12-2000

Effects of Neonatal Clomipramine Treatment on Photic and Non-Photic Circadian Phase Shifting in Rats

Suzanne M. Dwyer

EFFECTS OF NEONATAL CLOMIPRAMINE TREATMENT ON PHOTIC AND NON-PHOTIC CIRCADIAN PHASE SHIFTING IN RATS

By

Suzanne M. Dwyer

B.Sc. Saint Mary's University, 1993

Certificate of Honors Equivalency Saint Mary's University, 1994

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

(in Psychology)

The Graduate School

The University of Maine

December, 2000

Advisory Committee:

Alan M. Rosenwasser, Professor of Psychology, Advisor

Ziad Boulos, Research Scientist (New York State Psychiatric institute)

Marie 3. Hayes, Associate Professor of Psychology

Michael A. Robbins, Senior Research Associate and Cooperating Associate Professor of Psychology

D. Alan Stubbs, Professor of Psychology

EFFECTS OF NEONATAL CLOMIPRAMINE TREATMENT ON PHOTIC AND NON-PHOTIC CIRCADIAN PHASE SHIFTING IN RATS

By Suzanne M. Dwyer

Thesis Advisor: Dr. Alan M. Rosenwasser

An Abstract of the Thesis Presented

in Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

(in Psychology)

December, 2000

Neonatal exposure to monoaminergic antidepressant drugs produces a wide variety of effects on the behavior and physiology of adult rats which parallel those observed in human depression. Monoaminergic neurotransmitter systems are involved in the regulation of both affective and circadian behaviors, and alterations in circadian rhythmicity have been observed in depressed patients and in several other animal depression models. The purpose of the present study was to explore circadian phase shifting following neonatal antidepressant treatment. Neonatal rats were divided into three treatment groups; a clomipramine-treated group, a saline-treated group, and an unhandled group. Daily injections of clomipramine (25 mg/kg sc), a serotonin (5-HT) re-uptake inhibitor, or saline were given from postnatal day 8-21. The saline-treated group served as a control for the pharmacological effects of clomipramine, while the unhandled

group was included to control for any stressors associated with the injection procedure. As adults, rats participated in one of two separate experiments designed to assess non-photic and photic phase shifting. In the non-photic experiment, stimuli included injections of 8-OH-DPAT (5mg/kg ip), a 5-HT agonist, and six-hour dark pulses, both of which were presented during subjective day. A significant difference was found between the clomipramine and saline-treated groups in both the size and direction of 8-OH-DPAT-induced phase shifts, as the clomipramine-treated group displayed a small phase advance while the saline-treated group showed a larger phase delay. Following dark pulses, all groups displayed mean phase advances, but no significant group differences were observed. For both 8-OH-DPAT and dark pulses however, the pattern of responses across the three neonatal groups indicates that neonatal clomipramine and saline treatment may have opposite effects on non-photic phase shifting. In the photic experiment, rats were presented with light pulses during early and late subjective night, but no significant group differences were found in the magnitude of the resulting phase shifts. Taken together, results of this study suggest that neonatal clomipramine treatment alters non-photic, but not photic phase shifting, and comparisons of the present results with those reported previously, provide support for species differences in the role of serotonin in circadian regulation.

ACKNOWLEDGEMENTS

I would like to express my appreciation to the members of my advisory committee -- Marie J. Hayes, Ph.D., D. Alan Stubbs, Ph.D., Ziad Boulos, Ph.D., and Michael A. Robbins, Ph.D.-- for their contributions to this dissertation. A special thank you to my advisor, Alan M. Rosenwasser, Ph.D., for all his help and support over the course of my graduate school years. His lab was a fun and interesting place to work and learn about docks, and I truly value that experience.

I would also like to express my deep gratitude to my parents, Brian and Anne Marie Dwyer. They have been a constant source of support and encouragement for me, without which I could not have completed this project, and I sincerely thank them. Alice Taylor and my brothers, Sean and Mark Dwyer, have been great friends to me, and I would also like to acknowledge them here.

Finally, I would like to thank my fellow graduate students, particularly the Canadian contingent, for their support and friendship throughout this graduate school experience.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I
INTRODUCTION

The mammalian circadian timing system is a neural system responsible for the generation, regulation and temporal organization of daily biological and behavioral rhythms (Moore-Ede, Sulzman & Fuller, 1982). A fundamental feature of endogenous circadian rhythms is the continued expression of an approximately 24-hour period in the absence of external time cues (Pittendrigh & Daan, 1976a). The period of an output rhythm (e.g. locomotor activity) in the absence of external time cues is assumed to reflect the period of the underlying circadian pacemaker. Under natural conditions, the circadian pacemaker is usually entrained to environmental periodicities, particularly the light-dark (LD) cycle, although a number of non-photic cues such as the timing of social interactions, food availability and others, may also contribute to entrainment. Without external time cues, the circadian cycle comprises a subjective day, the phase during which diurnal activities occur, and a subjective night, the phase during which nocturnal activities occur (Meijer, 1991).

Fundamental Properties of Circadian Rhythms

Free-Running Period

Free-running period in constant darkness (DD) is considered to be a fundamental property of circadian rhythms. Mammalian species show characteristic free-running periods which tend to vary slightly from 24 hours, and stimuli found to affect free-running period are thought to have direct action on the circadian pacemaker or its input pathways (Pittendrigh, 1981). The effects of a stimulus on circadian perii can be evaluated by presenting the stimulus chronically to an animal while it is maintained in constant environmental conditions, and monitoring changes in the period of a particular circadian output rhythm. Circadian rhythms in locomotor activity are commonly used in such studies, as they are relatively easy to record and have dearly defined phase markers (Turek, Pinto, Viitema, Penev, Zee & Takahashi, 1995). For example, in nocturnal animals,

circadian time (CT) 12 represents the onset of subjective night, and is defined by activity onset. Changes in the timing of successive activity onsets during stimulus presentation can be used as a measure of the influence of that stimulus on the period of the circadian pacemaker.

An effective stimulus may cause the free-running period to shorten or lengthen (Aschoff, 1981). A shortening of free-running period would be evidenced in an activity rhythm when the onset of activity occurs progressively earlier each day than would be predicted from the pre-stimulus period. In contrast, a lengthening of free-running period occurs when the phase of the rhythm is progressively delayed with each successive circadian cycle. As will be discussed below, the most well-studied stimulus known to affect free-running period is constant light (LL), which can lengthen or shorten free-running period relative to DD conditions (Aschoff, 1981).

Phase-Response Curves (PRCs)

The ability of an acute stimulus to alter the phase (i.e. timing) of free-running circadian rhythms is also thought to reflect direct action on the circadian pacemaker or its input pathways (Pittendrigh, 1981). The magnitude and direction of phase shifts in response to a stimulus can be described through the use of phase-response curves (PRCs), which depict the relationship between circadian time of stimulus presentation, and the resulting change in steady-state phase of the free-running rhythm. An effective stimulus may result in a phase advance, phase delay or no change in the rhythm depending on the point in the circadian cycle at which it is presented (Smith, Turek & Takahashi, 1992). For example, the photic PRC shows the portions of the circadian cycle during which light exposure is effective in producing a phase shift. This PRC is obtained by maintaining an animal in constant darkness and exposing it to a brief pulse of light (generally from several seconds to several minutes, although both longer and shorter durations have been used) at different circadian times. Phase delays occur following light exposure in the early subjective night, and would be observed in an activity rhythm when activity onset for the circadian cycles following the stimulus occurs later than would be predicted by the pre-

stimulus phase. Phase advances occur following light exposure in the late subjective night, such that activity onset for the circadian cycles following the stimulus occurs earlier than would be predicted by the pre-stimulus phase. Light exposure has little or no effect when presented during the subjective day. At a given phase of presentation, the magnitude of a light-induced phase shift is a function of stimulus intensity and duration (Nelson & Takahashi, 1991). Although the photic PRC is derived from the effects of brief light pulses, it is thought that entrainment to an LD cycle occurs through daily re-adjustments, or phase shifts, of the circadian pacemaker by light exposure during sensitive phases near dawn and/or dusk, as defined by the photic PRC (Pittendrigh & Daan, 1976a; 1976b).

Often, the magnitude of the change in phase caused by an acute stimulus can be interpreted more accurately several circadian cycles following stimulus presentation. This is due to the presence of transients, or temporary states, before the final steady-state phase shift is observed (Moore-Ede et al., 1982). Transients are commonly observed following light pulses, particularly when the pulses are presented during the advance portion of the photic PRC (Moore-Ede et al., 1982; Nelson & Takahashi, 1991). However, double-pulse experiments, in which two light pulses are presented in the same circadian cycle, before a steady-state phase shift is observed, have shown that the primary pacemaker has a rapid response to appropriately timed photic stimulation (Moore-Ede et al., 1982). For example, a light pulse presented to hamsters at CT 13 and another two hours later results in a final phase delay (following several cycles of transients) of greater magnitude than that normally observed at CT 13. This would not be true if the second pulse fell at CT 15, a phase at which little or no photic phase shifting is observed. Instead, these results may be accounted for by assuming that a rapid phase delay induced by the first pulse resulted in the second pulse also falling on the delay portion of the PRC close to CT 13, such that the combination of the two phase delays is responsible for the large phase shift (Hastings et al., 1996). For further detail, imagine a photic PRC in which two hour phase delays are observed at CT 13 but no phase shifts are observed at CT 15. If the pacemaker had a slow response to stimulation, then a light

pulse presented at CT 13 and another two hours later (i.e. CT 15), would result in a total phase delay of two hours. If the pacemaker had a rapid response to stimulation, then a light pulse presented at CT 13 would immediately delay the pacemaker to CT 11. Thus, the second pulse presented two hours later would also fall at CT 13 and so the total phase shift would be four hours. Studies using this double-pulse technique find results similar to the latter scenario, supporting the idea that the pacemaker displays a rapid phase-shifting response to photic stimulation (e.g. Hastings et al., 1996).

Models of the circadian system predict that the primary pacemaker is composed of more than one component oscillator (Moore-Ede et al., 1982). For example, it has been proposed that the pacemaker of nocturnal rodents consists of two mutually coupled oscillators; an evening oscillator (E) controlling activity onset and coupled to dusk, and a morning oscillator (M) controlling activity offset and coupled to dawn (Pittendrigh & Daan, 1976c). These oscillators maintain a stable phase relationship with each other and with secondary oscillators downstream from the primary pacemaker. The relationship between the E and M oscillator can be temporarily disrupted by a light pulse falling on a different phase of each oscillator. In this model, transients are thought to represent a period during which the stable phase relationships are recovered.

Higher-Order Properties of Circadian Rhythms

Entrainment

As discussed above, free-running period in DD and the shape of the photic PRC are considered to be fundamental properties of the circadian pacemaker (Pittendrigh & Daan, 1976c). Other variables--such as steady-state entrainment phase under LD cycles, and the effects of exposure to LL on free-running period--can be predicted from these fundamental properties. Entrainment involves m-setting the circadian clock by external time cues, or zeltgebers. During entrainment, expressed periods come to match the period of the zeitgeber through daily phase shifts which correct for the difference between the zeitgeber period and the free-running period. Thus, for effective entrainment to occur, the zeitgeber must be present at a point in the circadian cycle that will result in an appropriate

phase shift. If the period of the zeitgeber is longer than that of the free-running rhythm, then entrainment will occur through daily phase delays, and the cue must be present at a point on the PRC resulting in phase delays. If the period of the zeitgeber is shorter than that of the free-running rhythm, then entrainment will occur through daily phase advances. Four requirements must be met in order to demonstrate entrainment by a particular zeitgeber: (1) the absence of another effective time cue; (2) period control (zeitgeber and entrained period are equal); (3) a stable phase relationship between the zeitgeber and circadian rhythm; and (4) phase control, such that following removal of the zeitgeber, free-running rhythms originate from the phase set by the zeitgeber (Moore-Ede et al., 1982).

