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Neonatal Monosodium Glutamate Treatment Alters the Circadian Response to Light in the Adult Rat

Kim Edelstein

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements

for the Degree of Master of Arts at

Concordia University

Montreal, Quebec

December 1993

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ABSTRACT

Neonatal Monosodium Glutamate Treatment Alters the Circadian Response to Light in the Adult Rat

Kim Edelstein

Continuous illumination disrupts rhythms controlled by a circadian pacemaker located in the mammalian suprachiasmatic nucleus (SCN). This disruption may be manifested as a loss of circadian rhythmicity, desynchrony of individual rhythms, or splitting of rhythms into two or more components. The neural mechanisms underlying this effect are not well defined. Although the pathway that provides input to the SCN from retinorecipient cells within the intergeniculate leaflet (IGL) and the ventrolateral geniculate nucleus (vLGN), the geniculohypothalamic tract (GHT), has been implicated, a role for the direct retinal projection to the SCN, the retinohypothalamic tract (RHT), has not been ruled out. The present study examines the effects of neonatal monosodium glutamate (MSG) treatment, known to lead to acute degeneration of retinal ganglion cells and demyelination of the optic tract on circadian rhythms and on light induced expression of Fos protein in retinorecipient brain regions involved in the regulation of circadian rhythms. Despite degeneration of the optic nerve, neonatal MSG treatment (2 mg/g SC on postnatal days 1,3,5,7,9) had no effect on circadian temperature or activity rhythms in the adult animal under 12-hour light, 12-hour dark (12:12 LD) cycles. However, the disintegration of circadian rhythms under constant light conditions observed in adult rats treated neonatally with 10% saline was prevented in MSG-treated rats. Furthermore, neonatal MSG treatment selectively prevented expression of Fos protein in the

IGL and vLGN, but not the SCN, following a one hour light pulse given during the dark phase of the LD cycle. These data suggest that cells in the IGL/vLGN of MSG-treated rats are not responsive to photic stimuli, and that cells within these regions may mediate the circadian response to constant light.

Acknowledgments

First and foremost, thanks to Dr. Shimon Amir for his guidance, support, patience, and sense of humour during the past two years.

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Biological rhythmicity is a dominant characteristic of most physiological and behavioral functions. Measures of heart rate, body temperature, corticosterone secretion, and activity all exhibit circadian rhythmicity with an oscillation period of approximately 24 hours (e.g., Bunning, 1973; Moore-Ede, Sulzman, & Fuller, 1982). Abnormalities in circadian rhythmicity have been associated with physical and mental illnesses such as fever, epilepsy, and manic depression (see Richter, 1965). Circadian rhythms have implications not only for the incidence and diagnosis of disease, but for treatment as well; both the toxic and therapeutic effects of drugs show circadian rhythms (Minors, 1985; Moore-Ede et al., 1982).

Mammalian circadian rhythms are generated by an endogenous clock, or pacemaker, located in the suprachiasmatic nucleus (SCN) of the hypothalamus. In addition to this internal timekeeping mechanism, circadian rhythms are entrained (regulated) by external cues, known as zeitgebers, such that synchrony between the internal clock and the external rhythmic world is maintained (see Moore, 1983; Moore & Card, 1985; Rusak & Zucker, 1979; Turek, 1985 for reviews). The most influential zeitgeber in rodents is the light-dark cycle, though nonphotic cues such as feeding, noise, ambient temperature, and social interactions may have entraining effects as well (Takahashi & Zatz, 1982; Turek, 1985; Zucker, Rusak, & King, 1976).

A great deal of research has focused on the physiological mechanisms underlying photic entrainment as a means of further understanding the nature of the circadian pacemaker. This work has included manipulations of the timing, type, and intensity of light that synchronizes the circadian system, as well as measurement of the extent of disruption of circadian rhythmicity observed under constant light conditions. In addition, the effects of damage to retinal pathways

on entrainment of circadian rhythms have been studied (see Moore & Card, 1985; Rusak & Zucker, 1979 for reviews).

The experiments described in this thesis were designed to examine the effects of neonatal monosodium glutamate (MSG) treatment on the circadian response to continuous illumination in the adult rat, using both behavioral and immunocytochemical measures. Neonatal treatment with MSG leads to acute degeneration of retinal ganglion cells as well as demyelination of the optic tract (Cohen, 1967; Lucas & Newhouse, 1957; Olney, 1969, 1971). Although previous work has shown that rhythms of adult rodents treated neonatally with MSG entrain to the light-dark cycle (Groos, 1981; Miyabo, Ooya, Yamamura, & Hayashi, 1982; Nemeroff et al., 1977; Pickard, Turek, Lamperti, & Silverman, 1982), the circadian response to continuous illumination or to brief pulses of light has not been examined in MSG-treated animals. In the following sections, the effects of different lighting schedules on the circadian system will be discussed and the role of the retinal pathways that mediate these effects will be considered. Expression of the immediate early gene, c-fos, and its protein, Fos, in response to light pulses will be examined. The effects of the monosodium glutamate syndrome as they pertain to circadian rhythms will conclude the Introduction.

Photic entrainment of the circadian system

Under a normal 12-hour light, 12-hour dark (12:12 LD) cycle, circadian rhythms entrain to the 24-hour day with one cycle per period. When the LD cycle is altered, the circadian timing system undergoes an adjustment to the new lighting schedule, and oscillations may be displaced for several cycles until reentrainment occurs. The rate of resynchronization of rhythms to the new zeitgeber can be taken as an indication of the effectiveness of the zeitgeber on

the system; the stronger the relationship between the zeitgeber and the circadian system, the more rapid the adjustment (Moore-Ede, et al., 1982). The state of an oscillation within a time period, called the circadian phase, may be shifted in response to a single stimulus, such as a brief pulse of light given during the animal's subjective night. The magnitude and direction of the phase shift induced by such a stimulus depends on the time at which the stimulus is applied during the circadian period. Phase delays occur when the light pulse is given during the early subjective night, whereas phase advances take place in response to light pulses given during the late subjective night. These differences may be depicted by generating phase response curves for different stimuli (Moore-Ede, et al., 1982).

In the absence of external time cues, such as under constant dark conditions, circadian rhythms are controlled exclusively by the pacemaker. Periods tend to drift , or free-run, to slightly longer or shorter than 24 hours, but synchrony between the various rhythms is maintained. Under prolonged continuous illumination conditions, however, a disruption of circadian rhythmicity occurs. Changes in amplitude and period, desynchronization (a loss of the phase relationship between different rhythms, so that rhythms free-run with different periods), arythmicity, or splitting of individual rhythms into two or more components, have all been observed in different species maintained under continuous illumination (e.g., Aschoff, 1965; Boulos & Terman, 1979; Czeisler, Weitzman, Moore-Ede, Zimmerman, & Knauer, 1980; Earnest & Turek, 1982; Eastman & Rechstaffen, 1983; Fioretti, Riccardi, Menconi, & Martini, 1974; Pickard, Kahn, & Silver, 1984; Pittendrigh & Daan, 1976; Turek, Earnest, & Swann, 1982).

The retinohypothalamic tract

Photic entrainment involves light transmission to the SCN. This occurs through a direct retinal projection, the retinohypothalamic tract (RHT), and an indirect projection, the geniculohypothalamic tract (GHT). The RHT is a bilateral, monosynaptic projection, arising from retinal ganglion cells and terminating in the ventrolateral region of the SCN (Moore & Lenn, 1972; Moore, 1973). In rats, approximately two-thirds of this innervation arises from the contralateral eye (Moore, 1973). The neurotransmitters involved in relaying information to the SCN from the RHT are thought to be excitatory amino acids, most likely glutamate or N-acetylaspartylglutamate (Cahill & Menaker, 1987; Mason & Rusak, 1991; Moffett, Williamson, Palkovits, & Namboodiri, 1990; Rusak, Abe, Mason, Piggins, & Ying, 1993; Shibata, Liou, & Ueki, 1986; van den Pol, 1991).

The RHT is the visual pathway, both necessary and sufficient, for photic entrainment of circadian rhythms (Moore, 1983). Transection of the RHT results in a loss of entrainment to the light-dark cycle (Johnson, Moore, & Morin, 1988). Stimulation of the RHT results in phase shifting of the free-running rhythm that resembles the circadian response to light pulses (Meijer & Rietveld, 1989).

The geniculohypothalamic tract

The GHT is a secondary visual pathway that arises from neurons of the IGL and the vLGN and projects to the SCN in a pattern that overlaps the RHT projection (Card & Moore, 1982; Moore, Gustafson, & Card, 1984; Hickey & Spear, 1976). The IGL and vLGN receive direct input from retinal ganglion cells, and at least some of those cells have bifurcating axons terminating in both the SCN and the IGL (Pickard, 1985). The GHT may play a role in the phase-shifting effects of light pulses on circadian rhythms. Lesions of the lateral

geniculate nucleus (LGN), as well as the primary and accessory optic tracts, have been shown to modify the phase of entrainment under LD cycles (Johnson, Moore, & Morin, 1989; Pickard, 1989; Rusak, 1977; Rusak & Boulos, 1981). Despite normal entrainment patterns under an LD cycle, hamsters with LGN lesions display slower rates of resynchronization following shifts in the LD schedule than nonlesioned animals (Zucker et al., 1976). Electrical stimulation of the IGL in hamsters housed under constant conditions causes phase-dependent shifts in circadian rhythms (Rusak, Meijer, & Harrington, 1989). Hamsters with IGL lesions display delayed phase advances in response to light pulses given during the late subjective night and prolonged phase delays in response to light pulses during the early subjective night (Harrington & Rusak, 1986; Pickard, Ralph, & Menaker, 1987).

The GHT may also mediate the responses of the circadian system to constant light (LL) exposure. Lesions of the lateral geniculate nucleus (LGN), and the primary and accessory optic tracts, alter the period of the free-running activity rhythm under constant light conditions (Rusak, 1977; Rusak & Boulos, 1981). Hamsters with IGL lesions are less likely to exhibit the change in period usually observed in normal or nonlesioned animals under LL conditions (Pickard et al., 1987). Furthermore, a decreased propensity toward rhythms splitting into two components has been reported in GHT-ablated hamsters, housed under LL (Harrington & Rusak, 1988).

Neurons of the GHT produce neuropeptide-Y (NPY). The effects of the GHT on the circadian pacemaker are presumably related to the release of NPY into the SCN (see Moore & Card, 1990 for review). NPY infused into the hamster SCN produces a phase response curve similar to that produced by electrical stimulation of the LGN, but quite different from that produced by light or RHT stimulation (Albers & Ferris, 1984; Albers, Ferris, Leeman, & Goldman,

1984; Boulos & Rusak, 1982). Bilateral destruction of the IGL eliminates the immunocytochemical staining of NPY in the SCN (Harrington, Nance, & Rusak, 1985; Johnson et al., 1989). Both excitatory and inhibitory effects of NPY on SCN neurons have been shown in in vitro preparations (e.g., Liou & Albers, 1991; Mason, Harrington, & Rusak, 1987; Shibata, & Moore, 1988, 1993). NPY immunoreactivity in the SCN exhibits a bimodal rhythm with peaks occurring during dark-light and light-dark transitions when animals are housed under a 12:12 LD cycle (Shinohara, Tominaga, Isobe, & Inouye, 1993). In response to brief light pulses given to animals housed under constant dark conditions, during the transition between the animals' subjective night and day, increased expression of NPY immunoreactivity in the SCN has also been observed (Shinohara, Tominaga, Fukuhara, Otori, & Inouye, 1993).

In addition to the IGL-SCN pathway, an enkephalinergic pathway between the two IGL's exists which does not appear to include cells of the SCN projection (Card & Moore, 1989; Pickard, 1985). Extracellular electrophysiological recordings have shown that separate IGL cells convey information about changes in the intensity of ambient light to the contralateral IGL and to the SCN (Zhang & Rusak, 1989). In addition, activation of cells in one IGL inhibited cell firing in the contralateral IGL, possibly acting to reduce sensitivity of the circadian system to transient changes in illumination (Zhang & Rusak, 1989).

