptor antagonists block the effects of light on circadian behavior in the mouse

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We report here the results of experiments designed to evaluate whether NMDA receptors mediate the phase shifting effects of light on the circadian rhythm of wheel-running activity in mice. Intraperitoneal administration of either the non-competitive NMDA receptor antagonist, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,b]cyclohepten-5,10-imine maleate (MK-801), or the competitive NMDA receptor antagonist, 3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) blocked light-induced phase advances and delays. Neither drug, by itself, caused any consistent effect on the phase of the rhythm. Furthermore, there was no significant difference between the effects of MK-801 on light-induced phase shifts in a retinally degenerate and retinally normal strain of C57 mouse. These data, coupled with previous findings, indicate that excitatory amino acid receptors play an important role in the transmission of light information from the retina to the circadian system.

INTRODUCTION

Circadian rhythms are endogenously generated oscillations with periods of approximately 24 h. Normally these biological oscillations are entrained (synchronized) by the environmental light–dark cycle. This entrainment is critical if the circadian system responsible for generating these oscillations is to function adaptively as a time-keeping system for the organism. Thus, elucidation of the mechanisms by which entrainment occurs is central to an understanding of circadian organization.

In mammals, although not in other vertebrates, the entraining effect of light on the circadian system is mediated by photoreceptors which are located in the retina[23]. These photoreceptors project to the hypothalamus via two paths: a direct projection from retinal ganglion cells (the retinohypothalamic tract or RHT), and an indirect projection from the intergeniculate leaflet known as the geniculohypothalamic tract (GHT)[17]. The hypothalamic site at which these fibers terminate, the suprachiasmatic nucleus (SCN), appears to function as the central pacemaker of the circadian system[16]. One approach to the study of entrainment is to examine the neurochemistry of the pathway by which external light cues reach the SCN.

Previous studies suggest that excitatory amino acids (EAAs) may be involved in mediating the effects of light on the circadian system. Stimulation of the optic nerve in an in vitro hypothalamic slice preparation that contains the SCN has been shown to induce a calcium-dependent release of [^{3}H]-glutamate[12]. Furthermore, EAA antagonists have been shown to block neural responses to optic nerve stimulation in the SCN in vitro[1,2,6,19]. Finally, an EAA receptor antagonist, MK-801 [(+)-5-methyl-10, 11-dihydro-5H-dibenzo[a,b]cyclohepten-5,10-imine maleate] and related compounds phenylcyclidine and ketamine, blocked light-induced phase shifts in the circadian rhythm of wheel-running in hamsters[3].

The EAA's L-glutamate and L-aspartate appear to be major excitatory neurotransmitters in the mammalian central nervous system. The postsynaptic receptors mediating the actions of these transmitters are generally classified into 3 major subtypes based on their activation by specific agonists: *N*-methyl-D-aspartate (NMDA), kainate, and amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)[24]. MK-801 is the most potent and selective of the non-competitive antagonists of the NMDA receptor[13,25]. Its mechanism of action is thought to involve blockade of the ion channel associated with the NMDA receptor[10]. On the other hand, 3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) is a potent and specific NMDA antagonist which acts competitively at the receptor site[4,8,11]. The demonstration that the competitive antagonist CPP blocked light-induced phase

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shifts would greatly strengthen our suggestion that NMDA receptors mediate the effects of light on circadian behavior[3].

In the studies reported here, we used a retinal degenerate strain of the C57 mouse[5]. Entrainment to light cycles in this mouse is indistinguishable from entrainment in normal mice despite the presence of a mutation which causes a profound loss of visual photoreceptors[7]. These findings suggest that this strain of mouse can serve as a 'reduced' preparation with which to examine questions about light input to the circadian system. Furthermore, the C57 mouse has already been used in brain slice experiments in which EAA antagonists have been shown to block neuronal responses to optic nerve stimulation in the SCN[1,2]. Accordingly, in the studies reported here, we have used the C57 mouse as a model system in which to determine whether the phase shifting effects of light pulses on the behavioral circadian rhythm of locomotor activity are mediated by NMDA receptors.