The phase relationship between a zeitgeber and an entrained rhythm is dependent on the period of the zeitgeber, as well as on the underlying free-running period, and is defined in terms of the difference between zeitgeber and rhythm reference phases in each cycle (Aschoff, 1981). For example, the phase relationship between the LD cycle and the activity rhythm of nocturnal animals is reflected in the difference between dark onset and activity onset. An advanced entrainment phase occurs when activity onset precedes dark onset, while a delayed entrainment phase occurs when activity onset follows dark onset. For a given zeitgeber period, entrainment phase can be predicted from the period of the free-running rhythm; entrainment phase of a free-running rhythm with a shorter period will be relatively advanced compared to that of a free-running rhythm with a longer period.

Mammals can entrain to zeitgeber cycles up to several hours less than, or greater than 24 hours (Pittendrigh & Daan, 1976b). The range of periods to which entrainment can occur is consistent within species, as it is limited by the ampliide of the PRC. Near the limits of the range of entrainment, relative coordination can occur when the zeitgeber modulates free-running period, but is not strong enough to result in stable entrainment (Pittendrigh, 1981). During relative coordination, phase shifts caused by the zeitgeber are not large enough to fully correct for the difference between the zeitgeber period and free-running period. With each successive cycle, the zeitgeber will fall at systematically shifting points on the PRC, causing the rhythms to alternately slow down and speed up in coordination with the zeitgeber.

Constant Light (LL)

As mentioned briefly above, constant light (LL) exposure systematically affects free-running period (Aschoff, 1981). During LL, length of free-running period is thought to reflect integration of the photic PRC. For nocturnal animals, LL generally results in an intensity-dependent lengthening of free-running period relative to that seen in DD. The extent of the period-lengthening can be predicted from the relative size of the delay and advance portions of the photic PRC (D/A ratio); the larger the D/A ratio, the greater the lengthening in period. Nocturnal animals also show reduced activity in LL, and a decrease in the duration of time spent active during each circadian cycle (alpha), as compared to DD (Aschoff, 1981). As the light intensity is increased, further lengthening of free-running period and reduction of alpha may be observed. Following termination of LL, free-running rhythms may continue to reflect the influence of LL (i.e. display a longer period than that seen prior to LL exposure) for a substantial length of time (Piiendrigh, 1981). These "aftereffects" are thought to represent residual effects of the stimulus on pacemaker period.

Splitting of activity rhythms can also occur under continuous light. In this state, the animal's activity pattern, normally occurring as one intense bout, splits into two or more separate bouts over the course of each circadian cycle (e.g. Boulos & Rusak, 1982b). In the hamster, these components often recouple at antiphase, thereby assuming a stable relationship 180^{o} out of phase. Splitting of free-running rhythms has been taken as evidence for the presence of multiple oscillators governing circadian rhythmicity. For example, in the dual-oscillator model of Pittendriih and Daan (1976c), splitting represents the uncoupling of the E and M oscillators due to their differential response to light intensity. In this model, the free-running period of the E oscillator is a positive function of light intensity, while that of the M oscillator is a negative function of light intensity. Splitting occurs when the difference between these intrinsic periods becomes too great for coupling to be maintained, and each free-runs with its own intrinsic period. In this model, the E and fvl components can maintain a stable phase relationship only when they are in phase or antiphase.

Prolonged exposure to LL, or even less prolonged exposure to particularly intense light, can result further in a breakdown of circadian rhythmicity (Aschoff, 1981). In this situation, no coherent circadian rhythms can be observed, as activity appears to be randomly distributed over time. It is hypothesized that this arrhythmic state represents the uncoupling of multiple secondary circadian oscillators from the primary pacemaker, or that the pacemaker itself dissociates into a multiplicity of uncoupled oscillators, manifested as a loss of overt rhythmicity (Moore-Ede et al., 1982; Rosenwasser & Adler, 1986).

Phase Shifting in Response to Behavioral Stimuli

A variety of behaviorally-arousing stimuli are capable of phase-shifting the circadian pacemaker in a pattern different from, but not opposite to, that of light. These stimuli are believed to exert their effects through changes in the animal's behavior; specifically, through increases in activity levels. The behavioral PRC shows large phase advances in the mid to late subjective day, and generally smaller phase delays in the late subjective night (Smith et al., 1992). Similar patterns of phase shifting are observed in response to many behaviorally-arousing events, including social interaction, cage cleaning, exposure to novel environments, and 'pulses" of darkness presented on a background of LL (Boulos & Rusak, 1982; Mrosovsky, 1996; Mrosovsky, Reebs, Honrado & Salmon, 1989). PRCs of the same general shape have also been observed for drugs acting on y -aminobutyric acid (GABA), serotonin (5-HT) and certain neuropeptide systems (e.g. neuropeptide Y) (Albers & Ferris, 1984; Huhman, Marvel, Gillespie, Mintz & Albers, 1997; Edgar, Miller, Prosser, Dean & Dement, 1993), as described more fully below.

In contrast to the photic PRC, there is much variability among these PRCs, particularly during the subjective night, when either phase delays, small phase advances, or no effects may be seen. In addition, the timing of the delay-to-advance transition is inconsistent across these PRCs, and although phase advances are always observed during the subjective day, the peak of the advance region can differ by as much as five hours (Smith et al., 1992). Although these differences provide evidence for possible subfamilies of behaviorally-derived PRCs, in general, these PRCs are deflned as such by

their dissimilarity to the photic PRC. Thus, although these PRCs have been referred to as D-type (i.e. dark-type; Smith et al., 1992) or Y-type (i.e. neuropeptide Y-type; Morin, 1991), this pattern of phase shifting and the stimuli that produce it are most commonly referred to simply as non-photic. In this context, the term non-photic refers to a particular PRC shape, and thus excludes the stimuli that mimic the phase-shifting pattern associated with light (see below).

Increased activity following a behaviorally-arousing stimulus is critical to the resulting non-photic phase shift. For example, phase shifts in response to dark pulses can be blocked by restraining the animal during the pulse (Reebs, Lavery & Mrosovsky, 1989). Moreover, the amount of activity observed in response to a behaviorally-arousing stimulus has been correlated with the size of the resulting phase shift Janik and Mrosovsky (1993) found that significant phase advances following dark pulses or novel wheel exposure in the mid-subjective day occurred only in those hamsters whose wheel running activii exceeded 4000 wheel revolutions in the three hours of stimulus exposure. Animals displaying less activity did not display significant phase advances. In this study, the amount of activity and the resulting phase shift followed a step function, in that activity needed to exceed a certain threshold before phase shifts were observed. Other studies have shown similar effects with a minimum duration (i.e. 3-4 hours) rather than amount of wheel-running activity or treadmill running as the critical factor in predicting phase shifts (Gannon & Rea, 1995; Marchant & Mistlberger, 1996). However, significant phase shifts have also been reported following non-photic stimulation accompanied by activity of lesser duration (e.g. Wickland & Turek, 1991) and intensity (e.g. Mistlberger, Marchant & Sinclair, 1996). In general, the effects of activity-inducing stimuli are thought to be mediated by increased arousal which is reflected in, but perhaps not perfectly correlated with, increased locomotor activii. It is the increase in arousal, or some as yet unidentified physiological correlate, that is believed to underlie the phase-shifting effects of many non-photic stimuli (Mrosovsky, 1996).

Conflicting evidence has been presented as to whether activity must be voluntary in order to produce a phase shift. For example, some hamsters do not voluntarily display

high levels of activity in response to novel wheel exposure, and even when activity is induced in these animals via exposure to cold temperatures, phase shifts are not observed (Mrosovsky & Biello, 1994). This result suggests that motivational state may be an important factor in the phase-shifting effects of activity. However, other studies have shown that activity in response to cold temperatures is effective in producing phase shifts in the majority of hamsters tested (Mistlberger et al., 1996), and that forced treadmill running produces phase shifts in mice (Marchant & Mistlberger, 1996). In addition, non-photic entrainment in mice has been demonstrated with both voluntary (e.g. Edgar & Dement, 1991) and involuntary (e.g. Marchant & Mistlberger, 1996) activity.

In addition to these phase-shifting effects, activity level also influences free-running period. For example, the total amount of activity over the course of the subjective night can be related to free-running period, as motor activity is negatively correlated with free running period in rats (Yamada, Shimoda, Takahashi & Takahashi, 1996). Thus, those animals displaying higher activity levels tend to have shorter periods than those displaying lower activity levels. In addition, in a study of locomotor activity of mice, Edgar, Martin and Dement (1991) found that mice with shorter periods tend to concentrate their activity in the early part of the subjective night, while those with longer periods tend to be most active in the latter part of the subjective night. This effect can be predicted from the non-photic PRC, in that early activity would tend to occur during the advance portion of the PRC resulting in shorter free-running periods, and later activity would tend to occur during the delay portion of the curve, resulting in longer free-running periods.

The fact that the circadian pacemaker is sensitive to behavioral feedback can complicate the interpretation of circadian studies in which the stimulus also has behavioral effects. For example, injections of the benzodiazepine, triazolam, result in non-photic phase shifts in hamsters, but, like those seen for dark pulses, these phase shifts can be blocked by restraining the animal following injection (Mrosovsky & Salmon, 1990; Van Reeth & Turek, 1989). Thus, in hamsters, the phase-shifting effects of triazolam appear to be mediated by induced activity. Although many stimuli that yield non-photic PRCs do have their effects through behavioral arousal, this is not true of all such stimuli. For

example, ttiazolam has a sedating effect on squirrel monkeys, but, like hamsters, these animals display non-photic phase shifts in response to ttiazolam (Mistlberger, Houpt & Moore-Ede, 1991). In addition, administration of the benzodiazepine chlordiazepoxide, neuropeptkle Y (NPY) or certain 5-HT agonists results in non-photic phase shifts without affecting observed activity levels (Biello, Janik & Mrosovsky, 1994; Biello & Mrosovsky, 1993; Bobrzynska, Godfrey & Mrosovsky, 1996). As discussed below, NPY and 5-HT neurons may actually mediate the effects of induced activity on the pacemaker, rather than the converse. Phase shifts in response to saline injections appear to be mediated through a stress-response, and activity levels are not increased following the injections (Hastings et al., 1992). However, this stimulus may be qualiitively different from other non-photic stimuli, as maximal phase advances are observed at CT 10 for saline injections, but typically at around CT 4-8 for other non-photlc stimuli. Finally, non-photic phase shifts have been observed in *vitro,* in pacemaker cell preparations, following various pharmacological treatments (e.g. Prosser, Bean, Edgar, Heller & Miller, 1993; Huhman, Babagbemi & Albers, 1995). Thus, although the circadian pacemaker must have a mechanism by which it receives behaviorallyderived feedback, non-photic stimuli are not exclusively dependent on this mechanism for their effects.

Interactions Between Photic and Non-Photic Stimuli

The studies discussed above indicate clearly that both photic and non-photic stimuli are capable of influencing the circadian pacemaker. Photic stimuli are most effective in producing phase shifts during the subjective night, while non-photic stimuli are most effective during the subjective day. In recent years, interest has arisen in the potential interactive effects of such stimuli on circadian rhythmicity. For example, a study of scorpions showed the importance of behavioral responses to light pulses in determining the resulting phase shifts. The typical photic PRC was observed for those animals remaining inactive during the light pulse, but a non-photic PRC was observed for those displaying behavioral activation during the light pulse (Honmann, Michel & Fleissner, 1990). Thus, changes in activity can interact with photic stimulation in influencing the

circadian system. Combining behavioral activation and photfc stimulation has also been found to influence both photic and non-phottc phase shifting, as well as entrainment, in mammals.

Light exposure during the subjective night generally inhibits the activity of nocturnal animals in the home cage environment (Mrosovsky, 1995). However, some animals will become active during a light pulse at this phase, when the light pulse is combined wlth a behaviorally-arousing stimulus. For example, Ralph and Mrosovsky (1992) presented hamsters with a light pulse in the late subjective night together with a novel wheel; importantly, novel wheel exposure does not normally result in phase shifts at this phase when presented alone. The number of wheel revdutions recorded during the light pulse was used as a marker of behavioral activation. The resulting photic phase shifts were significantly smaller for those animals with high levels of activity during the light pulse compared to those with lower activity levels. Thus, behavioral activation, or some biochemical correlate, had an antagonistic effect on photic phase shifting (Ralph & Mrosovsky, 1992). Presentation of a novel wheel does not alter photic phase delays in hamsters in the early subjective night (Mistlberger & Antle, 1997), but these animals are generally more active--as measured by wheel-running or general locomotor activity--during the early rather than late subjective night, so the lack of effect of induced activity at this phase may reflect a ceiling effect.