Cells forming the GHT have been shown to be sensitive to changes in general illumination over a narrow range corresponding roughly to twilight intensities (Harrington & Rusak, 1989). GHT neurons may therefore provide information about ambient light intensity to the SCN and may enable the entrainment system to respond preferentially to gradual changes in illumination

while filtering out high frequency changes (Harrington & Rusak, 1989; Zhang & Rusak, 1989).

Taken together, these studies suggest that the sensitivity of the circadian system to the effects of brief light pulses and to constant light conditions may be modulated by the GHT.

Glutamate neurotoxicity and the MSG syndrome

Research on the neural mechanisms underlying entrainment of the circadian system has focused on the roles of each of the anatomical pathways of the visual system, with emphasis on projections to the SCN. The monosodium glutamate (MSG) syndrome provides a model with which to study entrainment in rodents whose retinal inputs were severely damaged during the neonatal period. Treating neonatal rodents with MSG, the sodium salt of the excitatory amino acid glutamate, leads to acute degeneration of amacrine, bipolar, and ganglion cells in the inner layer of the retina, while sparing the photoreceptors in the outer layer of the retina (Cohen, 1967; Lucas & Newhouse, 1957; Olney, 1969, 1971). Destruction of virtually all myelinated axons in the optic nerve has been observed in MSG-treated animals by two-months of age (Cohen, 1967; Groos, 1981). Furthermore, nearly 90% of the neuronal perikarya in the arcuate nucleus are rapidly destroyed so that few traces other than a reduced population of neurons can be found in brains examined more than two to four days following treatment (Olney, 1971). Damage to hippocampal CA1 neurons in response to neonatal MSG treatment has recently been observed as well (Kubo, Kohira, Okano, & Ishikawa, 1993). In the adult animal, neonatal MSG treatment results in a number of behavioral, metabolic, and endocrine abnormalities, including stunted growth and obesity. A number of studies have suggested that neonatal MSG treatment may also

lead to a disruption of visually-guided behaviors (see Kizer, Nemeroff, & Youngblood, 1978; Meldrum, 1993 for reviews).

The impairment of visually-guided behaviors that may occur in MSG-treated animals is likely related to the destruction of retinal cells and/or demyelination of the optic nerve. The effects of such damage on circadian rhythms in MSG-treated animals is not clear. Previous work has shown that bilateral ablation of the optic chiasm in intact animals causes blindness or alters visually-guided behavior without altering entrainment of circadian rhythms, suggesting that a separation of function exists between retinal pathways mediating vision and those mediating entrainment of circadian rhythms (Klein & Moore, 1979). Furthermore, research has shown that food intake, locomotor activity, plasma corticosterone and pineal N-acetyltransferase rhythms of MSG-treated rodents entrain to the light-dark cycle, suggesting that despite extensive damage to the retina, the retinohypothalamic tract remains intact (Groos, 1981; Miyabo et al., 1982; Nemeroff et al., 1977; Pickard et al., 1982). Results of an anatomical study in hamsters treated with one injection of MSG on postnatal day 8 indicates that these animals have intact retinal projections to the SCN and the vLGN (Pickard et al., 1982). Electrophysiological studies have also shown that in response to light flashes, both the number of visually-responsive cells in the dorsal LGN, as well as the response latencies of those cells, remains normal, suggesting that there are sufficient intact primary optic fibers innervating this region (Groos, 1981). However, the effects of MSG on the input from the retina to the IGL, and its effects on the circadian response to constant light conditions, have not been studied in MSG-treated animals.

Immediate early gene expression and the circadian system

An important tool used to study cellular activation in response to photic

stimuli has been the expression of the c-fos and c-jun proto-oncogenes. Research has focused on the induction of these immediate early genes (IEG's) in the cascade of events that is involved in transducing photic stimuli into changes in the circadian system (Kornhauser, Nelson, Mayo, & Takahashi, 1990; 1992). Because of their putative role in the regulation of transcription, proto-oncogenes are thought to represent important components of the transduction cascade elicited by environmental stimuli. The c-fos proto-oncogene is an important example because this gene forms a nuclear phosphoprotein (Fos) that forms a heterodimeric complex with the protein product of the c-jun gene and interacts with DNA at the AP-1 binding site to regulate transcription (for reviews, see Doucet, Squinto, & Bazan, 1990; Morgan & Curran, 1991; Sheng & Greenberg, 1990). Although the basal level of c-fos expression is normally low, increases in c-fos mRNA and Fos proteins occur rapidly and transiently in response to many types of physiological stimuli (see Morgan & Curran, 1991, for review).

Researchers have recently shown a correlation between expression of immediate early genes c-fos and c-jun in the SCN and IGL regions, and the circadian response to light. Hamsters, rats, and mice exposed to brief light pulses during their subjective night all exhibited expression of Fos protein in the retinorecipient region of the SCN (Aronin, Sagar, Sharp, & Schwartz, 1990; Colwell & Foster, 1992; Earnest, Iadarola, Yeh, & Olschowka, 1990; Ebling et al., 1991; Kornhauser et al., 1990; Park, Baek, Kim, Kim, & Kim, 1993; Rea, 1989, 1992; Rusak, Robertson, Wisden, & Hunt, 1990; Rusak, McNaughton, Robertson, & Hunt, 1992; Sutin & Kilduff, 1992). Fos expression has also been observed in the IGL and vLGN regions in response to light pulses given during the dark phase of the LD cycle (Aronin et al., 1990; Park et al., 1993; Rea, 1989; Rusak et al., 1990). Electrical stimulation of the IGL during the dark phase of the

LD cycle also induces Fos-like immunoreactivity in the SCN (Abe & Rusak, 1992).

Photic induction of Fos protein in the SCN was found to be gated by the circadian pacemaker, in that Fos expression differed during the subjective day and night, with maximum induction occurring during the mid to late subjective night (Sutin & Kilduff, 1992). Furthermore, the amount of Fos expression was positively correlated with the effectiveness of photic stimuli to induce phase shifts in activity rhythms (Ebling et al., 1991; Rusak et al., 1990; Rusak et al., 1992). c-fos mRNA and Fos protein expression in the SCN have been shown to exhibit circadian variation in the absence of photic cues, as well as under constant light conditions (Earnest, Ouyang, & Olschowka, 1992; Koibuchi, Sakai, Watanabe, & Yamaoka, 1992; Kononen, Koistinaho, & Alho, 1990; Kornhauser et al., 1990; Rusak et al., 1992; Sutin & Kilduff, 1992). Because of the correlation between phase-shift inducing light pulses and induction of Fos in SCN, IGL, and vLGN regions, expression of Fos protein is a useful marker of light-induced cellular activation.

Objective

The experiments reported in this thesis were designed to explore the responsiveness of the circadian system to continuous illumination in rats treated neonatally with MSG. Specifically, circadian temperature and activity rhythms were measured under normal light-dark cycles as well as under continuous illumination using a telemetry system. In addition, activation of cells in the SCN, IGL, and vLGN regions in response to light pulses given during the animal's subjective night was examined in MSG- and saline-treated rats immunocytochemically, by measuring the expression of Fos protein in these areas.

Materials and Methods

Subjects. Male Wistar rats (from original stock provided by Charles River Breeding Farms, St. Constant, Quebec) were bred in the animal facility at Concordia University and housed in clear plastic cages (36x24x19) under a 12:12 LD cycle (lights on at 08:00), with ad libitum access to rat chow and water. On the day of birth (postnatal day 0 or PND0) litters were culled to 12 pups with a minimum of six males and three females each, and housed with their dams. Rats were weaned at 21 days of age and housed with their littermates for five days. On day 26, male rats were then separated from the females and housed in groups of two or three per cage.

Drug treatment. Pups from each litter received five subcutaneous injections of either 2 mg/g monosodium glutamate (Sigma) or 10% saline on PND 1,3,5,7,9. To prevent leakage of the drug, the injection site was sealed with Collodion (Fisher).

Apparatus. Body temperature and activity data were recorded and analyzed using a biotelemetry system (Mini Mitter Co. Inc., Sunriver, OR)

Procedure. At 90 days of age, MSG (n=5) and saline (n=5) treated animals were anesthetized with Metofane (methoxyflurane; Pitman Moore Inc., Washington Crossing, N.J.) and implanted intraperitoneally with precalibrated biotelemetry transmitters (Mini Mitter Co. Inc., Sunriver, OR). Animals were housed individually in white plastic cages (33x21x18 cm) and body temperature and activity data were recorded at ten minute intervals. Animals were maintained under a normal 12:12 LD cycle (lights on at 08:00) for 14 days. The

lighting schedule was then switched to continuous illumination (300 lux) for 21 days. During the final 14 days, the animals were housed under a reversed 12:12 LD schedule (lights on at 18:00).

Behavioral data analysis. Body temperature and activity data for individual animals were recorded every ten minutes and averaged across 90 and 120 minute intervals respectively, using the Dataquest III software package (Mini Mitter Co. Inc., Sunriver, OR). Fourier analysis was used to measure the number of cycles per day for each animal under each of the lighting schedules. Amplitude data were calculated using daily peak-trough differences, averaged across 180 minute intervals per day, for the final seven days of the 12:12 LD and LL schedules, for each animal and for each group. Two 2x2 between-within (neonatal drug treatment x lighting schedule) ANOVAs were used to analyze temperature and activity amplitude data. Post hoc analyses of significant interactions were conducted using Tukey's test.

Photic stimulation. On the fifteenth day of the reversed cycle, the MSG and saline treated animals described above were exposed to ambient light (300 lux) for 60 minutes during the fourth hour of the dark phase. All rats were deeply anesthetized with an overdose of urethane (2 mg/kg, IP) immediately afterwards. Three additional MSG-treated and three additional saline-treated animals, housed under the lighting schedules described above but who did not receive the light stimulus, were deeply anesthetized in a dark room, illuminated by a dim red bulb (< 5 lux).

Preparation of tissue. Anesthetized animals were perfused transcardially with 200 ml of cold physiological saline (0.9% NaCl) followed by 400ml of cold,

fresh 4% paraformaldehyde in a 0.1M phosphate buffer (pH 7.3). Brains were removed and postfixed for two hours in fresh 4% paraformaldehyde at 4°C, followed by storage overnight in a 30% sucrose solution at 4°C. Frozen coronal brain sections (30 mm) through the SCN and LGN regions, corresponding to plates 22-24 and 34-37, respectively, in the atlas of Paxinos and Watson (1986), were cut from each brain on a sliding microtome.

Fos immunocytochemistry. Alternate tissue sections were washed in cold 50 mM Tris buffered saline (TBS; pH 7.6) and incubated for 48 hours at 4°C with an affinity-purified mouse monoclonal antibody raised against the N-terminal sequence of Fos (corresponding to N-terminal residues 4-17 of human Fos protein; NCI/BCB Repository, Quality Biotech, Camden, NJ). The antibody was diluted 1:8000 with a solution of 0.05% Triton X 100 in TBS with 1% normal horse serum. Following incubation in the primary antibody, sections were rinsed in cold TBS and incubated for one hour at 4°C with a rat-adsorbed biotinylated anti-mouse IgG made in horse (Vector Labs), diluted 1:33 with 0.05% Triton X 100 in TBS with 1% normal horse serum. Following incubation with secondary antibody, sections were rinsed in cold TBS and incubated for two hours at 4°C with an avidin-biotin-peroxidase complex (Vectastain *Elite* ABC Kit, Vector Labs). Following incubation with the ABC reagents, sections were rinsed with cold TBS, rinsed again with cold 50 mM Tris buffer (pH 7.6), and again for 10 minutes with 0.05% 3,3'-diaminobenzidine (DAB) in 50 mM Tris-HCl. Sections were then incubated on an orbit shaker for 10 minutes in DAB/Tris-HCl with 0.01% H_2O_2 and 8% $NiCl_2$. After this final incubation, sections were rinsed with cold TBS, wet-mounted onto gel-coated slides, dehydrated through a series of alcohols, soaked in xylene, and coverslipped with Permount (Fisher).