MATERIALS AND METHODS

General methods

Adult male mice (*Mus musculus*, rd/rd or rd/+), obtained from our breeding colony at 60 days of age, were housed individually and their wheel-running activity recorded. The animals were exposed to a 12:12 light–dark cycle for two weeks and then placed in constant dark (DD) for 10 days to assess their freerunning locomotor activity pattern. Mice were then subjected to one of 5 treatments: (1) single quantified light pulse; (2) intraperitoneal (i.p.) injection of an experimental drug plus light pulse; (3) i.p. injection of vehicle plus light pulse; (4) i.p. injection of an experimental drug alone; (5) i.p. injection of vehicle alone. The light pulses were delivered either 4.0 h after the onset of activity [i.e. circadian time (CT) 16 where the onset of activity is defined as CT 12 for nocturnal animals] when light would normally induce a phase delay or 12 h after activity onset (i.e. CT 24) when light would normally induce a phase advance. Following each treatment, the animals were allowed to free-run undisturbed in DD for 10 days before receiving another treatment. No animal was treated more than twice.

The light stimulus used to induce phase shifts was a 15 min pulse of monochromatic light (515 nm) at an intensity of 4.6×10^{-2} $\mu W/cm^2$. The apparatus used to produce the stimulus has been previously described[21]. The stimulus parameters (duration, irradiance, and wavelength) were chosen to produce submaximal phase shifts. Stimulus intensity (irradiance) was measured before each trial with a UDT radiometer. Drugs, in either dimethylsulfoxide (DMSO) or isotonic saline, were administered by i.p. injection (injection volume was 0.1 ml). All handling and injection of animals was carried out in complete darkness with the aid of an FJW infrared viewer. Drugs were purchased from RBI, Natick, MA.

Experimental design

Three sets of experiments were performed. In the first experiment, the effect of (+)MK-801 on light-induced phase shifts at CT 16 and CT 24 was investigated. Experimental animals received an i.p. injection of MK-801 (1.5 mg/kg) in DMSO vehicle 60 min prior to the light pulse. The 4 control groups were treated as described in General methods. The stereospecificity of this drug's effect was investigated by using (–)MK-801 in combination with a light pulse. The dose–response function of the effect of MK-801 on light-induced phase delays was investigated. Five doses of MK-801 were tested (0.1, 0.5, 0.75, 1.0, 1.5 mg/kg). Drug alone controls were obtained at each dose. The ability of a low dose of MK-801 (0.15 mg/kg) to antagonize phase shifts induced by a dim light stimulus (1.0×10^{-2} $\mu W/cm^2$) was also investigated.

In the second experiment, the effect of the receptor antagonist CPP on light-induced phase advances (CT 24) and delays (CT 16) was assessed. Experimental animals received an injection of CPP (150 mg/kg) in saline 30 min prior to the light pulse. Four control groups were employed as described above. The dose response function of the effect of CPP (50, 75, 100, 125, and 150 mg/kg) on light-induced delays was investigated.

In the third experiment, the effects of MK-801 (0.75 mg/kg) on light-induced phase shifts in mice with degenerate retinas was compared with its effects on mice with normal retinas.

Data analysis

Phase shifts in the activity rhythm were determined by measuring the phase difference between eye-fitted lines connecting the onsets of activity for a period of 7 days before and 10 days after an experimental manipulation. In order to estimate the steady state phase shifts produced, transient cycles were excluded from the analysis (one day of data following treatments which caused phase delays and 4 days of data following treatments which caused phase advances). In other respects the method for calculating phase shifts was the same as that reported elsewhere[21]. The effects of the drugs on the phase-shifting effects of light pulses were evaluated using