Other examples of the modulation of photfc effects by non-photic stimuli can be seen in re-entrainment studies in which the entraining LD cycle is advanced by a number of hours. Re-entrainment to a new LD cycle typically occurs gradually, depending on the extent of the change. For example, up to several weeks may be required for hamsters to display stable entrainment following an 8-hour advance of the LD cycle (Mrosovsky & Salmon, 1999). However, rates of re-entrainment can be accelerated by manipulation of activii levels. Specifically, if high levels of activii are induced following the LD shift, then re-entrainment occurs rapidly (Mrosovsky & Salmon, 1990; Van Reeth & Turek, 1989). Particularly dramatic effects of this kind are seen when the induced activity is evoked by

an animal's initial exposure to a running-wheel, as this stimulus induces very high levels of activity in hamsters (Mrosovsky, 1996; Wickland & Turek, 1991).

In addition to non-photic modulation of photic phase shifting, there is evidence for photic modulation of non-photic phase shifting. For example, phase advances observed in response to novel wheel exposure during the mid-subjective day can be blocked by a subsequent light pulse (Ralph & Mrosovsky, 1992). Similarly, light pulses can block the phase-advancing effects of injections of NPY or 5-HT agonists during the mid-subjective day (Biello & Mrosovsky, 1995; Penev, Zee & Turek, 1997). Light pulses have little or no phase-shifting effects when presented alone at this phase, but these results show that acute light exposure can modify the response to non-photic stimuli during the subjective day. Exposure to LL may also modulate non-photic phase shifting, as certain pharmacological and behavioral stimuli have been shown to produce phase shifts during the subjective day when presented on a background of LL, but not DD (Cutrera, Ouarour & Pevet, 1994). Although the effects of light pulses and LL on non-photic phase shifting differ in direction, the results of these studies suggest an interaction between pathways communicating photic and non-photic information to the circadian pacemaker.

Taken together, these resutts show that in addition to a photic input pathway, the circadian pacemaker must have a mechanism through which it receives non-photic input, and further, that these pathways must ultimately converge. The effects of various photic, pharmacological and behaviorallyderived stimuli on circadian period and phase have contributed to the localization of many neural components of the circadian system, and have also provided insight into the signal transduction pathways through which photic and non-photic stimuli alter circadian rhythmicity.

Suprachiasmatic Nucleus (SCN)

The generation and regulation of circadian rhythms involves an elaborate multi-oscillator system, of which the suprachiasmatic nucleus (SCN) is the principal circadian pacemaker (Rosenwasser & Adler, 1966). The SCN is composed of a bilateral pair of nuclei located in the anterior hypothalamus, dorsal to the optic chiasm (Klein, Moore & Reppert, 1991).

Evidence implicating the SCN as the primary pacemaker includes the fact that destruction of the SCN results in a widespread loss of circadian rhythmicity (Rusak, 1977; Stephan & Zucker, 1972). In addition, a circadian rhythm in neuronal firing rate is observed in the SCN (with higher firing rates during the subjective day, and lower firing rates during the subjective night), and this circadian pattern of cellular activity is maintained in in vitro SCN preparations (Meijer, 1991). Finally, through fetal SCN transplants, rhythmicity can be restored to SCN-lesioned rats and hamsters (Lehman, Silver, Gladstone, Kahn, Gibson & Bittman, 1987; Ralph, 1991). Use of donor tissue from a mutant strain of hamster (*Tau*) expressing dramatically shortened free-running period has revealed that the transplanted SCN confers its period on the lesioned (non-mutant) host (Ralph, Foster, Davis & Menaker, 1990).

Based on its cellular organization, the SCN is divided into a dorsomedial region, referred to as the shell, and a ventrolateral region, referred to as the core (Moore, 1998). A large number of Interneurons are located in both regions and extensive projections between cells of the SCN are evident. Neurons of the shell contain arginine vasopressin (VP) and those of the core contain vasoactive intestinal polypeptide (VIP) or gastrin-releasing hormone (GRP). The neurotransmitter GABA is found in all SCN cells, co-localized with VP or VIP/GRP (Watts, 1991). Other SCN peptides include substance P, somatostatin, peptide histidine isoleucine (PHI), and cholecystokinin (Miller, Morin, Schwartz & Moore, 1998).

Several lines of evidence indicate that circadian rhythmicity may be generated by individual cellular oscillators. For example, dissociated SCN cells maintained in culture have been found to express independently phased circadian rhythms in electrical activity (Welsh, Logothetls, Melster & Reppert, 1995). In addition, fetal rat SCN cells display circadian rhythmicity in 2deoxyglucose utilization before synaptogenesis has occurred (Reppert, 1992), and application of tetrodotoxin, a sodium channel blocker, eliminates action potentials but fails to alter underlying circadian rhythm generation (Schwartz, Gross & Morton, 1987). Thus, although the coupling mechanisms by which SCN cells coordinate

their activity to function as a pacemaker have not been adequately determined, communication may be mediated by non-synaptic interactions (Miller et al., 1996).

The SCN receives major projections from the retina, the intergeniculate leaflet of the thalamus, and the serotonergic neurons of the midbrain raphe. These projections provide both direct and indirect pathways by which retinal photic input reaches the pacemaker. Retina-recipient SCN neurons change their firing rate as a function--either increasing or decreasing-of retinal illuminance, and the extent of this response appears to be phase specific (Meijer, Watanabe, Schaap, Albus, & Detari, 1998). Photic stimulation which results in a phase shift is correlated with the expression of immediate-early genes (IEGs) in the SCN (Komhauser, Ginty, Greenberg, Mayo & Takahashi, 1998). The induction of IEGs is observed throughout the nervous system in an anatomically-specific pattern following diverse forms of stimulation, and the protein products of these genes are believed to initiate signal transduction pathways. Thus, IEGs provide a method for coupling stimulation to gene expression, allowing IEG expression to be used as a marker for specific types of neuronal activation. For example, induction of the proto-oncogene, *c-fos*, is observed in retina-recipient regions of the SCN following photic stimulation during the early and late subjective night, but not in visual-perceptual target areas of retinal afferents (Rea, 1989).

The product of c-fos, Fos protein, dimerizes with Jun proteins in the nucleus, and forms a regulatory complex which influences DNA transcription. Following a light pulse, c-fos m-RNA and Fos protein expression are localized in the retino-recipient region of the SCN of the hamster, mouse and rat (Colwell & Foster, 1992; Komhauser et al., 1996; Rea, 1989). The amount of *c-fos* m-RNA induced in the SCN following a light pulse has been positively correlated with the magnitude of the resulting behavioral phase shift (Komhauser, Nelson, Mayo & Takahashi, 1999). Pm-treatment with antisense oliionucleotides to *c-fos,* which block *c-fos* expression, has been shown to prevent light-induced phase delays in rats, suggesting that *c-fos* expression is required for photic responses (Wollnik et al., 1995). The photic PRC of mice with a null mutation of the *c-fos* locus shows generally reduced phase shift responses, particularly in the delay region, but

neither phase advances nor phase delays are completely abolished (Komhauser et al., 1996). Thus, although c-fos expression may not be required for photic responses, at least in mice, it does appear to be an essential part of the process leading to normal photic responses (Ralph et al., 1996).

C-fos expression in the SCN is thought to be specific to activation of photic transduction pathways. In support of this idea, *c-fos* expression has not been observed in the SCN of hamsters following non-photic stimuli including novel wheel exposure, triazolam injections and saline injections, presented during the subjective day (Cutrera, Kalsbeek & Pevet, 1993; Janik & Mrosovsky, 1992; Mead et al., 1992). However, *c-fos* expression has been observed in the SCN of rats following exposure to non-photic stimuli, although the number of SCN cells expressing *c-fos* was lower than that observed following photic stimulation during the subjective night (Edelstein & Amir, 1995). In addition, *ofos* expression in the rat SCN following non-photic stimuli is not phase-specific and has not been correlated with phase shift magnitude. Thus, although SCN *c-fos* expression is possibly involved in both photic and non-photic transduction in the rat, it appears to be specific to photic pathways in the hamster.

Retinohypothalamic Tract (RHT)

The retinohypothalamic tract (RHT) is a direct bilateral projection from retinal ganglion cells to the ventrolateral region of the SCN (Moore, 1992). This projection is believed to be the primary source by which photic information reaches the SCN, and is distinct from pathways involved in vision. For example, in rats, specialized W-type retinal ganglion cells project exclusively to components of the circadian system (Moore, Speh & Card, 1995). These cells are distributed across the retina and are characterized by their small diameter, sparsely branching dendrites and slow, sustained response to light (Meijer, 1991; Moore et al., 1995). Bilateral enucleation or destruction of the RHT abolishes photic entrainment, resulting in free-running rhythms even under LD cycles (Johnson, Moore & Morin, 1988). Destruction of other visual projections does not have this effect, further supporting the primary role of the RHT in conveying photic information (Moore, 1992).

The excitatory amino acid (EAA) glutamate, appears to be the neurotransmitter of the RHT (Turek et al., 1995). RHT terminals in the SCN display glutamate immunoreactivity (DeVries, Cardoza, Van Der Want, DeWolf & Meijer, 1993), and both NMDA and non-NMDA type EAA receptors have been found in the SCN (Card & Moore, 1989). Application of selective agonists for these receptors results in increased neuronal activity which can be blocked with NMDA and non-NMDA antagonists (Meijer, 1991). Electrical stimulation of the RHT results in release of glutamate from terminals in the SCN (Liou, Shibata, Iwasaki, 1986) and application of glutamate to the SCN *in vitro*, or NMDA *in vivo*, results in a PRC similar to that of light (Ding et al., 1994; Mintz & Albers, 1997; Shirakawa & Moore, 1994). In addition, EAA antagonists block light-induced phase shifts and reduce light-induced *c-fos* expression in the ventral (retino-recipient) region--but not in the dorsal region--of the SCN *in vivo* (Colwell, Foster & Menaker, 1991; Rea, Buckley & Lutton, 1993; Vindlacheruvu, Ebling, Maywood & Hastings, 1992). Evidence indicates that the dorsal SCN receives afferents from a distinct RHT projection that innervates both the SCN and the intergeniculate leaflet of the thalamus (IGL), which in turn projects to the SCN (Treep, Abe, Rusak & Goguen, 1995). The putative non-EAA transmitter of this projection is as yet unidentified. Nevertheless, the results discussed above provide strong support for a primary role of glutamate in RHT transmission.

The peptide substance P is co-localized with glutamate in some RHT terminals at the SCN (Takatsuji, Miguel-Hidalgo & Tohyama, 1991). Application of substance P to *in vitro* rat SCN preparations results in light-type phase shifts in electrical activity (Shibata, Tsuneyoshi, Hamada, Tominaga & Watanabe, 1992a), an effect which may be glutamate-mediated, as substance P has been found to potentiate the effects of glutamate when both are applied *in* vitro (Shirakawa & Moore, 1994). However, no significant phase shifts in the wheel-running rhythm of hamsters are observed following substance P injections alone (Piggins & Rusak, 1997). This discrepancy may be due to species-differences, methodological differences between *in vitro* and *in vivo* studies, or may reflect a role for substance P as a modulatory peptide, rather than a neurotransmitter, in the photic pathway.