Immunocytochemical data analysis. The presence or absence of Fos protein was examined under a microscope (Leitz Laborlux S) and recorded for each region, in each animal. Brain sections through the SCN or IGL/vLGN were digitized using a Sony XC-77 Video Camera connected to a Scion LG-3 frame grabber using the NIH Image Software package (version 1.52) and printed on an Apple Laserwriter II NTX laser printer.

Results

Circadian rhythms under 12:12 LD. Circadian temperature and activity rhythms of all saline and MSG treated animals entrained to the 12:12 LD cycle. A Fourier analysis of the data indicated that both groups of animals exhibited one cycle per day. Figures 1-2 show circadian temperature and activity rhythms of representative saline- and MSG-treated rats, and their corresponding Fourier analyses. Figures 3 and 4 are actograms depicting the circadian rhythms of body temperature and activity, respectively, entrained to the 12:12 LD cycle in representative saline- and MSG-treated animals.

Circadian rhythms under LL. Under continuous illumination, all saline-treated animals displayed a progressive loss of circadian rhythmicity. In contrast, rhythms of MSG-treated rats did not respond to constant light. Figures 5 and 6 depict circadian temperature and activity rhythms of representative saline- and MSG-treated rats during the first week of continuous illumination. Fourier analyses of these data demonstrate that both groups of animals continued to exhibit one cycle per day during this period. During the second week of continuous illumination, the temperature rhythms of both MSG- and saline-treated animals continued to exhibit one major peak per day, while activity rhythms of saline-treated animals began to disintegrate at this time. The corresponding Fourier analyses confirm that temperature rhythms of both groups exhibited only one cycle per day, while activity rhythms of saline-treated animals displayed multiple cycles per day during the second week of continuous illumination (Figures 7-8). During the third week of continuous illumination, circadian rhythms of body temperature and activity were disrupted in saline- but not in MSG-treated animals. Fourier analyses of these data

demonstrate multiple peaks per period in both temperature and activity rhythms of the saline group only (Figures 9-10).

Circadian rhythms of body temperature and activity in all MSG-treated animals appeared to be free running by the third week of continuous illumination. Actograms depicting the period of temperature and activity rhythms in representative saline- and MSG-treated animals during the three week constant light period are shown in Figures 11-12.

Amplitude of circadian rhythms. Results of analysis of the temperature rhythm amplitude data showed significant main effects for both neonatal drug treatment and for lighting schedule (F (1,8) = 5.646, $p < 0.05$; F (1,8) = 57.449, $p < 0.01$, respectively). MSG-treated animals exhibited a decrease in amplitude as compared to saline-treated animals (MSG: M = 0.757, SD = 0.222; Saline: M = 0.936, SD = 0.364) and all animals exhibited a decrease in amplitude in LL as compared to LD (LL: M = 0.607, SD = 0.155; LD: M = 1.086, SD = 0.221) . A significant interaction between drug treatment and lighting schedule was found (F (1,8) = 5.400, $p < 0.05$). Post hoc analysis of the interaction revealed a significant blunting of amplitude in MSG- versus saline-treated animals under the 12:12 LD cycle, as well as a blunting of amplitude within each group under LL as compared to LD schedules. There was no difference between the amplitude of temperature rhythms of MSG-and saline-treated animals under LL. Analysis of the activity rhythm amplitude data indicated a significant main effect of lighting schedule (F (1,8) = 22.203, $p < 0.01$). All animals exhibited a decrease in amplitude in LL as compared to LD (LL: M = 38.540; SD = 15.766; LD: M = 67.144, SD = 25.319). However, there was no significant main effect for neonatal drug treatment, nor was there a significant interaction between neonatal drug treatment and lighting schedule (F (1,8) = 3.758, $p > 0.05$; F(1,8)

= 4.068, $p > 0.05$, respectively). Figure 13 depicts average amplitudes of the circadian temperature and activity rhythms in MSG- and saline-treated animals under 12:12 LD and LL schedules.

Fos immunoreactivity. Dense labeling of Fos immunoreactive cells was observed in the SCN of all MSG- and saline-treated animals who were exposed to a one-hour light pulse during the fourth hour of the dark phase of a 12:12 LD cycle. In the IGL and vLGN regions, Fos immunoreactive cells were observed in saline-treated rats, but not in MSG-treated rats. Examples of Fos expression in the SCN and IGL of the representative saline- and MSG-treated animals whose behavioral data are shown below, are shown in Figures 14 and 15, respectively. Control rats from both groups who did not receive a light pulse displayed no Fos expression in either of these regions (Figures 16 and 17). The extensive degeneration of the optic tract observed in all MSG-treated animals is shown in representative animals in Figures 14 and 16.

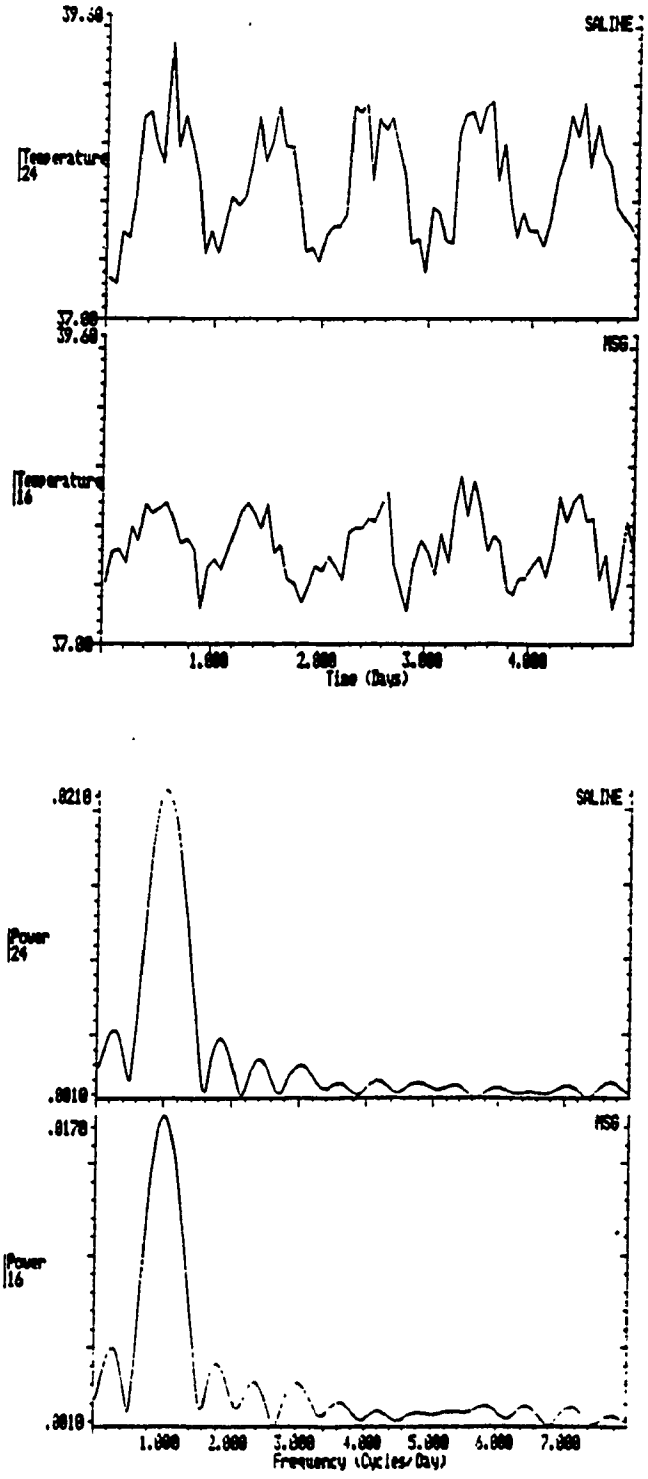

Figure 1. Circadian temperature rhythms (°C; top) and corresponding Fourier analyses (bottom) of representative saline- and MSG-treated rats under a 12:12 LD cycle (lights on 18:00-06:00).

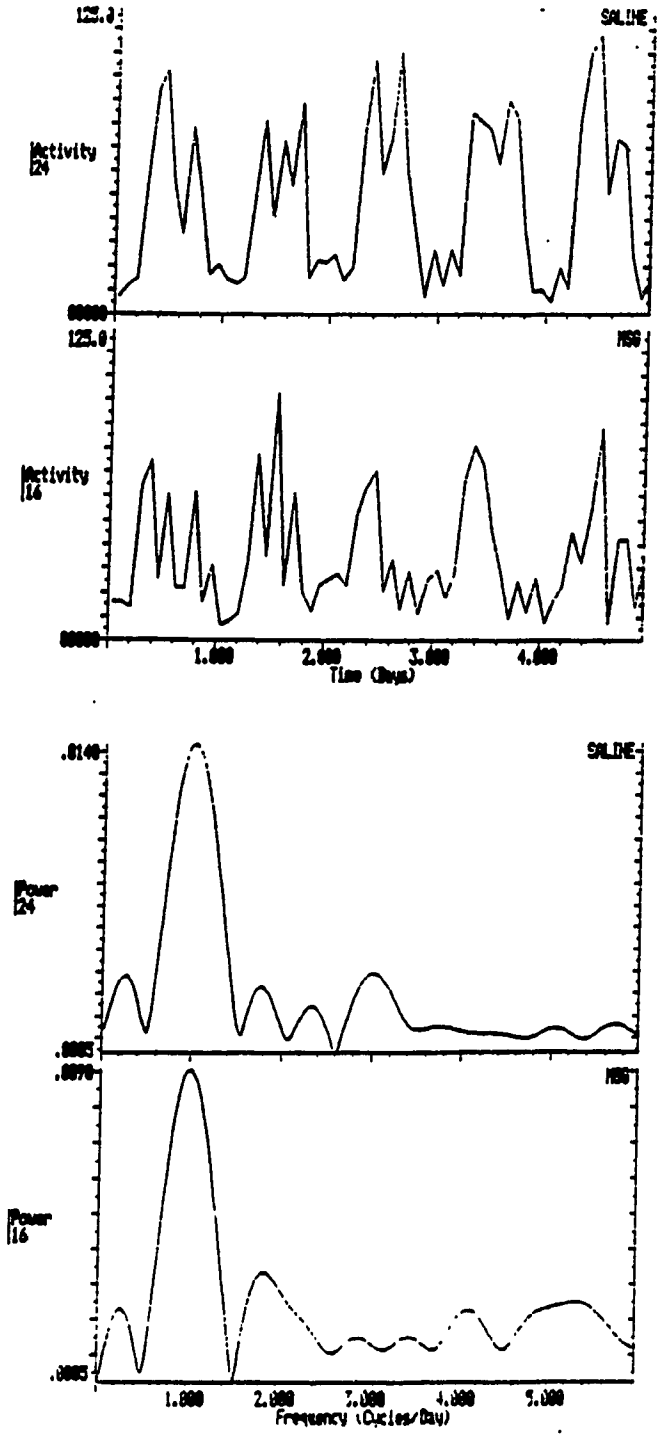

Figure 2. Circadian activity rhythms (counts; top) and corresponding Fourier analyses (bottom) of representative saline- and MSG-treated rats under a 12:12 LD cycle (lights on 18:00-06:00).