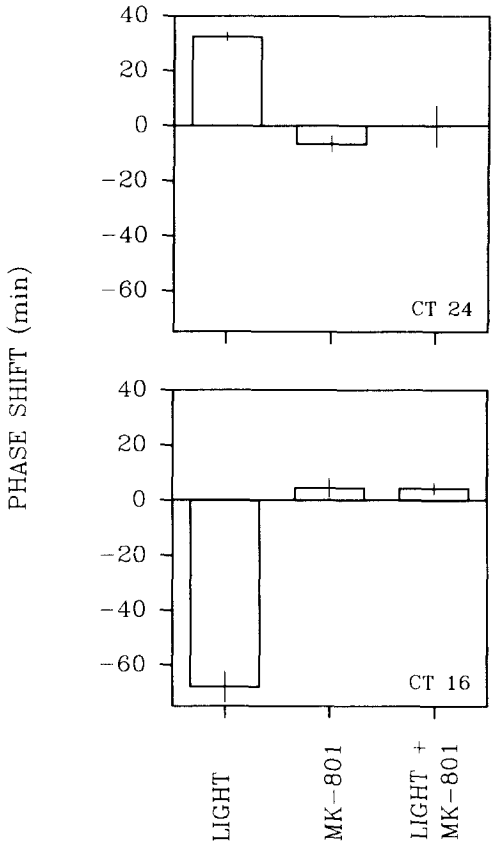

Fig. 1. Mean phase shift in the rhythm of locomotor activity in mice in constant darkness that received a treatment of either light + vehicle, MK-801, or light + MK-801. Top: MK-801 or vehicle was administered 11 h after the onset of activity. The light pulses were delivered 60 min later at CT 24. Bottom: MK-801 or vehicle was administered 3.0 h after activity onset while the light pulses were delivered 60 min later at CT 16. n = 6–8 for all points, verticle bar represents S.E.M.

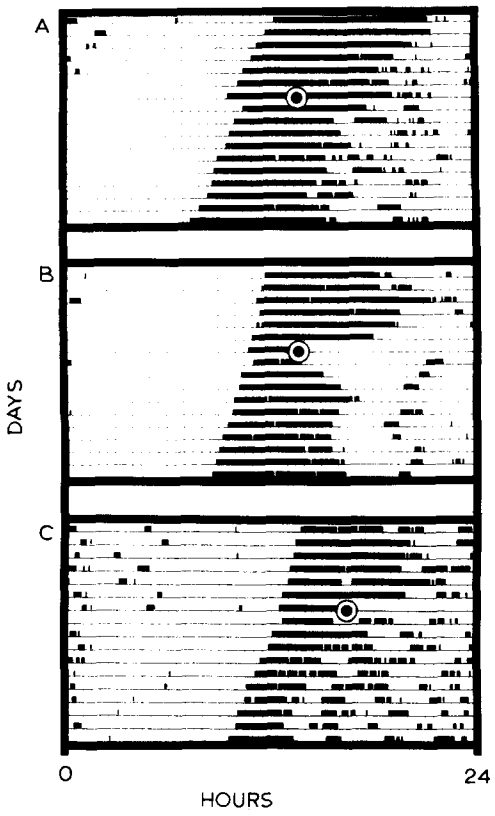

Fig. 2. Locomotor activity records from experimental and control animals maintained in constant darkness. Each horizontal line represents the activity record for a 24-h day and successive days are plotted from top to bottom. Circles represent the time of light and/or drug treatment. A: activity record illustrating the phase delaying effects of a standard light pulse given at CT 16. This animal also received an injection of vehicle (DMSO) one hour earlier as a control. B: activity record illustrating the lack of effect of an injection of MK-801 (1.5 mg/kg) at CT 15 on the phase of the circadian rhythm in locomotor activity. C: activity record illustrating the blockade of light-induced phase advances by an injection of MK-801 (1.5 mg/kg) 60 min prior to a light pulse given at CT 16.

ANOVA followed by Scheffe's multiple comparison procedures where appropriate. Values are shown as means ± standard error of the mean (S.E.M.) and were considered significantly different if $P < 0.05$.

RESULTS

The intraperitoneal administration of (+)MK-801 significantly reduced the magnitude of light-induced phase advances as well as light-induced phase delays in the circadian rhythm of wheel-running activity in mice (Fig. 1). Control injections of MK-801 or vehicle alone (data not shown) administered in the absence of a light pulse had no significant effect on the phase of the free-running rhythm. Furthermore, injections of vehicle alone had no significant effect on the amplitude of light-induced phase advances or delays. Examples of activity records from experimental and control animals are shown in Fig.