Another element in the transduction of photic/EAA input to the SCN appears to be nitric oxide (NO) synthesis. NO, a gaseous paracrine factor, is produced by nitric oxide synthase (NOS) in response to glutamatergic stimulation of NMDA receptors in several brain regions, including the SCN (Gillette, 1996). NO is highly diffusible, appears to play an important role in the photic transduction pathway, and may mediate non-synaptic communication among SCN neurons (Miller et al., 1996). NOS immunoreactivity is present in the SCN of rodents, and NOS inhibitors administered to hamsters in *vivo* block the phase-shifting effects of light, but not light-induced c-fos expression in the SCN (Wang & Morris, 1996; Weber, Gannon, Michel, Gillette & Rea, 1995). This result suggests that in the hamster, either NO and *c-fos* are activated through independent mechanisms or NO acts downstream from c-fos expression in the photic transduction pathway. In contrast, in the rat, photic phase shifting and c-fos expression are observed following *in vitro* application of a NO donor and application of a NOS inhibitor has been reported to block light-induced phase shifting and attenuate c-fos expression (Amir & Edelstein, 1997; Ding et al., 1994). These results may reflect either methodological differences between *in vi&o* and *in vivo* studies or species-differences, such that NO acts upstream from c-fos expression in the rat, but not the hamster.

Intergeniculate Leaflet (IGL)/Geniculohypothalamic Tract (GHT)

The intergeniculate leaflet (IGL) is a neurochemically distinct region of the lateral geniculate complex of the thalamus, intercalated between the dorsal and ventral nuclei of the LGN (Moore & Card, 1994). The IGL receives photic input via collaterals of the RHT (Pickard, 1985). Besides the retina, other sources of input to the IGL include the anterior hypothalamus, retrochiasmatic area, contralateral IGL, raphe nuclei, locus coeruleus and the SCN (Moore, 1992). Neurons of the IGL have been found to contain GABA, NPY, enkephalin (ENK) and substance P. As in the SCN, GABA has been found in all neurons of the IGL, co-localized in separate populations with NPY and ENK (Watts, 1991). In the rat, GABA/ENK neurons project exclusively to the contralateral IGL, while in the hamster, a proportion of these neurons also project to the SCN. In both species however,

GABA/NPY neurons project predominately to the SCN. This projection from the IGL to the SCN forms the geniculohypothalamic tract (GHT), providing a secondary pathway for photic information to reach the SCN, as well as a pathway by which information from a range of non-retinal sources can influence the circadian system (Harrington, 1997; Morin, 1994).

Role of the IGL/GHT in Photic Effects

Like neurons of the SCN, IGL neurons show sustained changes in firing rate in response to changes in illuminance, and expression of *c-fos* is induced in the IGL following photic stimulation (Harrington & Rusak, 1989; Peters, Aronin & Schwartz, 1996). Therefore, the IGL is responsive to photic input, but because ablation of the IGL/GHT does not abolish stable entrainment to the LD cycle, this structure is not required for photic entrainment of the circadian system (Johnson, Moore & Morin, 1989). However, distinct changes in circadian response to photic stimulation are observed following IGL/GHT ablation, suggesting that the IGL/GHT is involved in mediating photic input to the SCN.

Following IGL lesions, the period-lengthening and arrhythmicity-inducing effects of LL are blocked, and the magnitude of light-induced phase advances is reduced while that of phase delays may be either unaffected or increased (Duncan, Johnson, Sutin & Wehr, 1998; Johnson et al., 1989; Harrington & Rusak, 1986; Pickard, Ralph & Menaker, 1987). IGL/GHT lesions also slow the rate of re-entrainment to a change in the LD cycle (Johnson et al., 1989). However, it has been suggested that this effect may not be due to changes in photic responsiveness, but rather, to the reductions in activii levels following IGL/GHT lesions (Janik & Mrosovsky, 1994). As discussed earlier, in intact animals, the rate of m-entrainment to a change in the LD cycle can be accelerated if locomotor activity is induced at the new onset of darkness, so the slower rate of re-entrainment in lesioned animals may reflect the reduction of normal activity levels.

Role of the IGL/GHT in Non-Photic Effects

NPY appears to be a major transmitter in the IGL/GHT pathway, and NPY release is thought to communicate the effects of behaviorally-arousing stimuli to the SCN. Electrical stimulation of the GHT, or application of NPY to the SCN results in non-photic phase shifts (Albers & Ferris, 1984; Huhman & Albers, 1994; Rusak, Meijer & Harrington, 1989). *C-fos* expression is observed in the IGL of rats and hamsters following exposure to behaviorally-arousing stimuli during the subjective day but not during the subjective night (Edelstein & Amir, 1995; Janik & Mrosovsky, 1992). In hamsters, the number of IGL cells expressing *c-fos* following exposure to a novel running wheel is correlated wlth the amount of induced activity and the magnitude of the resulting phase shift (Janik & Mrosovsky, 1992). Thus, the IGL is responsive to behaviorally-arousing stimuli.

Following IGL/GHT lesions, free-running period of wheel-running activity in rats is lengthened, but it is unclear whether this finding is the result of the lesion itself, or the accompanying reduction in wheel-running activii (Kuroda, Fukushima, Nakai, Katayama & Murakami, 1997). IGL/GHT lesions also greatly reduce or block the phase-shifting effects of dark pulses, exposure to a novel running wheel, and triazolam administration (Harrington & Rusak, 1986; Janik & Mrosovsky, 1994; Johnson, Smale, Moore & Morin, 1988; Wickland & Turek, 1994). Lesioned animals generally fail to display the increases in activity associated with these stimuli in intact animals, so, like the effects of IGL/GHT lesions on period, reduced activity could be responsible for the lack of phase shifts. However, it is the loss of NPY input to the SCN following IGL/GHT lesions that is thought to be responsible for the lack of phase shifting in response to behavioral stimuli, and this idea is supported in studies in which non-photic phase shifts and behavioral arousal have been dissociated. For example, in mice, entrainment by forced treadmill running--in which activity levels are experimentally controlled-is abolished following IGL/GHT lesions (Marchant, Watson & Mistlberger, 1997). Further, although intra-SCN injections of NPY antiserum to hamsters reduce phase shifts in response to behaviorally-arousing stimuli, activity levels are unaffected (Blello et al., 1994). In addition, administratlon of the benzodiazepine, chlordiazepoxide, results in non-photic phase shifts in hamsters without

increased activity, and these phase shifts can be blocked by IGL/GHT lesions (Biello & Mrosovsky, 1993; Biello, Harrington & Mason, 1991; Meyer, Harrington & Rahmani, 1993). Benzodiazepines bind to benzodiazepine/$GABA_a$ receptors which do not appear to be localized at the IGL, so the site of action for chlordiazepoxide, and other benzodiazepines, may be on IGL terminals at the SCN, or afferent to the IGL, possibly in the midbrain raphe (Morin, 1991). With the contribution of these findings, evidence is converging on a primary role for NPY input to the SCN, arising in the IGL, in mediating the phase-shifting effects of activity-inducing stimuli.

More recent evidence suggests that the effects of NPY may be mediated through a GABAergic mechanism, although this issue is complicated by the fact that both the IGL/GHT and the SCN target cells are GABAergic. Pre-treatment with bicuculline, a $GABA_a$ antagonist, blocks NPY-induced phase advances both *in vitro* and *in vivo* (Biello, Golombek, Schak & Harrington, 1997; Huhman et al., 1995). Further, the $GABA_a$ agonist, muscimol, itself produces non-photic phase shifts similar to NPY, and application of tetrodotoxin to the SCN fails to block muscimol-induced phase advances *in vivo* (Huhman et al., 1997). These results suggest that a GABAergic mechanism acting directly on SCN pacemaker cells, mediates the effects of NPY. This conclusion is complicated however, by the findings that tetrodotoxin blocks NPY-induced phase advances in vivo (Huhman et al., 1997), but does not block NPY-induced phase advances *in* vitro (Biello, Golombek, Schak, et al., 1997). This discrepancy may reflect the methodological differences between *in vivo* and *in vitro* studies, or changes in the pharmacological response of *in vitro SCN* preparations following the loss of afferent input (Huhman et al., 1997). Although the mechanism underlying IGL/GHT communication to the SCN is as yet unclear, the non-photic effects resulting from activation of this pathway suggest that the IGL/GHT is a primary route by which non-photic stimuli affect the pacemaker.

Role of the IGL/GHT in the Interaction Between Photic and Non-Photic Effects

The evidence reviewed above indicates that the IGL is involved in mediating the effects of both photic and non-photic stimuli on the SCN circadian pacemaker. In addition, some evidence indicates that light-evoked RHT glutamate release, and NPY release from GHT neurons, may have opposing effects. For example, at the level of the SCN, glutamate and NPY have mutually antagonistic effects on phase shifting *in* vitro; co-application of glutamate and NPY blocks both glutamate-induced phase shifts in the subjective night and NPY-induced phase shifts in the subjective day (Biello, Golombek & Harrington, 1997). Antagonistic effects are also observed *in vivo*, as the magnitude of light-induced phase advances is reduced when the light pulse is followed by an injection of NPY into the SCN (Biello & Mrosovsky, 1995), and blocked when the NPY injection precedes the light pulse (Weber & Rea, 1997). In addition, injections of NPY antiserum into the SCN potentiate light-induced phase advances (Biello, 1995). However, in contrast to phase advances, photic phase delays are not blocked by NPY administration *in vivo* (Weber & Rea, 1997). Conflicting results for advance and delay phase shifts have also been reported for the effects of IGUGHT lesions, in that photic phase advances are reduced (Pickard et al., 1987) while photic phase delays may be unaffected (Harrington & Rusak, 1986) or increased (Pickard et al., 1987). IGUGHT lesions produce a chronic loss of NPY input to the SCN while NPY antiserum injection has an acute inhibitory effect, so results of studies using either of these methods may not be directly comparable. At this point, given the contradictory reports, the nature of the interaction between light and NPY remains unclear.

Raphe Nuclei / Serotonin (5-HT)

The third major input pathway to the SCN arises from the serotonergic neurons of the midbrain raphe (Moore, Halaris & Jones, 1978). These projections can influence the SCN directly, or indirectly through projections to the IGL, as neurons of the median raphe project to the SCN while those of the dorsal raphe project to the IGL (Meyer-Bernstein & Morin, 1996). Integrity of the serotonergic system is not required for the generation of circadian

rhythmicity (Kawai, Yokota & Yamawaki, 1994; Levine, Rosenwasser, Yanovski & Adler, 1986), but there is considerable evidence for a rote of 5-HT in mediating responsiveness to non-photic stimuli, and in modulating photic input.

5-HT-Induced Phase Shifting

Administration of 5-HT and certain 5-HT agonists during the subjective day results in phase advances characteristic of non-photic stimuli both *in vitro* and *in wivo* (Edgar et al., 1993; Medanic & Gillette, 1992; Prosser et al., 1993; Prosser, Miller & Heiier, 1990; Shibata, Tsuneyoshi, Hamada, Tominaga & Watanabe, 1992b; Tominaga, Shibata, Ueki, & Watanabe, 1992). 5-HT is thought to act directly on SCN ceils, as the phase-shifting effects of the non-specific 5-HT agonist quipazine, in SCN slices, are not blocked by tetrodotoxin and therefore do not depend on synaptic transmission (Prosser, Hefter & Miller, 1992). initially, the 5-HT_1A receptor subtype appeared to be responsible for these effects, as both the non-specific agonist quipazine, and the 5-HT_1A agonist 8-OH-DPAT, were effective in producing phase advances (Prosser et al., 1993). More recent evidence indicates that the phase-advancing effects of 5-HT agonists may be mediated through 5-HT_7 receptors, which have been localized at the SCN (Lovenberg et at., 1993). 8-OH-DPAT has a high affinity for both 5-HT_{1A} and 5-HT_7 receptors, and *in* vitro 8-OH-DPAT-induced phase advances can be blocked by a selective 5-HT_7 antagonist (Prosser et al., 1993). in addition, phase advances following *in vitro* application of c-AMP analogs are similar to those of 8-OH-DPAT, and because 5-HT_7 receptors, but not 5-HT_{1A} receptors, are cAMP-coupled, the 5-HT_7 receptor subtype is believed to mediate the phase-shifting effects of 5-HT agonists during the subjective day (Miller et al., 1996; Prosser & Gillette, 1989).