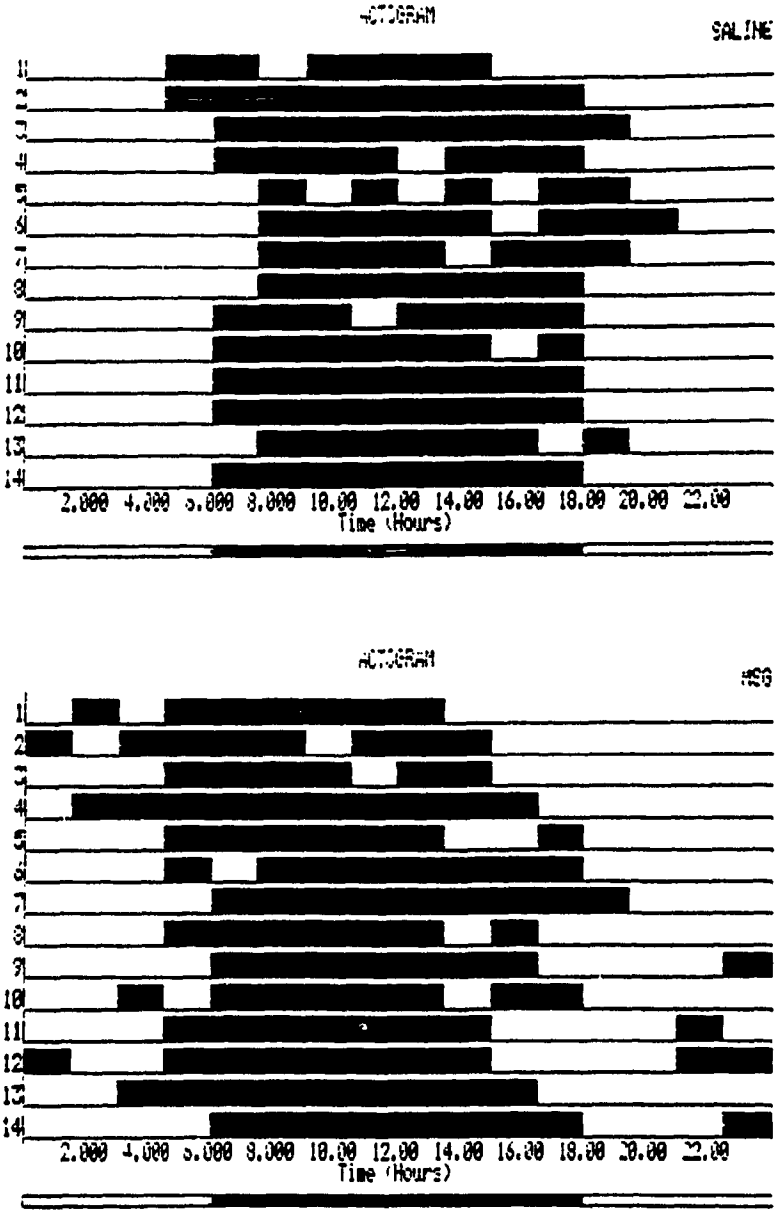

Figure 3. Actograms of body temperature data, averaged over 90 minute intervals, across 14 days of the 12:12 LD cycle in representative saline-treated (top) and MSG-treated (bottom) animals.

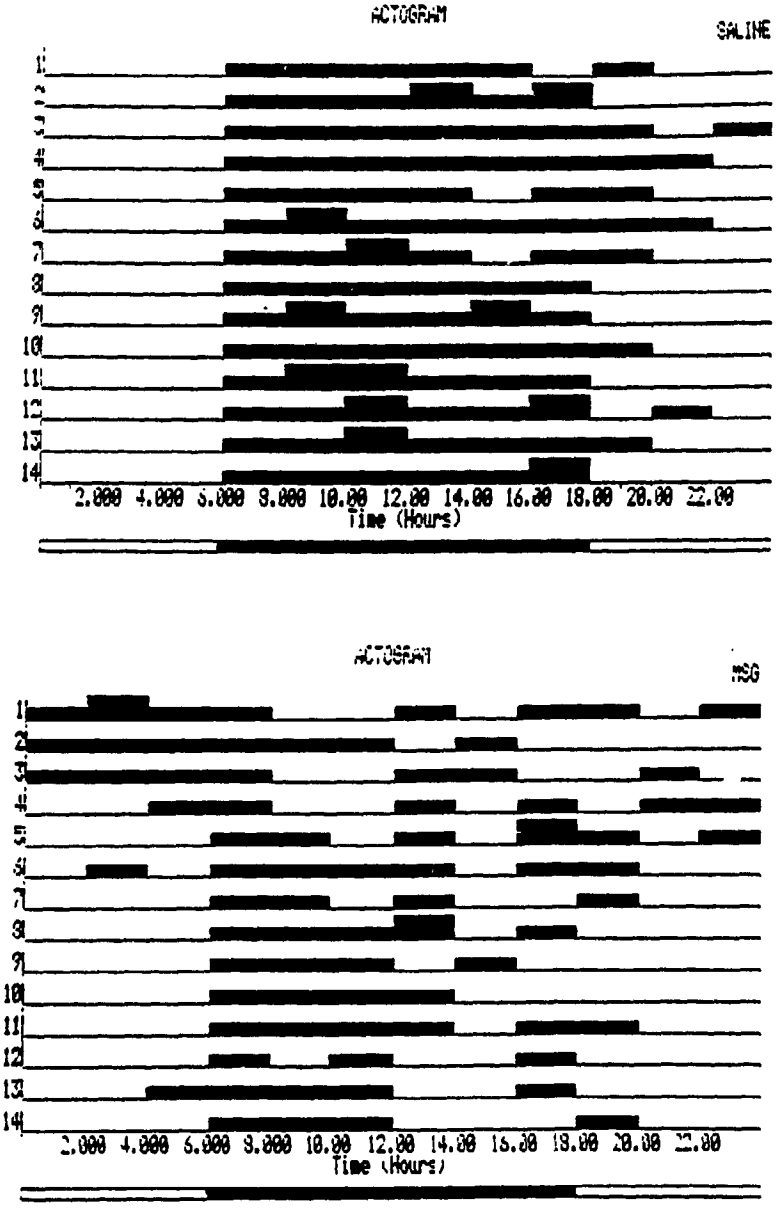

Figure 4. Actograms of activity data, averaged over 120 minute intervals, across 14 days of the 12:12 LD cycle in representative saline-treated (top) and MSG-treated (bottom) animals.

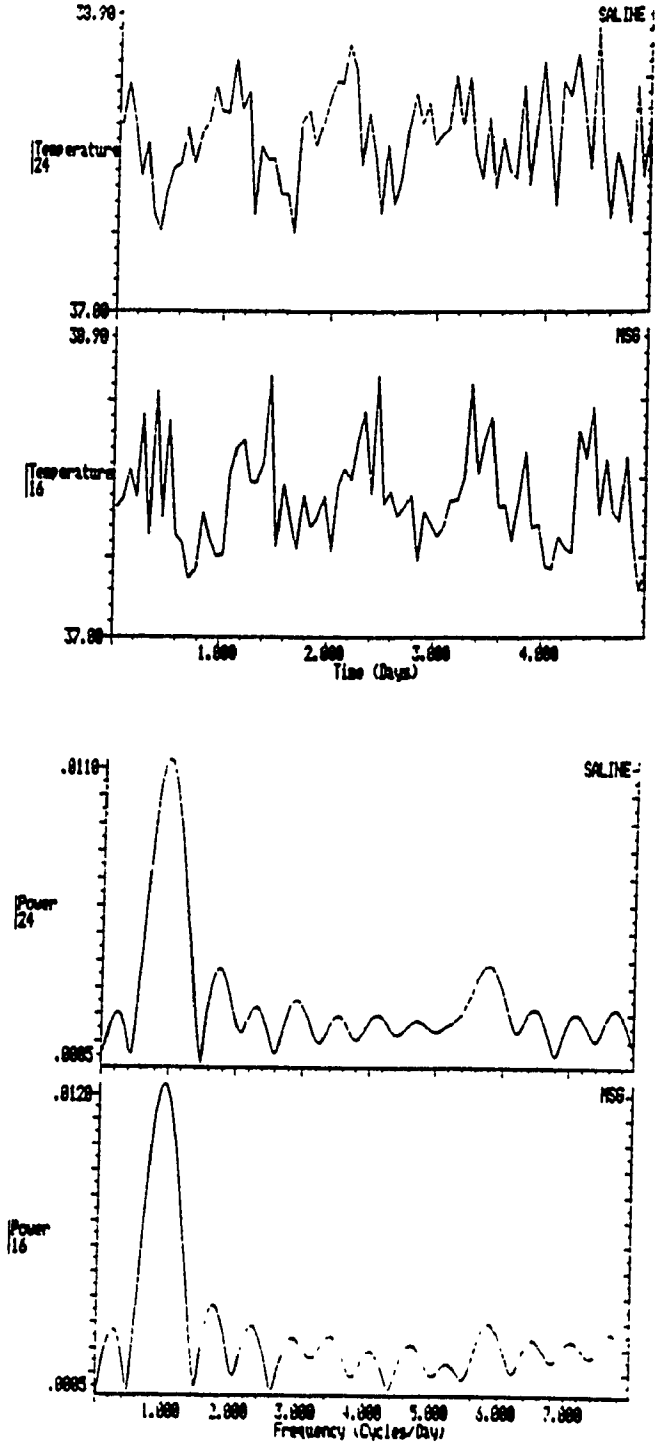

Figure 5. Circadian temperature rhythms (°C; top) and corresponding Fourier analyses (bottom) of representative saline- and MSG-treated rats during the first week of exposure to continuous illumination.

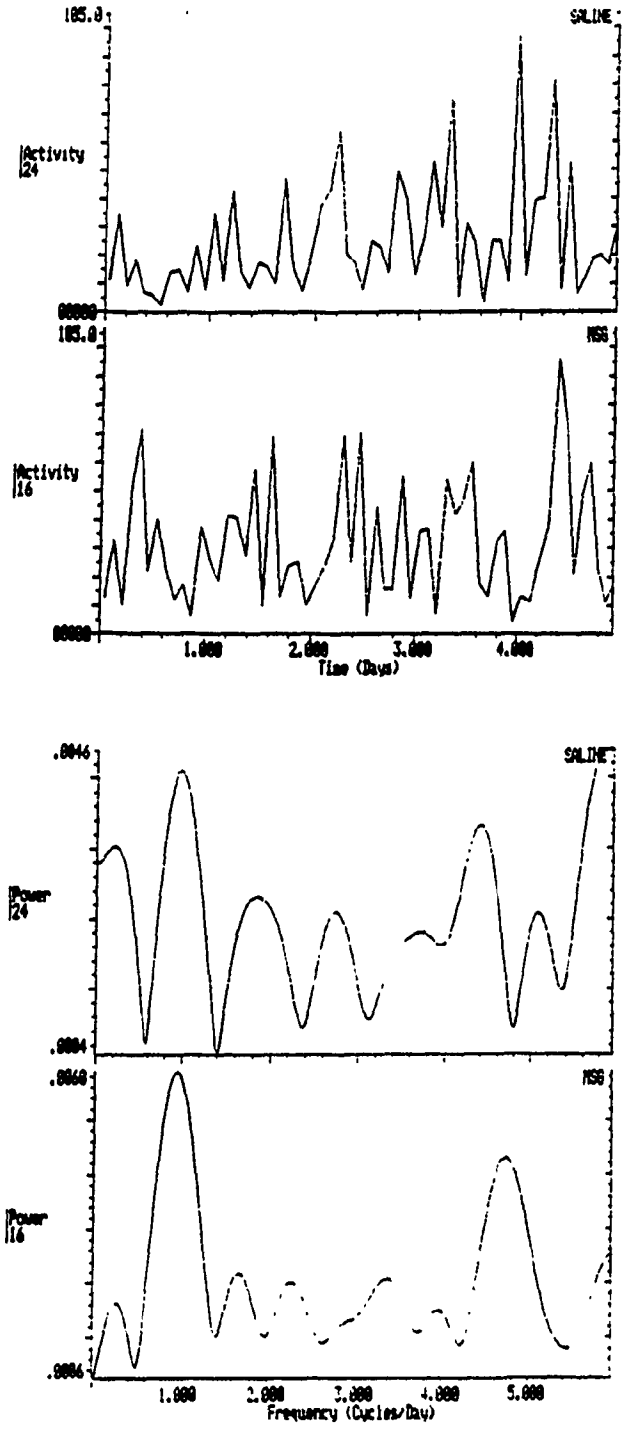

Figure 6. Circadian activity rhythms (counts; top) and corresponding Fourier analyses (bottom) of representative saline- and MSG-treated rats during the first week of exposure to continuous illumination.