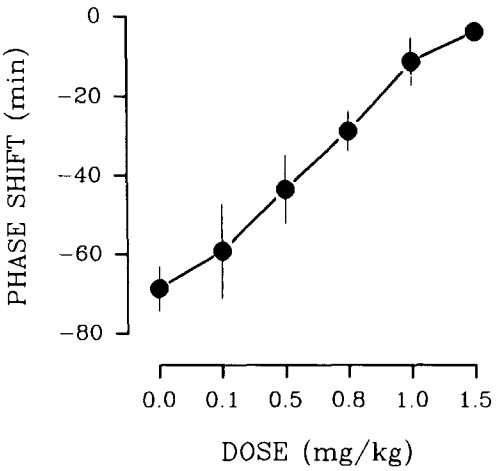

Fig. 3. Dose–response curves for the blockade of light-induced phase delays by MK-801. The effects of light + vehicle injection are shown at 0 mg/kg. n = 6–8 for all points, verticle bar represents S.E.M.

2A–C. Light-induced delays were not affected by treatment with the optical isomer (–)MK-801. The phase shift

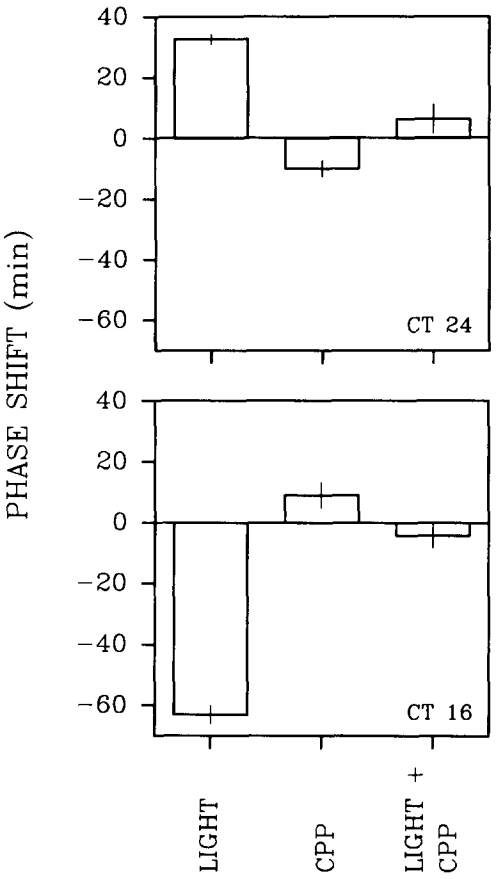

Fig. 4. Mean phase shift in the rhythm of locomotor activity in mice in constant darkness that received a treatment of either light + vehicle, CPP, or light + CPP. Top: CPP or vehicle was administered 11.5 h after the onset of activity. The light pulses were delivered 30 min later at CT 24. Bottom: Drug or vehicle was administered 3.5 h after activity onset while the light pulses were delivered 30 min later at CT 16. n = 6–8 for all points, verticle bar represents S.E.M.