Despite consistent *in vitro* evidence for 5-HT-induced phase advances during the subjective day, conflicting evidence has been reported for the phase-shifting effects of 5-HT agonists during the subjective night. *In vitro* administration of 8-OH-DPAT has no phase-shifting effects at this phase (Prosser et al., 1993; Shibata et al., 1992b) but

Prosser et al. (1993) have reported phase delays following administration of either 5-HT or quipazine at CT 15. These effects were not blocked by the same 5-HT antagonists which were effective in blocking 5-HT and quipazine-induced phase advances, leading to the suggestion that serotonergic phase delays are mediated by an as yet unidentifii 5-HT receptor (Prosser et al., 1993; Miller et al., 1996). However, this issue is complicated by the fact that Medanic and Gillette (1992) did not find phase shifts in response to 5-HT application during the subjective night, and so it remains to be seen whether the phase delays are a replicable result.

Administration of quipazine *in vivo* has been reported to produce phase advances during the subjective day in rats but not in hamsters, and it is unclear whether this discrepancy is due to species or methodological differences (Bobrzynska, Godfrey, et al., 1996; Edgar et al., 1993). However, in agreement with *in* vitro studies, 8-OH-DPAT produces phase advances in both rats and hamsters when injected during the subjective day (Bobrzynska, Godfrey, et al., 1996; Edgar et al., 1993; Tominaga et al., 1992). Administration of 8-OH-DPAT *in vivo* to rats and hamsters, or quipazine to rats, has no phase-shifting effects during the subjective night Because endogenous 5-HT levels are elevated during the subjective night, Edgar et al. (1993) suggest that the lack of effects of these agonists may be due to receptor competition, as endogenous 5-HT has a greater afflnity for serotonergic receptors than 5-HT agonists. Of course, such competition would not be expected to be a factor in the *in* vitro studies discussed above.

5-HT and Non-Photic Phase Shifting

Increases in behavioral arousal are associated with increased neuronal discharge in the raphe, and a strong positive correlation has been found between motor activity and 5-HT levels in the SCN (Shiori, Takahashi, Yamada & Takahashi, 1991). Given the similarity between the PRCs for serotonergic drugs and behavioral activity, these results suggest that the effects of non-photic stimuli may be mediated through serotonergic input to the SCN. Evidence supporting this idea is found in studies of non-photic phase shifting after

lesions of the raphe-SCN projection, monoamine depletion, or 5-HT antagonists, as discussed below.

Complete destruction of 5-HT afferents to the SCN with intra-SCN administration of the selective serotonergic neurotoxin, 5,7-DHT, reduces the phase-advancing effects of triazolam--believed to be mediated by induced locomotor activity--in the mid-subjective day (Cutrera, Kalsbeek & Pevet, 1994). Systemic treatment with the non-specific monoamine depletor, reserpine, also reduces phase advances in response to triazolam (Penev, Zee & Turek, 1994). However, these results are difficult to interpret, as both procedures reduce triazolam-induced activity, and reserpine reduces dopamine and norepinephrine levels in addii to 5-HT. Treatment with another serotonergic neurotoxin, pchloroamphetamine (PCA) reduces triazolam-induced phase shifts without as great a reduction in activity as that seen with 5,7-DHT and reserpine, but activity is still somewhat reduced (Penev, Turek & Zee, 1995). Benzodiazepine/$GABA_a$ receptors have been localized at the raphe, and because IGL lesions block phase shifts in response to benzodiazepines, it is possible that 5-HT projections from the dorsal raphe to the IGL may constitute the route through which the effects of benzodiazepines are communicated to the SCN (Johnson, Smale, et al., 1988; Morin, 1991). However, at present, the arousal-inducing versus the phase-shifting effects of benzodiazepines have yet to be adequately diierentiated, and the site of action for the non-photic phase-shifting effects of the benzodiazepines is still unclear.

Several studies have examined the role of 5-HT in the phase-shifting effects of other non-photic stimuli. For example, pre-treatment of hamsters with 5-HT antagonists has been shown to reduce, but not block, phase shifts in response to saline injections at CT 10, although it is not dear whether this result was due to reductions in arousal, or in arousal-induced phase shifting (Sumova, Maywood, Selvage, Ebling & Hastings, 1998). In contrast, intra-SCN 5,7-DHT lesions have no effect on activity-induced phase advances to novel wheel exposure in hamsters (Bobrzynska, Vrang & Mrosovsky, 1998). Although these experimental paradigms may not be directly comparable, the results suggest that 5-HT may not play a primary role in mediating the phase-shifting

effects of arousing stimuli in hamsters. 5-HT may be more important to non-photlc responses of mice, as entrainment by forced treadmill running was abolished following intra-SCN 5,7-DHT lesions, although slight modulation of free-running period by this stimulus remained evident (Marchant et al., 1997). The entraining effects of treadmill running are also blocked by IGL/GHT lesions, suggesting that non-photic entrainment in this species may require integration of input to the SCN from both the raphe and the IGL.

5-HT and Photic Phase Shifting

More consistent evidence has been reported supporting the role of 5-HT in the modulation of photic input to the circadian system. Administration of &OH-DPAT to hamsters, reduces the magnitude of light-induced phase delays at CT 14 and phase advances at CT 19, suggesting that 5-HT may provide inhibitory modulation of photic input (Rea, Glass & Colwell, 1994). Light-induced *c-fos* expression in the SCN is also reduced by pretreatment with either quipazine or 8-OH-DPAT (Glass, Selim & flea, 1994; Selim, Glass, Hauser & Rea, 1994). Complementary to these findings, the magnitude of light-induced phase delays is increased following intraventricular administration of 5,7-DHT (Morin & Blanchard, 1991). In addition, both reserpine treatment and the serotonergic drug, NAN-1 90 significantly potentiate the magnitude of light-induced phase shifts (Penev, Turek & Zee, 1998; Rea, Barrera, Glass & Gannon, 1995). Evidence suggests that NAN-1 90 may be a potent 5-HT autoreceptor agonist, resulting in reduced 5-HT transmission Thus, reductions in available 5-HT result in potentiation of the effects of light. Similarly, the behavioral attenuation of photic phase advances during the late subjective night can be blocked by pre-treatment with a non-selective 5-HT antagonist, further supporting the role of 5-HT in inhibiting photic responsiveness (Mistlberger & Antle, 1997).

The mechanisms through which 5-HT exerts these effects may involve action at 5-HT receptors located both post-synaptically on retlno-recipient SCN cells and pre-synaptically on RHT terminals. Quipazine administration lowers the activity of photically-responsive SCN cells, and localized application of 5-HT, quipazine or 8-OH-DPAT to the

SCN reduces the extracellular concentration of glutamate, believed to be the primary RHT neurotransmitter (Miller & Fuller, 1990; Selim, Glass, Hauser & Rea, 1993; Srkalovic, Selim, Rea & Glass, 1994) Thus, 5-HT may act to suppress EAA transmission at the SCN. Evidence also suggests that activation of 5-HT$_{1}$B receptors located on RHT terminals results in pre-synaptic inhibition of glutamate release (Pickard, Weber, Scott, Riberdy & Rea, 1996). For example, application of a 5-HT$_{1}$B agonist reduces extracellular glutamate, although to a lesser degree than 8-OH-DPAT (Srklalovic et al., 1994). Application of 5-HT$_{1}$B agonists also reduces light-induced *c-fos* expression in the SCN as well as the magnitude of light-induced phase shifts (Pickard et al., 1996). These effects can be blocked by a non-selective 5-HT$_{1}$ antagonist, but not by antagonists selective for other 5-HT receptor types. Therefore, 5-HT acts at the SCN both pre- and post-synaptically, and taken together, this evidence suggests that 5-HT has a tonic inhibitory effect on photic input to the SCN.

Further support for this role of 5-HT is found in studies of 5-HT depletion on entrainment to LD cycles and free-running period in LL. Both reserpine and intraventricular 5, 7-DHT treatment result in advanced activity onset, delayed activity offset, and a prolonged duration of the active phase in hamsters under LD 14:10 (Morln & Blanchard, 1991; Penev et al., 1993). However, this prolonged activity pattern persists in DD, suggesting that the atterations in entrainment may be reflective of alterations in circadian regulation, rather than a decrease in visual sensitivity. More conclusive evidence for serotonergic inhibition of photic input to the SCN is found with the effects of 5,7-DHT treatment on rhythmicity in LL. Following 5,7-DHT treatment, the period-lengthening effects of LL are greatly enhanced, a result which could be predicted from the relatively larger delay region in the photic PRC of 5,7-DHT-treated animals (Morln & Blanchard, 1991). As discussed previously, the larger the D/A ratio, the greater the extent of period-lengthening in LL (Aschoff, 1981). Following U-induced period-lengthening in 5,7-DHT treated hamsters, coherent rhythmicity is rapidly lost These results are opposite to those found following IGL lesions, and appear to represent an exaggeration of the normal response to

LL, as though 5-HT depletion removed a source of inhibitory input (Morin & Bianchard, 1991). These effects are likely due to 5-HT depietion in the median raphe, because local application of 5,7-DHT to the median, but not dorsal, raphe produces the same effects on photic entrainment and rhythmicity in LL as intraventricuiar application (Meyer-Bernstein & Morin, 1999). Lesions specific to the dorsai raphe have no effects on these parameters. This is consistent with the fact that 5-HT projections to the SCN arise from the median raphe, and taken together, these results suggest that a primary roie of the serotonergic input to the SCN involves inhibitory modulation of photic input.

SCN Output

Much of the research on the anatomy, physiology and pharmacology of circadian rhythms has concentrated on the SCN and its input pathways. Reiativeiy little is known about the mechanisms by which the neural circadii system regulates and coordinates the diversity of functions under circadian control (Turek et al., 1995). The SCN does not have extensive efferent projections, but it is possible that higher-order projections from a reiativeiy small number of primary SCN output areas communicate the circadian signal to a wider range of secondary projection sites (Moore, 1995).

A substantial portion of the output of SCN neurons is to the other neurons of the SCN (Moore, 1996; Watts, 1991). The most substantial non-SCN target for synaptic input from SCN neurons is the subparaventricuiar zone (SPVZ), an area of the anterior hypothalamus lying between the SCN and paraventricuiar nucleus (PVN). Many of the projections from the SPVZ overlap with smaller SCN projections, suggesting that the roie of these SPVZ efferents may be to amplify the circadian signal (Moore, 1996). Other areas receiving less extensive SCN innervation inciude the IGL, paraventricuiar thaiamic nucieus (PVT), zona incerta, preoptic area, retrochiasmatic area (RCA), ventromediai and dorsomediai nucleus, paraventricuiar hypothalamic nucieus, and periaqueductal gray (Moga, Weis & Moore, 1996; Watts, 1991). Many of these areas also receive innervation from the IGL and retina, indicating that photic and circadian information may be integrated at many levels of the circadian system (Moore, 1996; Moga & Moore, 1997).

Examination of the output patterns of these SCN target regions has provided some insight into the mechanisms underlying circadian modulation of a diverse range of functions. For example, the PVT projects to the nucleus accumbens, an area involved in motor integration and reinforcement mechanisms, and to the hippocampus, amygdala, septum and cingulate cortex, areas involved in memory and the regulatlon of affective behaviors (Moga et al., 1996; Watts, 1991). Projections from the SPVZ and RCA to the hypothalamus may provide circadian regulation of the autonomic and neuroendocrine functions medii by this region. Autonomic, behavioral and sensorimotor functions may also be influenced by efferent fibers from the RCA to areas of the basal forebrain, brainstem and spinal cord and from the IGL to thalamic nuclei (Moore, 1996). Thus, although the SCN has relatively few efferent projections, widespread projections from SCN target areas may possibly underlie the circadian regulation of many physiological and behavioral functions.

Circadian Dysfunction, Neurotransmitters and Depression

Although the direction of causation is unclear, ample evidence exists to show a relationship between circadian rhythm dysregulation and affective disorders (Rosenwasser & Wirz-Justice, 1997'). Numerous methodological diiculties are encountered in studies of circadian rhythmicity of depressed patients, but there is considerable evidence for disturbances in both amplitude and phase of circadian rhythms in these individuals. Decreased rhythm ampliide is one of the most consistent findings in such studiis and increased amplitude often accompanies remission of depressive symptoms (Duncan, 1996; Souetre et al., 1989). If reflective of a low amplitude pacemaker, this difference may possibty underlie alterations in other circadian parameters observed in depression (Rosenwasser & Wirz-Justice, 1997). For example, a low amplitude pacemaker may display decreased sensitivii to photic stimuli or alterations in the photic PRC.