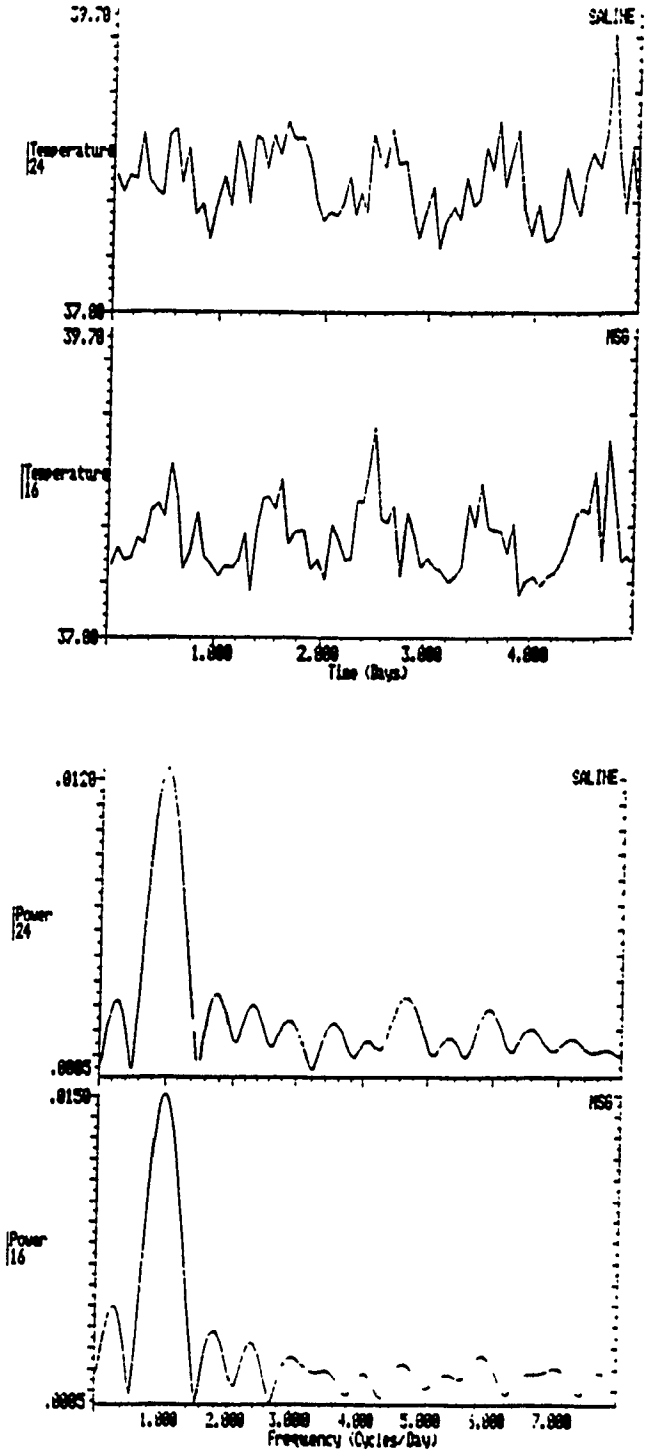

Figure 7. Circadian temperature rhythms (°C; top) and corresponding Fourier analyses (bottom) of representative saline- and MSG-treated rats during the second week of exposure to continuous illumination.

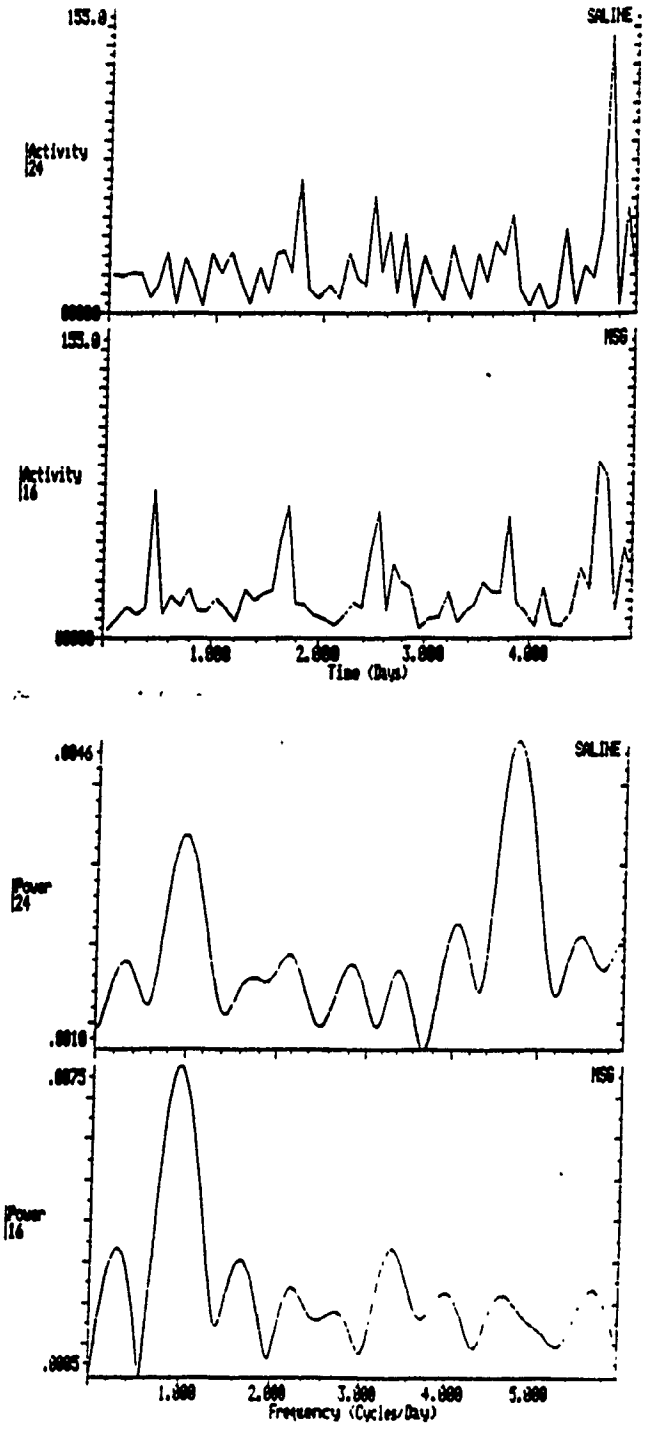

Figure 8. Circadian activity rhythms (counts; top) and corresponding Fourier analyses (bottom) of representative saline- and MSG-treated rats during the second week of exposure to continuous illumination.

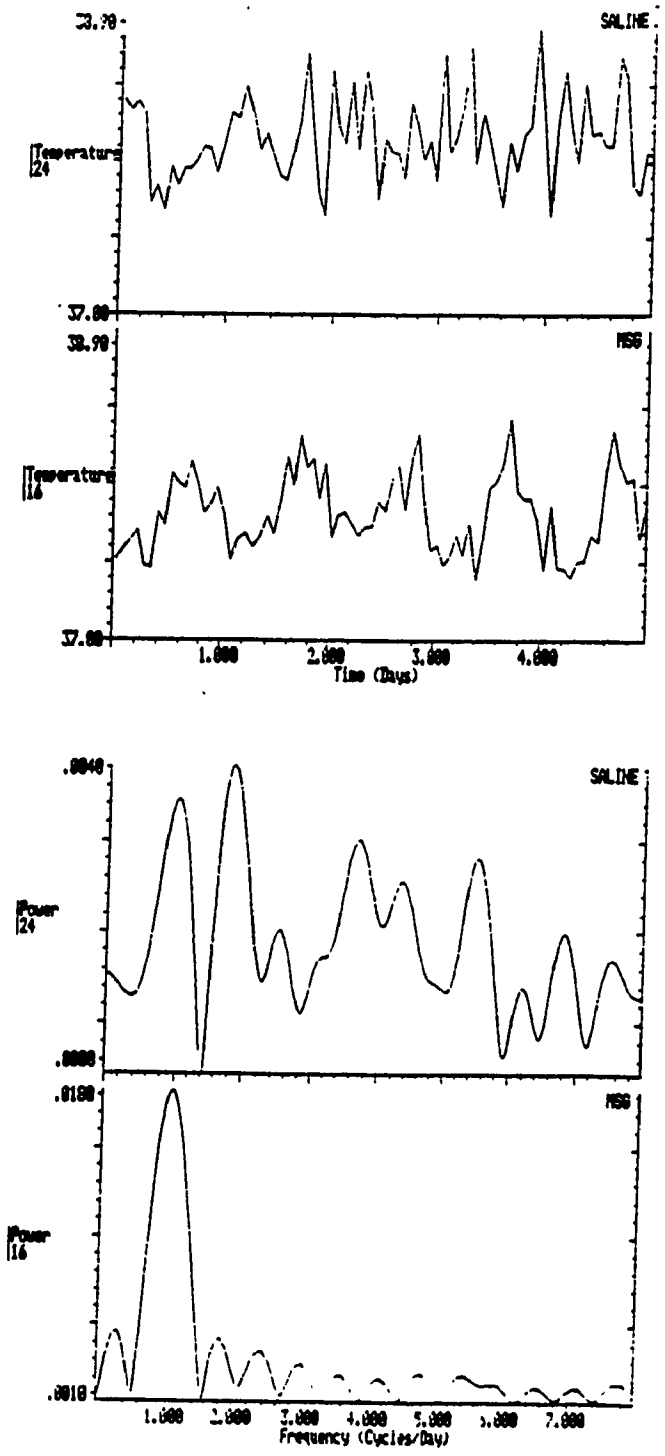

Figure 9. Circadian temperature rhythms (°C; top) and corresponding Fourier analyses (bottom) of representative saline- and MSG-treated rats during the third week of exposure to continuous illumination.

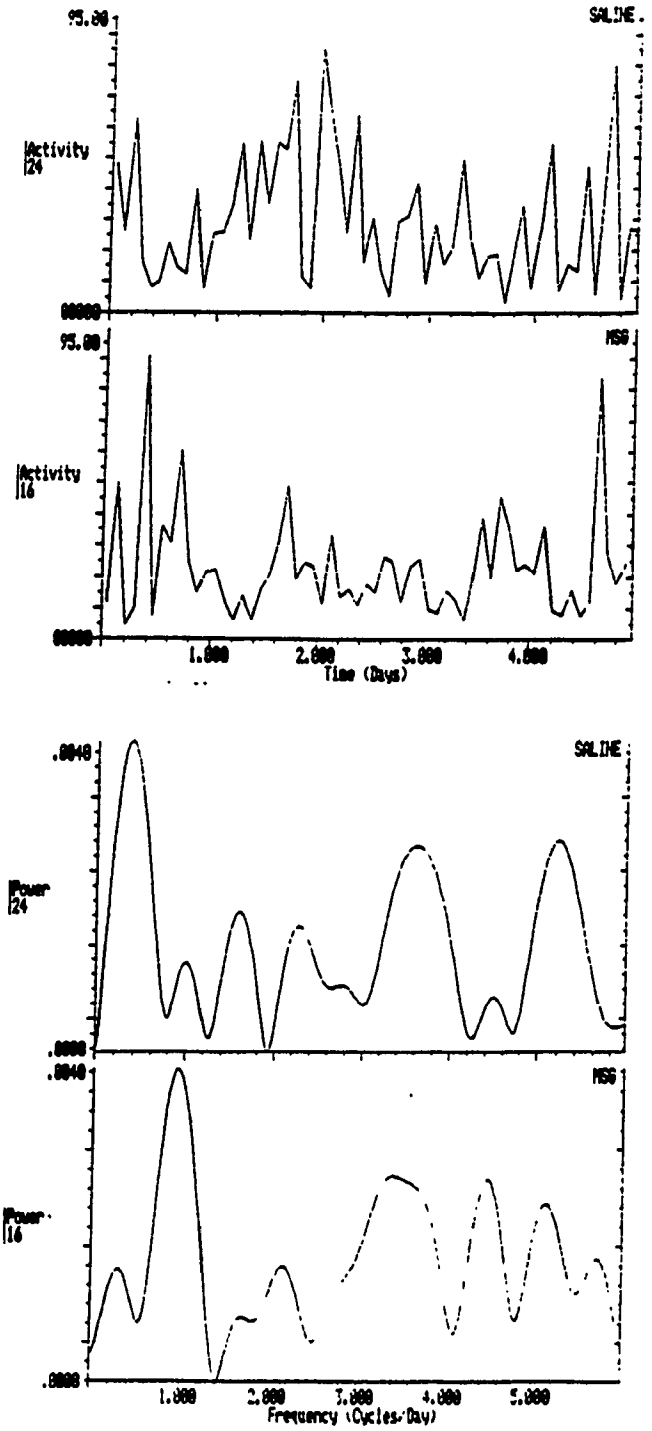

Figure 10. Circadian activity rhythms (counts; top) and corresponding Fourier analyses (bottom) of representative saline- and MSG-treated rats during the third week of exposure to continuous illumination.