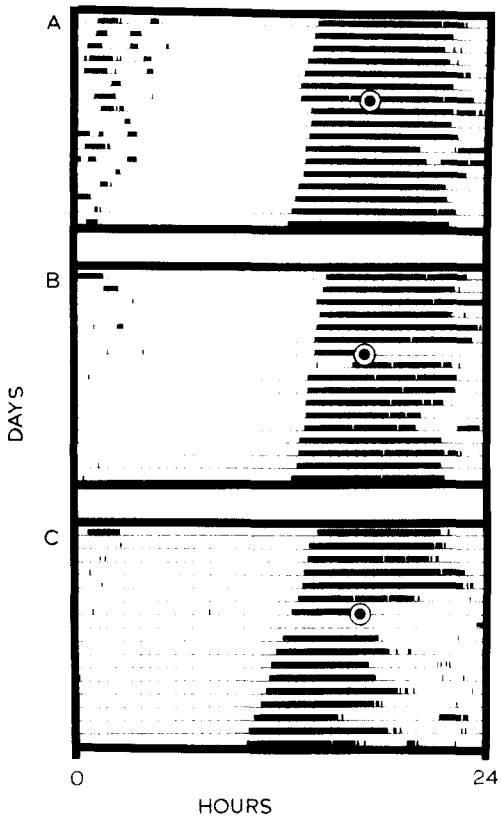

Fig. 5. Locomotor activity records from experimental and control animals maintained in constant darkness. Each horizontal line represents the activity record for a 24-h day and successive days are plotted from top to bottom. Circles represent the time of light and/or drug treatment. A: activity record illustrating the phase delay which results from a standard light pulse given at CT 16. This animal also received an injection of vehicle (saline) one hour earlier as a control. B: activity record illustrating that an injection of CPP (150 mg/kg) at CT 15.5 had no effect on the phase of the circadian rhythm of locomotor activity. C: activity record illustrating the blockade of light-induced phase delay by an injection of CPP (150 mg/kg) 30 min prior to a light pulse given at CT 16.

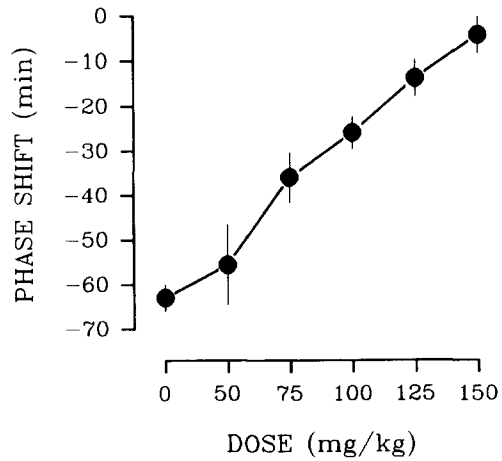

Fig. 6. Dose–response curve for the blockade of light-induced phase delays by CPP. The effects of light + vehicle injection are shown at 0 mg/kg. n = 6–8 for all points, verticle bar represents S.E.M.

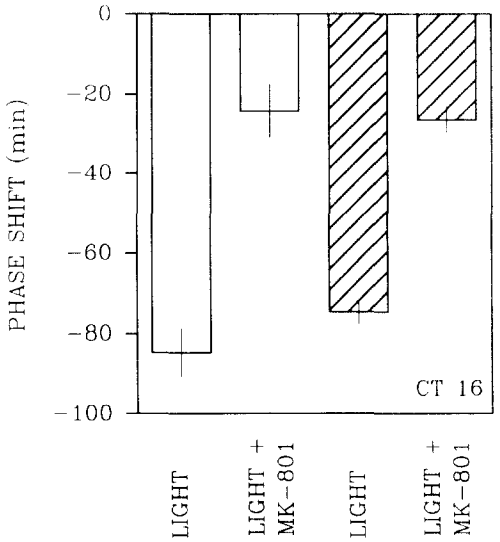

Fig. 7. A comparison between the effects of MK-801 on light-induced phase shifts in a retinal degenerate and normal strain of C57 mouse. Mean phase shifts in the rhythms of animals which received light, or light + MK-801 (0.75 mg/kg). The open histograms represent +/rd mice while the striped histograms represent rd/rd. n = 6–8 for all points, verticle bar represents S.E.M.

induced by light plus vehicle at CT 16 was 68.7 ± 5.5 min [(mean ± S.E.M.), n = 12] and the phase delay induced by light plus (–)MK-801 at a dose of 1.5 mg/kg was 70.7 ± 8.8 min (n = 7).

The blockade of light-induced phase delays by MK-801 was dose dependent between 0.1 and 1.5 mg/kg with an ED_{50} of 0.6 mg/kg (Fig. 3). Lowering the intensity of the light stimulus (from 4.6×10^{-2} $\mu W/cm^2$ to 1.0×10^{-2} $\mu W/cm^2$), decreased both the magnitude of the light-induced phase delay and also decreased the dose required to block the response. The phase delay induced by the lower intensity of light and by light plus MK-801 (0.15 mg/kg) were 55.0 ± 8.5 (n = 6) and 7.8 ± 2.4 (n = 5), respectively.

Treatment with CPP also significantly reduced the magnitude of light-induced phase shifts (Fig. 4). Control injections of CPP or vehicle administered in the absence of a light pulse had no significant effect on the phase of the free-running rhythm; and injections of vehicle alone had no significant effect on the amplitude of light-induced phase delays. Examples of activity records from experimental and control animals are shown in Fig. 5A–C. The reduction of phase delays was dose dependent between 50.0 and 150.0 mg/kg with an ED_{50} of 87.5 mg/kg (Fig. 6).