Phase advances In core body temperature, hormone secretion and sleep-wake cycles have also been reported frequently in depressed patients (Duncan, 1996). The circadian

sleep rhythm in particular has been a major focus of research on the relationship between depression and circadian rhythmicity, as depressed patients commonly display sleep disturbances including early morning awakening, changes in the density and pattern of rapid eye movement (REM) sleep, and an unusually short interval between the onset of sleep and the first REM period (i.e. REM latency) (Goodwin & Jamison, 1990). These results are consistent with a phase advance hypothesis of depression in which depression reflects a phase advance of the circadian pacemaker either with respect to objective environment time, or with respect to the sleep-wake cycle (Rosenwasser & Wirz-Justice, 1997). According to this hypothesis, the circadian pacemaker displays a sleep-dependent depressogenic phase, such that sleep during this phase promotes the expression of depressive symptoms. Non-depressed individuals are thought to experience this sleep-sensitive phase during waking hours, but due to a phase-advanced pacemaker, depressed individuals experience this phase in the latter part of the night, during sleep, and thus, experience depression. Treatments designed to avoid sleep during this potentially sensitive phase can have antidepressant effects, supporting a causal role of sleep on depression. For example, total sleep deprivation, REM sleep deprivation, or shifting steep to an earlier phase, have all been shown to induce remissions in depressive patients (Wehr & Wirz-Justice, 1982; Wu & Bunney, 1990). In addition, although antidepressant drugs have multiple effects, most of the clinically useful antidepressants also act to suppress REM sleep, which has been suggested as a mechanism of antidepressant efficacy (Vogel, Minter & Woolwine, 1988). However, although the results discussed above support a relationship between circadian rhythm dysfunction and depression, considerable inconsistencies are found in clinical studies of depressed patients, as neither phase advances nor blunted amplitude have been found in all such studies (Duncan, 1996). At present, it is unknown whether these inconsistencies reflect methodological differences across studies, low statistical power within particular studies, or the general variability seen in the expression of depressive symptoms.

Biiogical theories of depression emphasize the importance of monoaminergic and cholinergic neurotransmitter dysfunction in the etiology and maintenance of depressive disorders (Golden & Janowsky, 1990). Although the interactive nature of neurotransmitter systems makes it diffiilt to specify disturbances in a particular system as a causal factor, these theories generally incorporate either deficiencies or imbalances among monoaminergic and cholinergic systems. In general, deficiency hypotheses suggest that affective disorders are the result of reduced norepinephrine or serotonin neurotransmission (Golden & Janowsky, 1990). The strongest support for these hypotheses is found in the clinical efficacy of monoamine oxidase inhibii and monoaminergic m-uptake inhibitors, both of which increase the synaptic availability of these transmitters (Duncan, 1996). The antidepressant effects of these monoaminergic drugs also provide support for neurochemical imbalance theories, in whii alterations in neurotransmitter ratios underlii affective disorders. For example, Janowsky, El-Yousef, Davis and Sererke (1972) suggest that depression results from alterations in the ratio of cholinergic to monoaminergic activity; specifically, that up-regulation of cholinergic activity together with down-regulation of monoaminergic activity results in the manifestation of a depressed state. Increases in the severity of depressive symptoms following treatment with choiinomimetic drugs together with the important role of cholinergic mechanisms in the generation of REM sleep, provide support for this hypothesis (Golden & Janowsky, 1990; Mirmiran & Van Someren, 1993).

Depressive disorders are characterized by a constellation of symptoms involving disturbances of mood, activity, sleep, appetite, and cognition. These behaviors are closely associated wlth cholinergic and monoaminergic actlvity, and are regulated by the circadian system (Goodwin & Jamison, 1990; Rosenwasser & Wirz-Justice, 1997). Examination of SCN input and output pathways can provide a possible neuroanatomical basis for these relationships, the functional basis for which can be explored through pharmacological manipulation of cholinergic and monoaminergic systems.

Cholinergic Effects on Circadian Rhythmicity

The SCN receives input from cholinergic neurons of the brainstern and basal forebrain, and acetylcholine (ACh) concentration in the SCN is increased following photic stimulation (Bina, Rusak & Semba, 1993; Murakami, Takahashi & Kawashirna, 1964). Several cholinergic drugs are capable of aitering circadian period and phase, supporting an interaction between the chotinergic and circadian systems, For example, chronic administration of the cholinergic agonist, carbachol, has been shown to shorten free-running period of rats (Furukawa, Murakami, Takahashi & Etoh, 1967). Acute administration of carbachol in *vivo* has been shown to mimic the phase-shifting effects of light; phase delays are observed in the early subjective night, and phase advances are observed in the fate subjective night (Earnest & Turek, 1965; Zatz & Herkenham, 1961). Like phase shifts in response to light pukes, carbachol-induced phase shifts can be blocked by pre-treatment with an NMDA antagonist (Colwell, Kaufman & Menaker, 1993). Taken together, these results provide evidence for a possibie rote of ACh in the transduction of photic input to the SCN. However, unlike light exposure, carbachol administration does not induce *c-fos* expression in the SCN. Therefore, if carbachol does act on the photic transduction pathway, this action must occur downstream from *c-fos* expression. Also unlike light exposure, carbachd-induced phase advances have been observed *in vivo* during the subjective day (Bina & flusak, 1996; Meijer, Van Der Zee & Dietz, 1988; Wee & Turek, 1969). Conflicting results have been provided for *in vitro* carbachd-induced phase shifting during the subjective night; phase advances in the late subjective night only (Trachsel, Heller & Miller, 1995), and phase delays throughout the entire subjective night (Liu & Gillette, 1996) have been reported. Thus, at present, the effects of carbachoi during the subjective night remain to be darified.

Several studies have attempted to identify the chotinergic receptor subtype through which the phase-shifting effects of carbachoi are mediated. Cholinergic receptors are broadty classified as either rnuscarinic or nicotinic, and evidence indicates that the muscarinic receptor may be of particular importance to the effects of carbachol. For example, aithough nicotinic antagonists fail to block carbachoi-induced phase shifts either

in vivo or *in vitro*, atropine, a muscarinic antagonist, has been found to Mock *in vivo* phase advances during the subjective day and late subjective night (Bina & Rusak, 1996; Liu & Gillette, 1996). Atropine fails to block *in vivo* carbachof-induced phase delays (Bma & Rusak, 1996), but Liu and Gillette (1996) found that atropine was effective in blocking *in vitro* phase delays during the subjective night.

Although some of the effects of carbachot may be mediated through muscarinic receptors, the selective nicotinic choiinergic agonist, nicotine, also can induce circadian phase shifts, so the involvement of the cholmergic system in circadian phase-shifting may invoive both receptor types. The complex effects of the nonselective chofinergic agonist, carbachoi, are mirrored in studies of the phase-shifting effects of nicotine, which also acts at nicotinic receptors. Nicotine administration mimics the phase-shifting effects of light *in* vivo (O'Hara et al., 1998) but *in* vitro studies have reported either phase advances during the late subjective night only (O'Hara et al., 1998) or phase advances throughout the entire subjective night (Trachsel et al., 1995). Thus, like carbachoi, the effects of nicotine during the subjective night are as yet unclear. in further support of the idea that both chofinergic receptor types are involved in circadian phase shifting, mecamyiamine, a nicotinic antagonist, blocks the phase-shifting effects of nicotine *in vitro*, and pre-treatment with mecamylamine, but not atropine, blocks light-induced phase shifts in locomotor activity of hamsters (Keefe, Earnest, Nelson, Takahashi & Turek, 1987; Pauly & Horseman, 1985), and in electrical activity of rat SCN slices (Miller, Murakami & Fuller, 1987). Thus, although the relationships between the cholinergic and circadian systems are complex and remain to be fully understood, the results discussed above certainly support a role for cholinergic input to the circadian pacemaker.

Monoaminergic Effects on Circadian Rhythmicity

Both the SCN and IGL reciprocally innervate a number of structures which are known to receive monoaminergic input (Moore, 1996). As discussed in detail above, serotonergic cells of the raphe constitute a major source of input to the circadian system via projections to both the SCN and IGL, and extensive evidence supports a role for 5-HT in circadian

regulation. Evidence also suggests that another monoamine, noradrenaline, influences circadian rhythmicity, as both the IGL and SCN are known to receive input from noradrenergic cells of the locus coeruleus (Moore, 1996). Studies have shown that administration of the alpha-adrenergic agonist, ckmidine, an antihypettensive drug with potentially depressogenic effects, can alter both circadian period and phase, as well as photic responsiveness. Lie carbachol, chronic donidine administration has been found to shorten free-running period of rats, white acute administration mimics the phase-shifting effects of light in hamsters (Rosenwasser, 1989; 1990; 1996; Rosenwasser, Vogt & Pellowski, 1995). in contrast, chronic donidine administration appears to antagonize the effects of tight in hamsters, as treatment with clonidine reduces the magnitude of photic phase advances, and impairs re-entrainment to an advance of the LO cycle (Dwyer & flosenwasser, 1998a). These effects suggest that donidine acts either directly or indirectly on the photic input pathway, and supports a role for noradrenaline in the regulation of circadian rhythmicity.

Effects of Antidepressant Treatment on Circadian Rhythms: Animal Studies

The effects of monoaminergic antidepressants on circadian parameters are of particular interest in attempts to elucidate the relationship between affective behaviors and circadian rhythmicity. Although many of these drugs do alter rhythmicity, no consistent pattern in the direction of effects has emerged. Chronic administration of desipramine, a noradrenergic re-uptake inhibii, and moclobemide, a monoamine oxidase inhibitor (MAOI), shortens free-running period and increases circadian amplitude of rats (Wollnik, 1992). The effects of moclobemide on period are opposite to that of another MAOI, clorgyline, which has been found to lengthen free-running period in hamsters (Duncan, Tamarkin, Sokolove & Wehr, 1988). Clorgyline treatment also results in delayed entrainment phase, reduced magnitude of photic phase shifts, and promotion of dissociation of rhythmicity in LL (Duncan et al., 1988; 1998; Wirz-Justice & Campbell, 1982). IGL lesions block the effects of clorgyline on free-running period and on promotion of dissociation in LL, suggesting that these effects of dorgyline may be mediated by me IGL.

Fluoxetine, a preferential serotonergic re-uptake inhibitor, shortens the free-running period of mice, but has no effect on either period or amplitude of rats (Possktente, Lumia, McEldowney & Rapp, 1992; Wollnik, 1992). Similarly, clomipramine, another preferential 5-HT m-uptake inhibitor, does not alter free-running period of rats or hamsters (Wollnik, 1992; Yannielli, Cadeiras, Cardinali & Golombek, 1993). However, chronic clomipramine potentiates, and acute domipramine inhibits, the magnitude of photic phase advances in hamsters (Yannielli, Cadeiras, et al., 1993). Clomipramine increases the synaptic availability of 5-HT, so chronic administration would be expected to result in down-regulation of post-synaptic 5-HT receptors, white the acute effects of this drug would be to increase synaptic 5-HT levels. As such, the effects of acute and chronic clomipramine on light-induced phase advances are in agreement with other hamster studies showing an antagonistic interaction between 5-HT and light (e.g. Morin & Blanchard, 1991; Rea et al., 1995). However, neither acute nor chronic clomipramine administration affects photic phase delays.

Animal Models of Depression

A number of putative animal models of depression have been developed to explore the biological basis of this disorder. These models involve genetic, surgical, pharmacological or behavioral procedures designed to produce changes in neurochemistry and behavior that may be analogous to those seen in endogenous depression. Theories regarding the biological basis of human depression emphasize monoaminergic and cholinergic neurotransmitter systems, and it is hypothesized that dysregulation in these systems may be the underlying cause of depression (Golden & Janowsky, 1990; Goodwin & Jamison 1999). Thus, methods employed to produce animai models of depression typically target these systems.