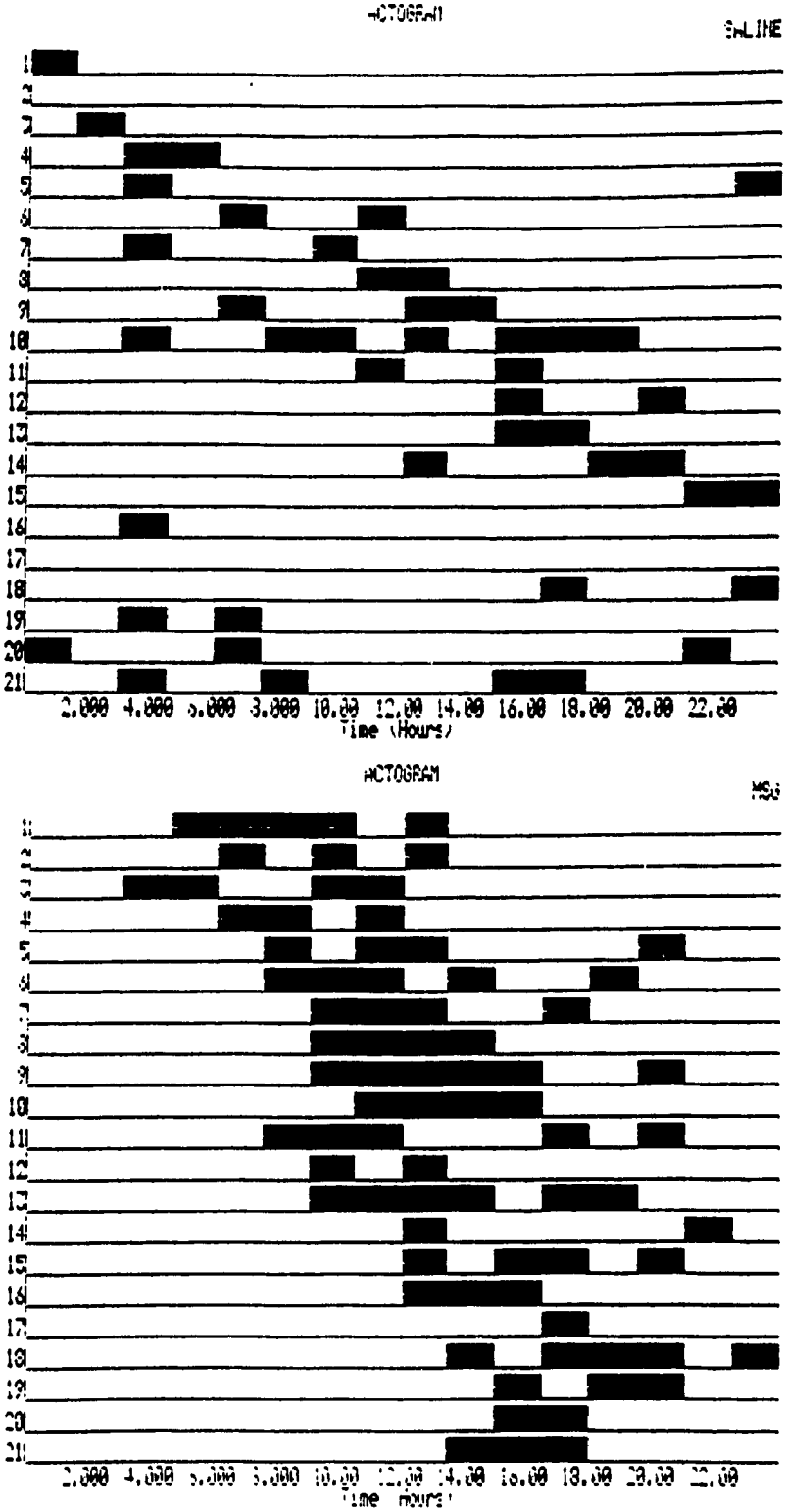

Figure 11. Actograms of body temperature data, averaged over 90 minute intervals, across 21 days of continuous illumination in representative saline-treated (top) and MSG-treated (bottom) animals.

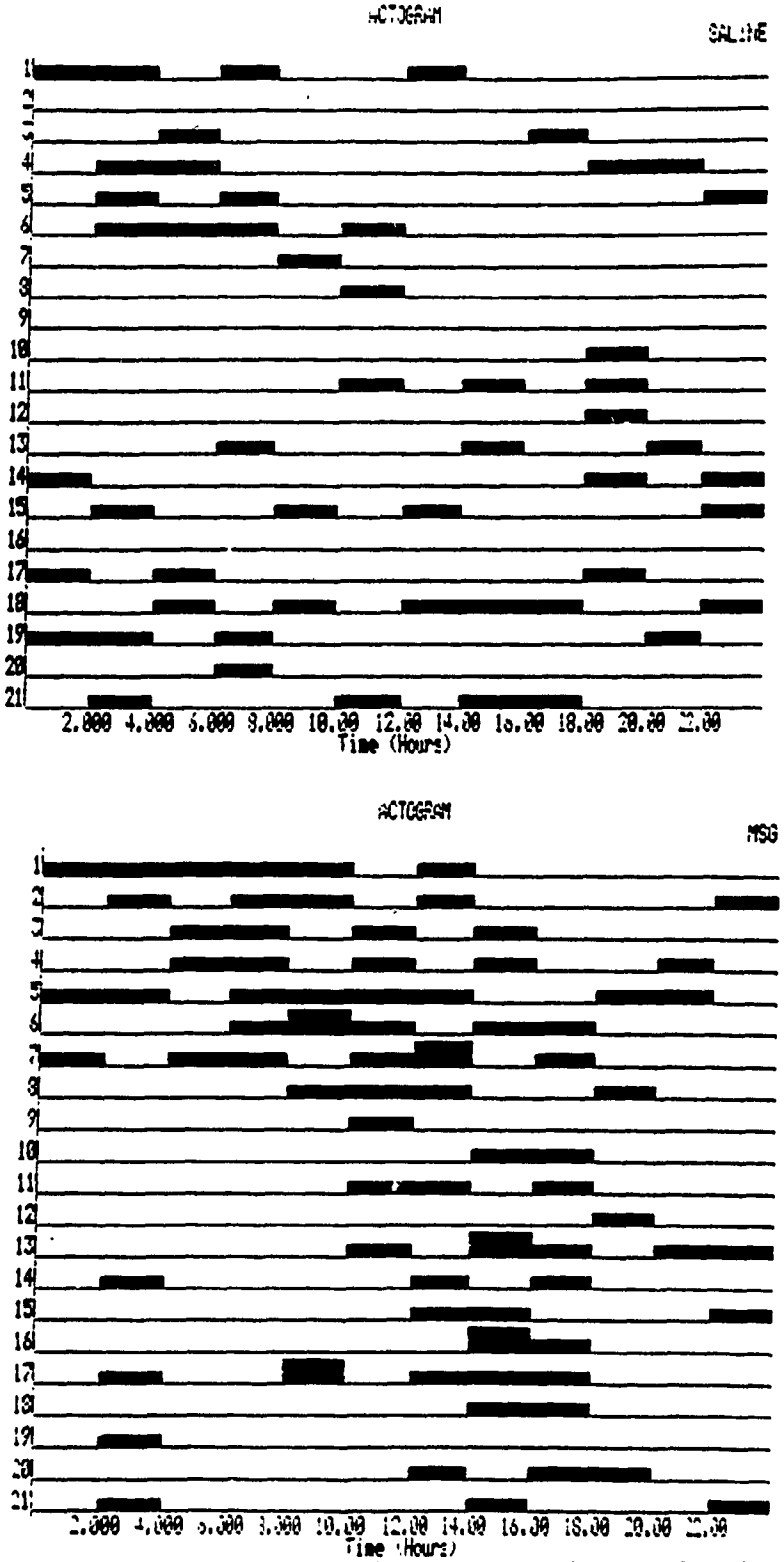

Figure 12. Actograms of activity data, averaged over 120 minute intervals, across 21 days of continuous illumination in representative saline-treated (top) and MSG-treated (bottom) animals.

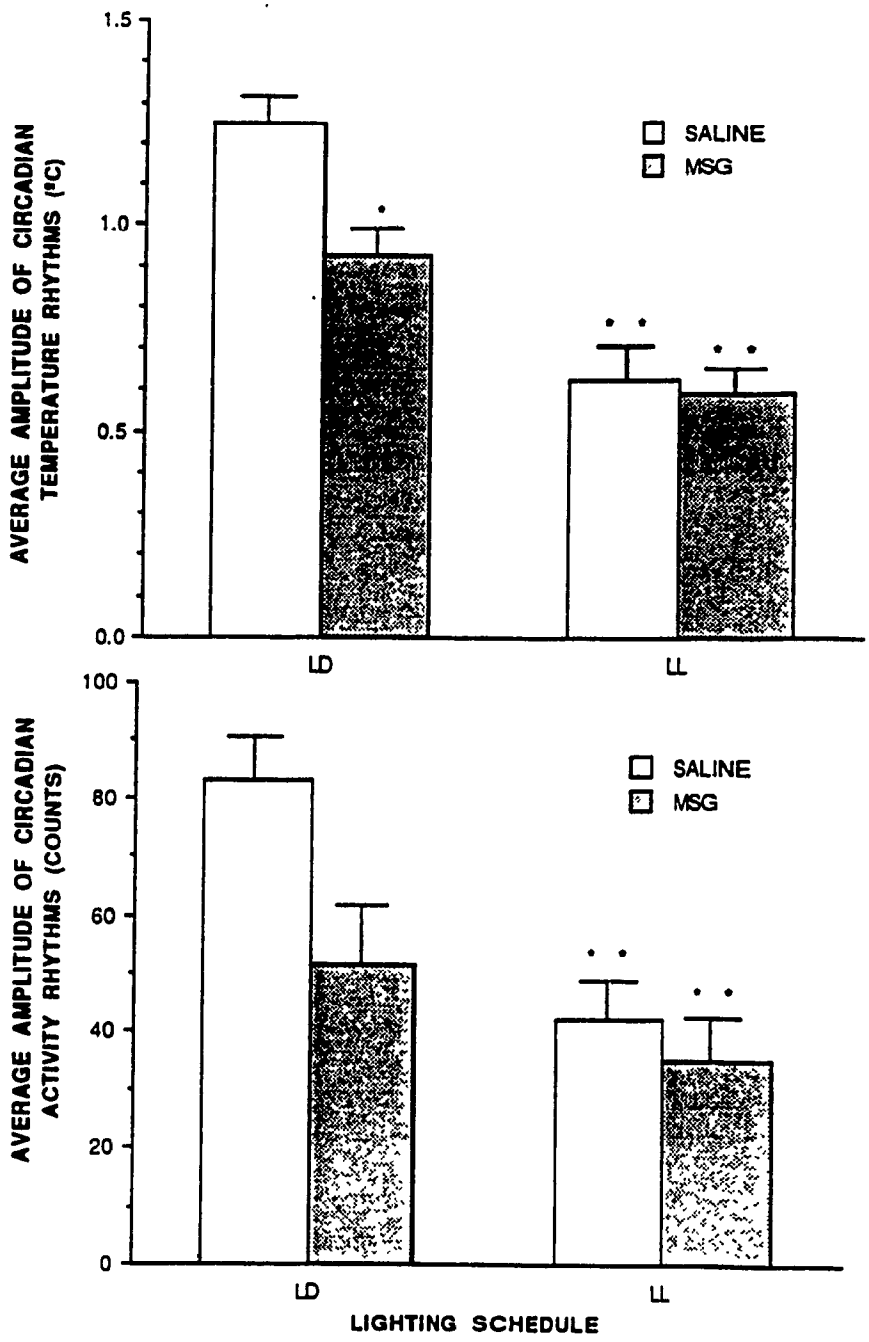

Figure 13. Average amplitude of circadian temperature (top) and activity (bottom) rhythms in MSG and saline treated rats during the 12:12 LD and LL cycles (mean± sem).