Finally, a submaximal dose of MK-801 reduced light-induced phase delays in both strains of C57 mice. Figure 7 shows the phase shifts that resulted when light and light plus MK-801 (0.75 mg/kg) were administered to both rd/rd and +/rd mice. There was no significant difference

in the response of the two genotypes either to light alone or to the effect of MK-801.

DISCUSSION

Our results demonstrate that EAA receptor antagonists can block the phase shifting effects of light on the circadian rhythm of locomotor activity in the mouse. These results extend the findings from an earlier study which demonstrated that MK-801 blocked light-induced phase shifts in the locomotor rhythm of hamsters[3], to a new model system — the circadian rhythm of wheel-running activity in the C57 (rd/rd) retinally degenerate mouse. This model system was then used to show that competitive (CPP) as well as non-competitive (MK-801) NMDA antagonists block the phase shifting effects of light. EAA antagonists have now been shown to block both the phase shifting effects of light on behavioral rhythmicity and the field potentials recorded in the SCN in response to optic nerve stimulation in vitro in the mouse[1,2]. These findings and those of others[6,12,14,19] support the suggestion that glutamate or a related EAA mediate the effects of light on the circadian system in rodents.

MK-801 and CPP are known to be potent and selective antagonists of the NMDA receptor class and it is likely that this is the mechanism by which these agents act on the circadian system. In the present study, the effects of both drugs were dose dependent over a range higher than that reported to block NMDA mediated responses specifically in other in vivo systems[4,11,15,18,22]. However, in the current study, the dose–response relationships were determined using a light stimuli which produced a near maximum response in the circadian system. Our data also demonstrate that lower doses are effective in blocking responses to weaker light stimuli. It may be that blockade of strong sensory inputs requires higher doses than does the blockade of such responses as NMDA-induced convulsions. Additionally, the behavioral effects of MK-801 appear to be stereoselective. (–)MK-801 completely failed to block light-induced phase shifts at the same dose at which (+)MK-801 completely blocked the effects of light. Finally, although both MK-801 and CPP antagonize NMDA-mediated responses, they act at quite different molecular sites; MK-801 acts by blocking the NMDA receptor-associated ion channel while CPP acts by interfering with the binding of the ligand to the NMDA receptor itself. The fact that both compounds block light-induced phase shifts strongly supports our hypothesis that NMDA receptors are involved in mediating the effects of light on the circadian system.

The neuroanatomical site(s) of action of the NMDA antagonists' effect on light-induced phase shifts is not known. In the present study, we found that MK-801 and CPP blocked both light-induced phase advances and delays but did not, by themselves, produce phase shifts. The simplest explanation for these findings is that these drugs are acting on a light input pathway to the circadian oscillator(s) in the SCN. In some cases, administration of MK-801 and CPP disrupted wheel-running activity (see Figs. 2 and 5). Pilot studies indicated that high doses of MK-801 (1.5 mg/kg) immobilized the mice for 1–3 h. However the antagonists' disruptive effect on wheel running was not correlated with their effects on light-induced phase shifts. For example, the administration of MK-801 at a dose of 0.15 mg/kg blocked light-induced phase shifts ($I = 1.0 \times 10^{-2}\ \mu W/cm^2$) yet had no obvious effect on wheel-running activity.

Evidence for EAA neurotransmission has been previously reported in the light input pathway to the circadian system of the C57 mouse. Using a brain slice preparation, Cahill and Menaker found that EAA antagonists blocked the field potentials recorded in the SCN in response to optic nerve stimulation[1,2]. However, their data indicated that NMDA receptors were not involved in mediating this response. In light of the behavioral data reported here, the slice preparation should perhaps be re-examined under conditions which favor expression of NMDA mediated responses (e.g. antagonism of GABA receptors, high rates of stimulation). It is interesting to note that NMDA receptors also appear to be involved in the visual response of dLGN neurons[9,20]. It may be that both NMDA and non-NMDA receptors are involved at these synaptic connections between retinal ganglion cells and their targets. However, it is also possible that MK-801 and CPP are acting at some other site in the light input pathway.

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