Flinders Sensitive (FSL) Rats

The Flinders Sensitive (FSL) line of rats has been put forth as a model of hypercholinergic depression (Overstreet, 1986). This strain was bred for hypersensitivity to a particular anticholinesterase, but aiso displays increased sensitivity to certain choiinergic agonists and a generalized hyperchoiinergic tone (Overstreet, 1986). These effects are thought to be related to up-regulation of muscarinic receptors in the brainstem, hippocampus and cortex (Overstreet, Russell, Crocker & Schiller, 1984). FSL rats display a constellation of alterations in behavior which resemble many features of clinical depression. For example, FSL rats dispiay reduced actiiity, increased REM sleep, and shorter REM latency as compared to control rats (Overstreet, 1986; Shiromani et al., 1988). FSL rats also show exaggerated responses to stressors, further supporting the idea that this strain models a genetic vulnerability to depression (Overstreet, 1986). Alterations in circadian rhythmicity are also a feature of this model, as free-running period is shorter in FSL rats compared to controls, and FSL rats have shown advanced entrainment of the temperature rhythm (Shiromani, Klemfuss, Lucero & Overstreet, 1991; Shiromani & Overstreet, 1994). Alterations in rhythmicity of FSL rats is not surprising, given the evidence for choiinergic influences on the circadian system of normal rats discussed previously. Although the mechanisms underlying chdiergic influences on free-running period and photic responsiveness have yet to be elucidated, these effects support the hypothesis that alterations in acetylcholinergic transmission provide a neurochemicai link between affective and circadian regulation.

Olfactory Bulbectomy

Olfactory bulbectomy of rodents has been suggested as a putative surgical model for hyposerotonergic depression. Removal of the olfactory bulbs of mice, hamsters and rats results in characteristic neurochemical and behavioral changes reflecting those seen in anxious or agitated depression (Jesberger & Richardson, 1988; Richardson, 1991). olfactory buibectomy resutts in a generalized decrease in monoaminergic, particularly serotonergic, transmission together with changes in behavior including increased activity,

aggression and emotionaiity, and decreased sexual activity. This collection of effects is referred to as 'bulbectomy syndrome' and its behavioral resemblance to depression, as well as the successful reversal of these effects with antidepressant treatment, has led to the use of olfactory buibectomy as an animal model of depression (Giardina & Radek, 1991; Jesberger & Richardson, 1966; Richardson, 1991).

The SCN receives input from the olfactory bulbs via intermediate structures, and increases in cAMP content in the SCN have been observed following olfactory bulbectomy, which may be related to the drcadii effects of this procedure (Moga & Moore, 1997; Vagell, McGinnis, Possidente, Narasimhan & Lumia, 1991). Mice, hamsters and rats display lengthening of free-running period and delayed entrainment phase following olfactory butbectomy (Pieper & Lobocki, 1991; Lumia, Teicher, Salchli, Ayers & Possklente, 1992; Possidente et al., 1999). This procedure may have less consistent effects on the amplitude of circadian activity rhythms, as both increases (Lumia et al., 1992) and decreases (Giardina & Radek, 1991) have been reported. Treatment with ffuoxetine, a serotonin re-uptake inhibitor has been shown to block the period-lengthening effects of olfactory buibectomy, but not the delay in entrainment phase, suggesting that the tatter effect may be mediated through a non-serotonergic mechanism (Possidente, Lumia, McGinnis, Rapp & McEldowney, 1996).

Chronic Stress

Afthough the exact role of stress in the etiology of human depression is controversial, a number of studies have shown the depressogenic effects of chronic stress on animal behavior (Willner, 1984; 1997). Features of this model include alterations in activity, impaired cognitive performance, anhedonia, and sleep abnormalities, each of which are closely associated with alterations in monoaminergic systems (Willner, 1964; 1997; Rosenwasser & Wirz-Justice, 1997). Many of these behavioral alterations have also been shown to be reversibie with antidepressant treatment (Wifiner, 1997). Although little information is available on the circadian effects of chronic stress, lengthening of free-

running period has been observed in some rats foitowing repetitive stress exposure using inescapable shock (Stewart, Rosenwasser, Hauser, Volpicelli & Adler, 1990).

Developmental Models: Prenatal Stress

Another stress-induced depression model involves the effects of maternal stress during pregnancy on the neurochemistry and behavior of the offspring (Levine, 1970; Weinstock, Fride & Hertzberg, 1988). Prenatal stress in rats produces permanent changes in monoaminergic transmission and increases in stress-induced corticosterone secretion (Henry, Kabbaj, Simon, LeMoal & Maccari, 1994; Peters, 1984; 1986). Behavioral effects include altered activity, impaired cognitive performance, increased emotionali and exaggeration of stress-induced responses (Wakshlak & Weinstock, 1990). These effects are thought to be initiated by stress responses during a critical period of neural development. For example, Peters (1990) suggests that stress-induced increases in maternal tryptophan and 5-HT, transmitted to the fetus as the 5-HT system emerges, may underlie some of the behavioral alterations seen in the aduit offspring. in addition, critical periods for the development of the hypothalamo-pituitary-adrenal (HPA) axis occur both pre and postnatally, and stressors presented during either the prenatal or postnatal (via handling) period have opposite effects on both stress-induced corticosterone secretion and behavior during adulthood (Fride, Dan, Feldon, Halevy & Weinstock, 1986; Ogawa et al., 1994). indeed, several studies have shown that when both prenatal stress and postnatal handling are administered, the behavioral effects of prenatal stress are reversed {e.g. Fride et al., 1986; Wakshlak & Weinstock, 1990).

Few studies have explored the circadian effects of prenatal stress, but advanced entrainment of the circadian corticosterone rhythm has been observed in prenatally stressed male rats, and this effect can be reversed by postnatal handling (Koehl, Barbazanges, Le Moal & Maccari, 1997). Advanced entrainment of both corticosterone and locomotor rhythms have also recently bean repotted for prenataffy stressed female rats (Maccari et al., 1997). Although further study is required, advanced entrainment

implies that prenatal stress may resuit in shortened free-running period, or alterations in the photic PRC.

Developmental Models: Neonatal Antidepressant Treatment

Another putative animal model of depression has been developed through administration of antidepressant drugs during the neonatal period (Femandez-Pardai & Hilakivi, 1939; Hilakivi & Hilakivi, 1997; Vogei, Neill, Hagler & Kors, 1990; Vogel & Vogel, 1992). Drug treatment during early postnatal life can result in permanent alterations in both neural function and behavior, similar to those observed following other depressogenic procedures. like prenatal stress models, the changes observed in response to neonatal drug treatment are thought to reflect perturbation of neural systems during critical periods of development.

Bats have a 21 day gestation period and monoamines are first detected during the latter hatf of this period. The noradrenergic cells of the locus coeruleus differentiate on days 1 O-1 3 of gestation and the serotonergic cells of the raphe nuclei differentiate on days 1 1 - 15 of gestation (Mirmiran, 1986). At birth, rats are in an altricial state displaying immature neural function. Brain maturation occurs postnatally during the fii two to three weeks, a period considered to be roughly equivalent to the tatter half of gestation through the first two years of human brain development (Borella, Bindra 8t Whitaker-Azmitia, 1997; Mirmiran, 1996).

As monoaminergic systems mature, these transmitters are also thought to influence the development of other brain regions (Borella et al., 1997; Mirmiran, 1986; Peters, 1990). For example, in the developing brain, serotonin serves a regulatory role, influencing the maturation of both the neurons producing serotonin, and the target tissues to which these neurons project (Mazer et al., 1997). Evidence supporting this role includes the fact that treatment with the tryptophan hydroxylase inhibitor, parachtorophenylaline (PCPA) from postnatal days 1 O-20 delays the timing of synaptogenesis in 5-HT projection areas and results in a significant loss of dendrites in the hippocampal region of adult rats, reflected in behavioral deficits in tasks assessing learning and memory (Mazer et at., 1997).

Neonatal antidepressant models have employed preferential noradrenergic (e.g. desipramine) or serotonergic (e.g. domipramine, zimeldine) m-uptake inhibitors, and these treatments produce remarkably simii alterations in adult behavior. These alterations include increased immobility in the forced swim test, and decreased aggressiveness, reward-seeking, and sexual activity (Nell, Vogel, Hagler, Kors 8 Hennessey, 1996; Velazquez-Moctezuma & Ruiz, 1992; Vogel, Hartley, Neil, Hagler & Kors, 1990; Vogel, Neill, Hagler & Kors, 1990). Reduced REM latency and changes in the pattern of REM sleep also result from neonatal antidepressant treatment, and these effects have been shown to be reversed with adult antidepressant treatment, thus providing further support for neonatal antidepressant treatment as a model of depression (Vogel, Neill, Kors & Hagler, 1996).

Increases in voluntary alcohol intake are a consistent finding in studies of neonatai antidepressant treatment (Dwyer & Rosenwasser, 1998; Hiikivi, Sinclair & Hilakivi, 1984; Hiiakii, Stenberg, Sinclair & Kiianmaa, 1967; Rosenwasser & Hayes, 1994). increased alcohol intake is suggestive of decreased 5-HT function (LeMarquand, Pihl & Benkelfat, 1994), so this result provides indirect evidence of alterations in 5-HT activity in response to antidepressant treatment during the neonatal period. More direct evidence has been found in electrophysiological studies of neonatal ciomipramine treatment, which have shown decreased hypothalamic 5-HT levels and hyposensitivity to the inhibitory effects of acute 5-HT re-uptake blockade (Feenstra, Van Galen, Te Riele, Botterblom & Mirmiran, 1996; Maudhuit, Hamon & Adrien, 1996). Studies of the effects of neonatal domipramine treatment on the spontaneous firing rate of neurons in the dorsal raphe nucleus are less conclusive, as both unaffected (Maudhuit, Hamon & Adrien, 1995) and decreased (Yavari, Vogel & Neill, 1996) firing rates have been reported.

The mechanism by which neonatal antidepressant treatment produces these behavioral and physiological changes is as yet unclear. Monoaminergic m-uptake inhibitors elevate the synaptic availability of monoamines, which may induce a permanent down-regulation or decreased sensitivii of monoaminergic receptors when administered during a critical period for neural development. However, these drugs also have immediate

behavioral effects which may ultimately be responsible for the abnormalities observed during adulthood. For example, monoaminergic antidepressants act to inhibit REM sleep (Mirmiran, 1983). Norepinephrine and 5-HT inhibit cholinergic neurons in the brainstem and midbrain which are important in the generation of REM sleep, and thus, by elevating levels of norepinephrine and 5-HT, monoaminergic re-uptake inhibitors also indirectly reduce the amount of REM sleep (Frank, Page & Heller, 1997; Jones, 1991; Mirmiran, 1988). Several authors have argued that suppression of active sleep, a state which precedes the emergence of REM sleep in neonates, mediates the physiological and behavioral alterations observed in adulthood (Mirmiran, 1986; Mirmiran & Van Someren, 1993; Vogel & Vogel, 1982; Vogel et al., 1990). However, recent evidence suggests that active and REM sleep are neither homologous states, nor generated by the same mechanisms (Frank et al., 1997). In addition, behavioral effects of non-pharmacological inhibition of active sleep are not as extensive as those of neonatal antidepressant treatment (Mirmiran et al., 1983). Thus, the mechanism underlying the effects of neonatal drug treatment is unclear, but may be due to permanent changes in neurotransmitter systems, the disruption of early sleep states, or a combination thereof.

The alterations in circadian rhythmicity observed in other animal models of depression suggest that such changes may also occur in models using neonatal drug treatment. Although few studies have examined this issue, circadian rhythm disturbances have been observed in response to neonatal treatment with both domipramine and desipramine. Administration of desipramine during the neonatai period has been shown to lengthen free-running period and increase the amplitude and coherence of circadian drinkii rhythms of adult male rats (Rosenwasser & Hayes, 1994). In contrast, neonatal domipramine has not been found to affect free-running period (rats: Dwyer & Rosenwasser, 1998b; hamsters: Yannielli, Cutrera, Cardinaii & Golombek, 1998), but like desipramine treatment, domipramine administration results in increased rhythm coherence in both mate and female rats, and increased rhythm amplitude in females (Dwyer & Rosenwasser, 1998b). Thus, the effects on rhythm amplitude and coherence are consistent for desipramine and domipramine in rats, but increased amplitude is opposite in direction to what would be

predicted from the rather consistent blunting of circadian amplitude observed in human depression (Souetre et al., 1989). Although circadian amplitude has not been explored systematically in other animal depression models, neonatal handling typically produces behavioral effects in adulthood opposite to those reported for neonatal antidepressant treatments, and has been shown to reduce the amplitude of the circadian temperature rhythm (Amir & Schiavetto, 1990). The increased rhythm amplitude seen following putative depressogenic treatments is consistent with this result, but the reasons for the lack of correspondence between these effects and those seen in clinical depression are as yet unclear.