* significant differences between drug treatment groups ($p<0.05$)

** significant differences across lighting schedules ($p<0.01$)

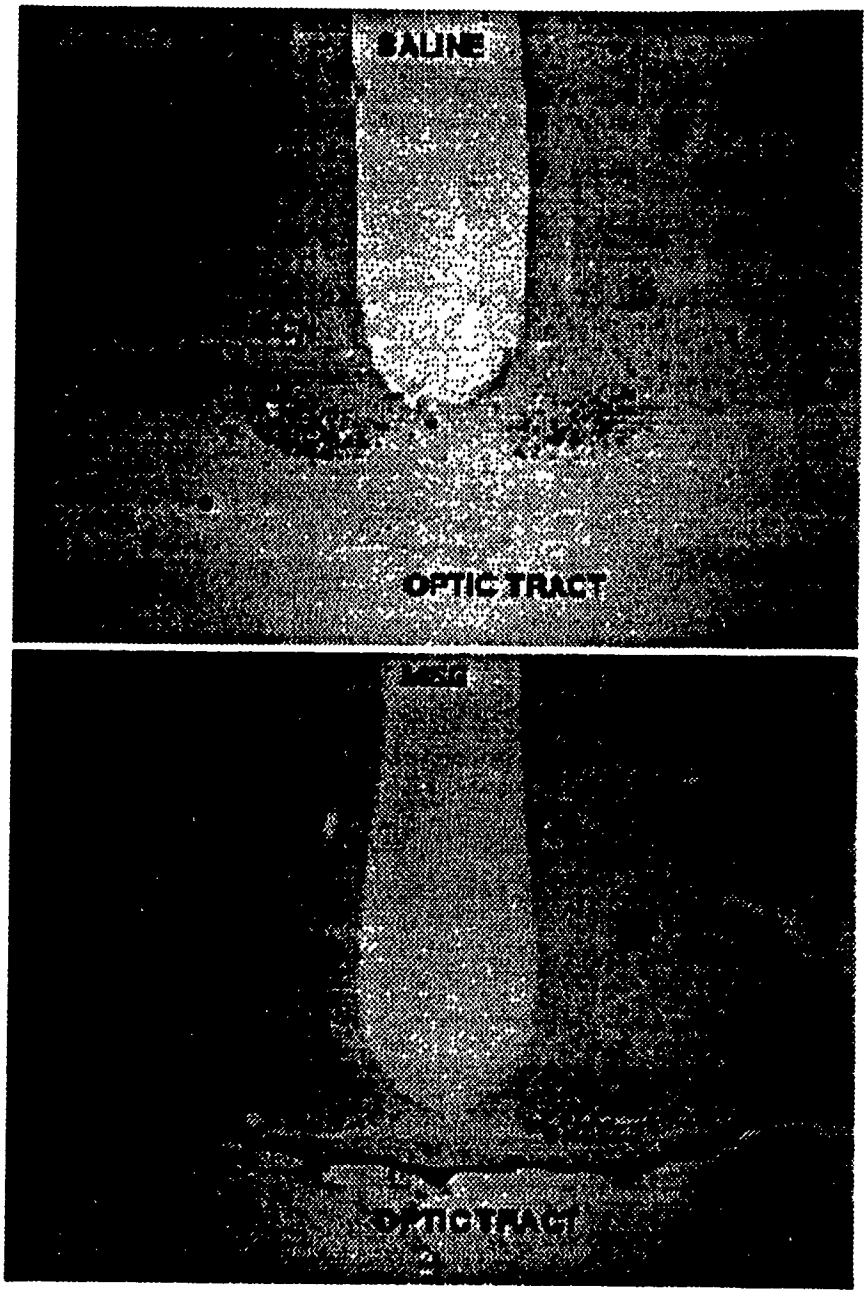

Figure 14. Fos-like immunoreactivity in a coronal section through the SCN of representative saline and MSG treated rats exposed to a one hour light pulse during the fourth hour of the dark phase of the 12:12 LD cycle.

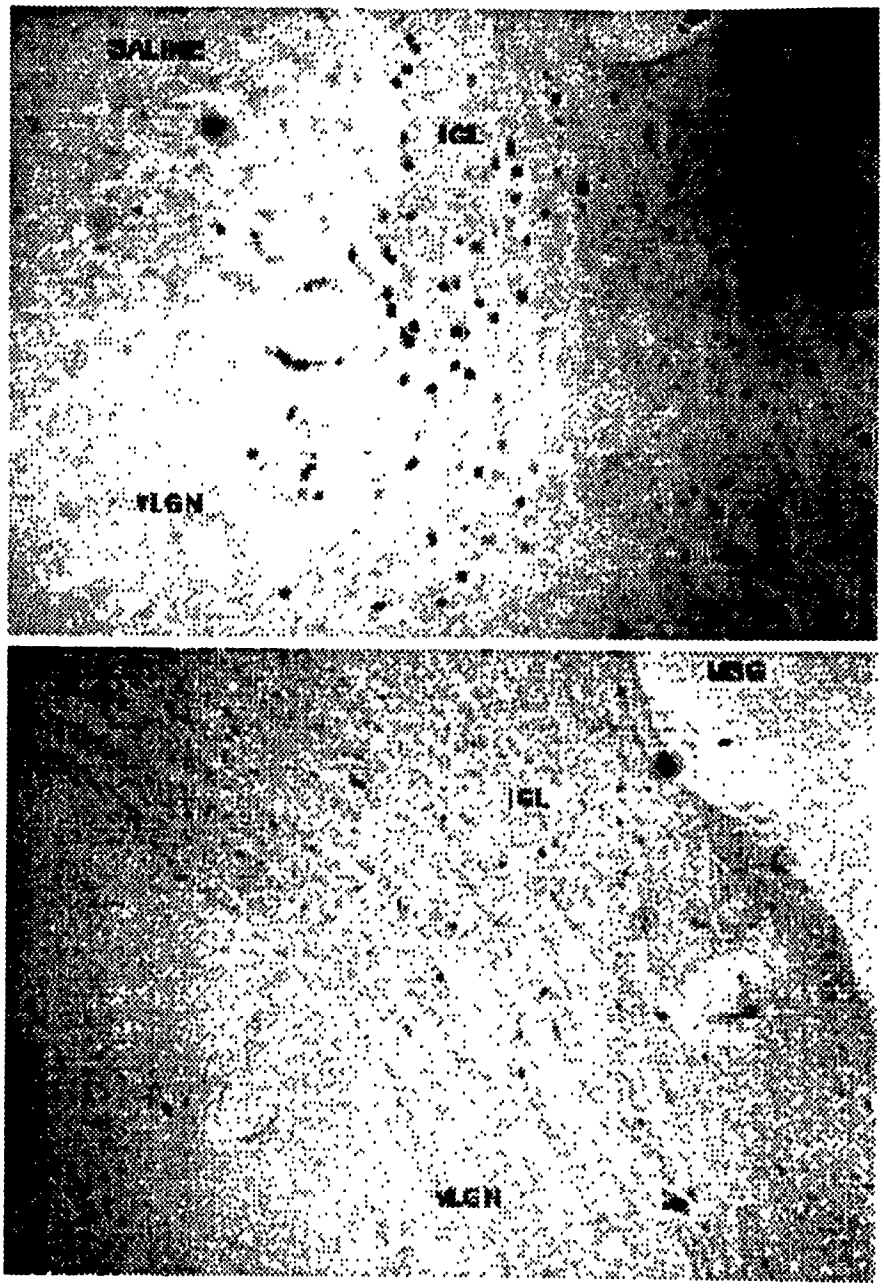

Figure 15. Fos-like immunoreactivity in a coronal section through the IGL of representative saline and MSG treated rats exposed to a one hour light pulse during the fourth hour of the dark phase of the 12:12 LD cycle.

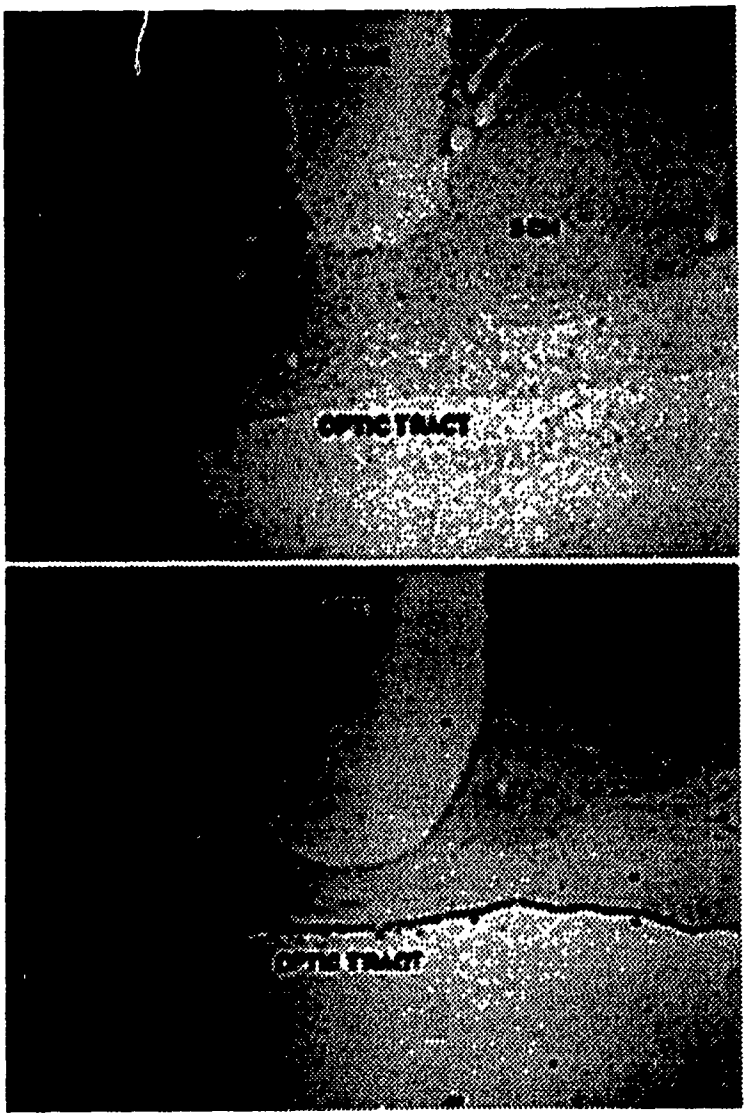

Figure 16. Fos-like immunoreactivity in a coronal section through the SCN of representative saline and MSG treated rats who did not receive a light pulse.

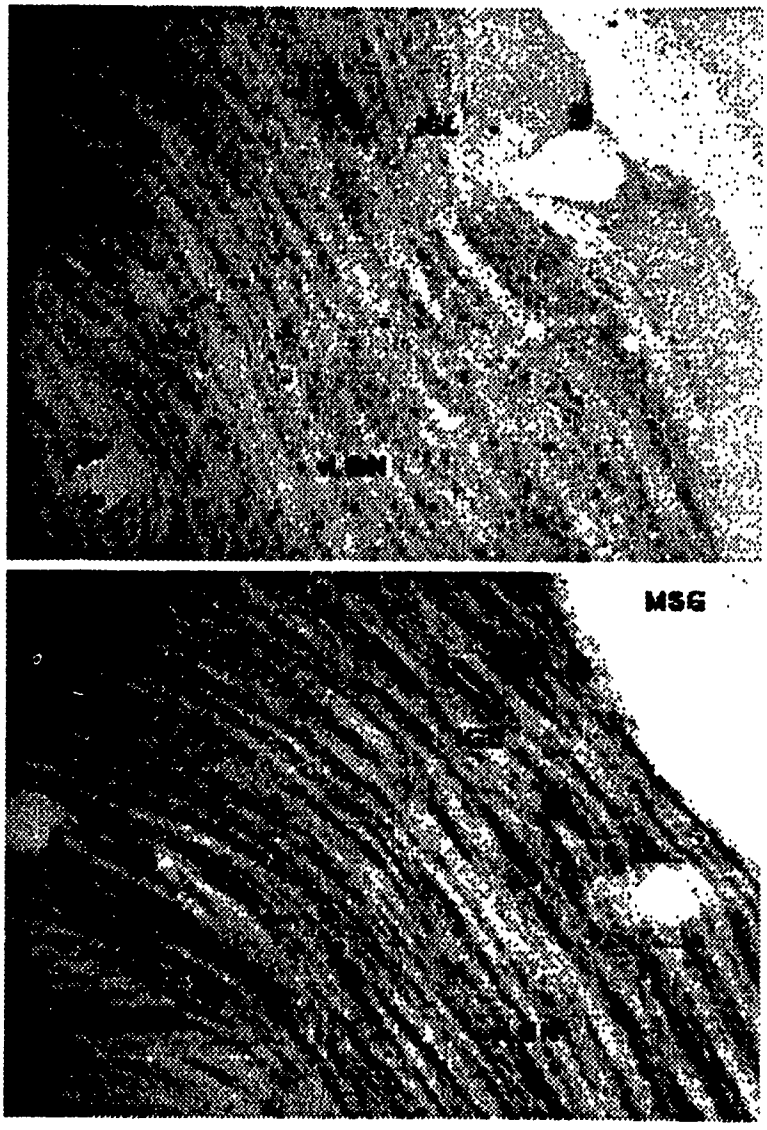

Figure 17. Fos-like immunoreactivity in a coronal section through the IGL of representative saline and MSG treated rats who did not receive a light pulse.

Discussion

The results of this study demonstrate that although circadian temperature and activity rhythms of rats treated neonatally with MSG entrain to 12:12 LD cycles, they do not degenerate under constant light conditions as do the rhythms of saline-treated rats. Furthermore, although both MSG- and saline-treated rats exhibit Fos expression in the SCN in response to photic stimulation, a light pulse that induces Fos in the IGL and vLGN regions of saline-treated rats does not induce Fos in the IGL/vLGN regions in MSG-treated animals. The lack of Fos expression in the IGL/vLGN regions in response to photic stimulation in these animals suggests that retinal input to the GHT is altered by MSG treatment. Taken together, these data suggest that the disruption of circadian rhythmicity observed under constant light conditions may depend on the integrity of the GHT.

It is likely that the retinohypothalamic tract remains intact in MSG-treated animals. This pathway is necessary for entrainment to the light-dark cycle (Moore, 1983), and MSG-treated animals have been shown to exhibit rhythms entrained to the 12:12 LD cycle. Previous research on the circadian system in MSG-treated animals has indicated that these animals display normal circadian rhythms of food intake, locomotor activity, plasma corticosterone and pineal N-acetyltransferase under light-dark cycles (Groos, 1981; Miyabo et al., 1982; Nemeroff et al., 1977; Pickard et al., 1982). Furthermore, anatomical studies have demonstrated intact retinohypothalamic projections in the MSG-treated hamster, providing further support for the present results (Pickard et al., 1982).

The immunocytochemical data of the present study provide further support for the functional integrity of the RHT in MSG-treated animals. Previous research has shown that induction of Fos protein in cells of the SCN and

IGL/vLGN regions occurs in response to light pulses that induce phase shifts. Fos protein has therefore been used as a marker for neuronal activation in these regions. The light-induced expression of Fos in the SCN of MSG-treated rats indicates that retinal input to the SCN via the RHT remains intact in these animals.

In the present study, the robust circadian temperature and activity rhythms observed in MSG-treated animals under constant light conditions suggests that MSG treatment alters the responsiveness of the neural pathway that mediates the circadian response to constant light. The disruption of circadian rhythmicity in normal animals under continuous illumination conditions has been attributed to the input from the IGL to the SCN, although the mechanism by which this disruption occurs is not known. In hamsters, lesions of the IGL or GHT alter the sensitivity of the circadian activity rhythm to constant light exposure (Harrington & Rusak, 1988; Pickard et al., 1987). The present work provides new data indicating that the GHT also mediates the circadian response to constant light in rats.

The immunocytochemical data reported in this study provide further support that MSG treatment influences the responsiveness of the IGL and vLGN to photic input. MSG-treated animals do not exhibit Fos expression in the IGL or vLGN regions in response to a light pulse that consistently induces Fos in these regions in saline-treated rats. This finding suggests that cells in the IGL/vLGN of MSG treated rats are not responsive to photic stimulation. Neonatal treatment with MSG most likely affects the GHT by damaging the retinogeniculate projection, although anatomical evidence for such damage remains to be established.

The previous finding of intact retinal projections to the vLGN in MSG-treated hamsters (Pickard et al., 1982) possibly results from methodological or

species differences. Pickard et al. (1982) treated hamsters with one injection (8 mg/g) on postnatal day 8. Because neonatal animals show increasing resistance to the excitotoxic effects of MSG during the first two weeks of life (see Kizer et al., 1978, or Meldrum, 1993), it is conceivable that pups treated on day 8 could have been protected from some of the excitotoxic effects of the drug by this age, and therefore did not experience damage to the same extent as animals treated from the day after birth .

In the present study, neonatal drug treatment significantly altered amplitude of the circadian temperature rhythm in the adult animal under a 12:12 LD cycle. Although this effect was not significant for the activity rhythm, trends in the amplitude activity data suggest that MSG-treated animals tend toward blunted amplitudes as compared to saline-treated rats as well. The lack of a statistically significant difference in this case may be due to the high degree of variability of activity data within each group. These data suggest that the components of the circadian system involved in circadian amplitude were affected by neonatal MSG treatment. Reduction in amplitude of rhythms has been associated with a decreased effectiveness of environmental zeitgebers, damage to the pacemaker or to the transmission pathways, or pathology of the tissues in which the overt rhythmicity is seen (Moore-Ede et al., 1982). The observed decrease in amplitude in the rhythms of MSG-treated animals may be related to the lack of adequate input from the GHT to the SCN in response to light-dark transitions.

Taken together, the behavioral and immunocytochemical results of the present study confirm and extend results from previous work implicating the GHT in the circadian response to constant light. Furthermore, these results underscore the separation of function between the two neural pathways that mediate entrainment. The retinohypothalamic tract is necessary for entrainment

to the light-dark cycle, whereas the geniculohypothalamic projection appears to mediate responsiveness of the circadian system to constant light conditions. MSG treatment, therefore, provides a useful model to study the role of the retinohypothalamic tract in the absence of input from the geniculohypothalamic tract in the adult rat.

Alternate explanations for the differences between MSG- and saline-treated animals could include attributing the differences to damage in either the arcuate nucleus or the hippocampal CA1 region. The development of endocrine and metabolic abnormalities seen in MSG-treated animals has been associated with the decrease in neuropeptide-Y fibers in the paraventricular nucleus of those animals. These NPY fibers arise from the arcuate nucleus where a reduction in cell bodies has also been found (Kerkerian & Pelletier, 1986; Abe, Saito, & Shimazu, 1990). Damage to CA1 neurons in the hippocampus has been associated with impaired performance on visual discrimination tasks (Kubo et al., 1993). The mechanism by which either of these regions could exert effects on the circadian system, however, is not known. There is no evidence that entrainment of circadian rhythms under normal light-dark cycles, or the disruption of rhythms under constant light conditions, is associated with the functional integrity of either the arcuate nucleus or the hippocampus. Moreover, the immunocytochemical detection of Fos in the arcuate nucleus or hippocampal CA1 regions in response to light pulses that induce Fos in SCN, IGL, and vLGN regions, has never been reported. Preliminary examination of these regions in subjects used in the present study suggests that cells in the arcuate nucleus and hippocampus do not express Fos protein in response to light.

Future Considerations

The full extent of damage to retinohypothalamic and retinogeniculate projections in MSG-treated animals has not been verified. Previous work has indicated that acute degeneration of retinal ganglion cells and demyelination of the optic tract occurs as a result of MSG treatment. Those findings appear to conflict with research demonstrating that entrainment to light-dark cycles occurs in MSG-treated animals. Tract tracing work should be conducted to examine the functional integrity of both the primary visual pathways and the secondary circadian pathways in animals treated neonatally with MSG.

The extent of damage to retinal projections to the IGL and vLGN should correlate with the protective effect of MSG in the lack of disruption of circadian rhythmicity in MSG-treated animals. To verify that this response is truly prevented due to MSG treatment, MSG-treated animals should be exposed to constant light conditions over a longer time frame. It is possible that the disruptive effect of constant light on rhythmicity is merely delayed and not prevented in response to MSG treatment.

The immunocytochemical work should be further analyzed as well. The expression of Fos in the IGL and vLGN regions in response to a one hour light pulse has not been found consistently in other laboratories. For example, Park et al. (1993) have shown that a minimum of two hours of light is required in order to obtain Fos expression in these regions. Methodological differences, such as use of a different primary antibody, may account for these inconsistencies. Examination of the expression of Fos in the IGL and vLGN regions in response to light pulses of different lengths, and at different times in the circadian cycle, would be appropriate to ascertain the lack of response of this region in MSG-treated animals.

Correlation between immunocytochemical staining for neuropeptide-Y in the IGL and behavioral data during exposure to prolonged constant light should also be examined in MSG- and saline-treated animals. Previous researchers have implicated NPY in the transmission of photic information to the SCN via the GHT. Differences in NPY immunoreactivity across the circadian cycle in animals treated with MSG could provide insight into the mechanism by which the IGL exerts an influence over the circadian timing system.

Further insight into the molecular mechanisms of entrainment may be gained by examining the role of immediate-early genes in the cellular response to light. For example, use of antisense oligonucleotides against the c-fos gene would be expected to directly block the induction of Fos protein in response to light. If Fos plays a causal role in the circadian response to light pulses, then blocking expression of Fos should alter the behavioral response to brief pulses of light. The role of the IGL may then be further clarified by selectively blocking Fos in IGL and vLGN regions, and examining the behavioral response to photic stimulation.

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Appendices

Appendix A

Source Table for the ANOVA for Amplitude of the Circadian Temperature Rhythm

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Drug Treatment	1	0.160	0.160	5.646	0.0448
Subjects	8	0.227	0.028		
Lighting Schedule	1	1.145	1.145	57.449	0.0001
Light x Drug	1	0.108	0.108	5.400	0.0486
Light x Subjects	8	0.159	0.020		

Appendix B

Source Table for the ANOVA for Amplitude of the Circadian Activity Rhythm

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Drug Treatment	1	1848.195	1848.195	3.758	0.0886
Subjects	8	3934.795	491.849		
Lighting Schedule	1	4091.001	4091.001	22.203	0.0015
Light x Drug	1	749.651	749.651	4.068	0.0784
Light x Subjects	8	1474.061	184.258		