Neonatal clomipramine treatment in hamsters appears to have inhibitory effects on circadian responses to photic stimuli. Although no effects were found on free-running period in DD, these hamsters did show shorter free-running periods in LL (Yannielli, Cutrera, et al., 1998). Delayed re-entrainment following an advance of the LD cycle, and reduced photic phase advances were also observed. Neonatal domipramine treatment also affected non-photic phase shifting, as these hamsters displayed increases in the magnitude of 8-OH-DPAT-induced phase advances during the subjective day. At present, the effects of neonatal domipramine treatment on photic and non-photic phase shifting in rats have yet to be reported.

Present Study

The purpose of this study was to examine the effects of neonatal clomipramine treatment on photic and non-photic phase shifting in adult rats. Rats treated neonatally with clomipramine participated in two separate experiments; one experiment assessed the phase-shifting effects of light pulses presented during the early and late subjective night, white the other experiment assessed the phase-shifting effects of dark pulses and 8-OH-DPAT administration during the subjective day.

CHAPTER II

METHOD

Subjects and Neonatal Treatment

Forty-eight male Long-Evans rats derived from a total of 28 litters participated in this study. Due to equipment limitations, only 24 adult rats could be tested in parallel, so two age-matched squads ware run consecutively. Each squad was composed of rats from 14 litters. In general, most studies of circadian phase shifting in rats employ only males. Daily activity onset is often used in such studies as a reference point for measuring phase shifts in response to a particular stimulus and was also used in this study (see below). The timing of activity onset of unperturbed male Long-Evans rats is very stable and predictable from day to day, but activity onset of female rats changes over the course of the four-day estrous cycle, making it diilt to measure stimulus-induced phase shifts. Therefore, in the present study, only the male offspring of the 28 litters received the neonatal treatments, and participated in the circadian phase-shifting experiments as adults.

For each squad, 14 pregnant Long-Evans rats were obtained from Charles River Laboratories at mid-gestation and maintained in individual plastic bottom cages under a 12:12 LO cycle. Fourteen mothers per squad were chosen for thls study because 14 litters provided enough male pups to compose genetically diverse experimental groups, and to adequately address the possibility of litter effects. These affects are a significant concern in developmental studies of multiiarous species such as the rat, and refer to the fact that animals within a litter are less variable than animals from different litters. This reduced varlabii among littermates may be due to prenatal factors, including similar genetic inheritances and early development within the same intrauterine environment, or postnatal factors, including exposure to similar maternal behaviors (Holson & Pierce, 1992; Zorrilla, 1997). Thus, animals within a litter are not considered to be statistically independent observations. In light of this issue, it is generally recommended that, within an experiment, only one pup par titter be assigned to each treatment group (Spear & File,

1996). The present study was performed in accord with this recommendation. The male pups in this study were divided into three neonatal treatment groups (see below). As adults, four rats from each treatment group participated simultaneously in each of two independent experiments, thus requiring a total of 24 male rats per squad (12 per experiment), and a minimum of 12 litters per squad. Two additional titters were included in each squad in case any of the pregnancies failed or maternal care was inadequate. With a total of 14 litters per squad, some treated pups were not assessed as adults, but the larger number of treated pups was necessary in order to allow for genetically diverse treatment groups, in case some pups failed to survive to adulthood, and to maintain uniform litter composition (see below).

In order to avoid confounding prenatal litter effects with postnatal maternal effects, pups were cross-fostered within four days of birth. Cross-fostering involved removing the pups from their mothers and then distributing them among the different mothers such that only one male per original litter was placed with a particular mother. This procedure was done to separate the genetically similar pups, and to allow for uniform composition of foster litters. To maintain a uniform litter size, litters were cuffed such that each included four males. Although female pups did not participate directly in this study, one female pup was also included with each of the 28 foster litters. Several other studies involving male rats treated neonatally with domipramine have retained females in the fitters (e.g. Hilakivi & Hilakivi, 1987) and it has been suggested that this procedure prevents behavioral and physiological aiterations which can occur when pups are reared in same-sex litters (Spear & File, 1999). As in a previous study invoiving neonatal clomipramine treatment (Feenstra et al., 1994), the present study included only one female rat with each foster litter, in order to keep foster litter size relatively smail. A small litter size was thought to help ensure that all pups received adequate maternal care.

The male pups were divided into three neonatal treatment groups; a clomipramine-treated group, a saline-treated group and an unhandled group. Using a procedure commonly employed in studies of neonatal domipramine treatment (e.g. Mirmiran et al., 1983; Vogel & Vogel, 1982) domipramine was administered through daily subcutaneous

injections at 25mg/kg delivered in a volume of .01ml saline per gram body weight. Injections were given on postnatal days 8-21, during the early light phase of the LD cycle. As discussed previously, this period was chosen to coincide with the maturation of the serotonergic system which occurs throughout the second and third postnatal weeks in rats. Saline injections were administered at the same time and in the same volume as that of clomipramine.

The adult behavior of the clomipramine-treated group was of primary interest in this study, and a saline-treated group was included in order to control for non-pharmacological aspects of the drug administration procedure. For example, saline treatment controlled for the stressful nature of the drug administration procedure, which included brief maternal separation, handling by the experimenter, and injection. The third group consisted of unhandled pups, included to detect any effects of the injection procedure per se, and thus represented a comparison for the saline-treated group. In order to distribute potential differences in maternal behavior across treatment groups, each foster litter was composed of two clomipramine-treated, one saline-treated and one unhandled male pup as well as one female pup. The larger number of domipramine-treated pups was in anticipation of a potentially higher mortality rate within this group, compared to that of the saline-treated and unhandled pups (cf. Vogel, Hagler, Hennessey 4 Richard, 1996). All animals were marked with indelible ink to identify group membership.

At approximately one month of age, animals were weaned and males from each foster litter were housed together. At approximately seven weeks of age, animals were placed in individual experimental cages equipped to monitor either circadian drinking or wheel-running rhythms (see below). Animals in each cage type participated in a separate series of experiments involving presentation of either non-photic or photic stimuli respectively. In each squad, twelve rats, divided into three groups (4 clomipramine-treated, 4 saline-treated, 4 undisturbed) were placed in cages equipped with running wheels for the experiment on non-photic phase shifting. Another twelve rats, divided into three groups (4 domipramine-treated, 4 saline-treated, 4 undisturbed) were placed in cages equipped to monitor circadian drinking rhythms for the experiment on photic phase shifting. Each of the

four rats within a treatment group was taken at random from a different foster titter, and each foster fitter contributed no more than one rat to each experiment. Following completion of testing of squad 1, the same treatment protocol was repeated with a second squad of animals, and the data within each experiment (non-photic vs. photic phase shifting) were pooled across squads.

It should be noted that because it was unfeasible to track original littermates following cross-fostering, it was impossible to know for certain if rats from the same biological litter were included in the same group in either experiment. However, given the large number of pups distributed among three treatment groups and the relatively few chosen for each experiment, this possibility appears very unlikely. Indeed, the neonatal protocol outlined above was designed to keep this possibility to a minimum, while still controlling for postnatal litter effects and avoiding excessive numbers of unused pups.

Apparatus and Procedure

Experiment 1: Effects of neonatal domipramine treatment on circadian phase shifting induced by dark pulses and serotonergic stimulation

Animals were maintained in standard running wheel cages with attached home cage compartments (Lafayette Instruments). The running wheels were housed within ventilated, light- and sound-attenuating enclosures with one, two or four cages per enclosure. Each wheel revdution dosed a microswitch, and all switch closures were monitored and stored in 10 min bins using Dataquest III. Food and water were freely available.

Initially, animals were maintained in 12: 12 LO for approximately three weeks in order to estabiish entrainment to the LD cycle. Animals were then exposed to LL for 10 days at an intensity of 40 lux. This intensity was chosen so as to be intense enough to provide a suffiit level of contrast between LL and darkness such that the dark pulse would be a behaviorally-salient event, but less intense than that expected to induce arrhythmicity. After approximately 10 days of LL, animals were transferred individually along with their

cages to a separate dark pube cabinet, for six hours beginning at CT6. The cabinet was equipped such that the number of wheel turns displayed during the dark pulse could be recorded. The phase and duration of the dark pulse were based on dark pulse PRCs of hamsters (e.g. Boulos & Rusak, 1982a; Reebs et al., 1989), as comparable PRCs have not been reported for rats. Following the dark pulse, animals remained in LL for a further 15 days in order to measure changes in steady-state phase.

Following 15 days of LL after the dark pulse, animals were re-entrained to a 12:12 LO cycle. Animals were then returned to LL at an intensity of approximately 5 lux, selected to be the miniial intensity required to allow injections to be performed without the aid of additional fight sources. After 10 days in LL, animals received an intraperitoneal (i.p.) injection of 8-hydroxy-2-[di-n-propylamino](8-OH-DPAT) at 5mg/kg. This dose was the same as that used in other studies of the circadian effects of 8-OH-DPAT in both rats (e.g. Kennaway, Rowe & Ferguson, 1996) and hamsters (e.g. Cutrera, Saboureau & Pevet, 1996). Injections were given at CT 8, the phase at which maximal phase advances have been observed in hamsters in response to serotonergic agonists (e.g. Bobrzynska, Godfrey, et al., 1996; Tominaga et al., 1992). Although daily activity was continuously recorded, the number of wheel-turns during the three hours following injection was quantified separately, in order to examine any 8-OH-OPAT-induced increases in activity. Foliowing injections, animats were kept in LL for an additional 15 days to allow measurement of changes in steady-state phase.

Experiment 2: Effects of neonatal clomipramine treatment on circadian phase shifting induced by photic stimulation

Individual wire-mesh experimental cages (35 x 22 x 18 cm) were housed three per shelf within a fight and sound-shielded ventilated supply cabinet. Circadian drinking rhythms were continuously monitored throughout the experiment using contact-sensing drinkometers (Lafayette Instruments). AH drinkii tube contacts were recorded and stored in 10-min bins using the Dataquest III hardware-software system (MiniMitter Co.). Food (Agway Prolab 3000) and water were freeiy available. Drinking activity was monitored at

all times, except during light pulse presentation. For delivery of light pulses, animals were transferred individually, along with their home cages, to a separate cabinet. Light within the cabinet was supplied by a standard incandescent bulb and light intensity was controlled by attenuation of the supply voltage. Intensity measurements were made in lux with a photometer (Tektronix) held within the cage.

In this experiment, animals were initially maintained in the home cages under 12:12 LD for approximately three weeks in order to establish entrainment to the LD cycle. Next, animals were exposed to constant darkness (DD) for 10 days prior to, and for 15 days following, presentation of a light pulse. On day 10 of DD, animals received a 10min 100 lux light pulse at CT 14. Approximately 15 days after this light pulse, rats received a 10 min 50 lux light pulse at CT 14, followed by an additional 15 days of DD. This phase was chosen because previous studies have shown that the peak of the delay portion of the photic PRC of rats occurs near CT 14 (Honma, 1985; Summer, Ferraro & McCormack, 1984). As discussed previously, the magnitude of a photic phase shift is a function of both the intensity and duration of the light pulse (e.g. Nelson & Takahashi, 1991). However, within a particular species, both photic phase delays and phase advances display a characteristic maximum magnitude which will not be exceeded by any further increases in light intensity or duration. A light pulse which results in an asymptotically large phase shift is referred to as a saturating light pulse. In the present study, the 10min 100 lux light pulse appeared to be saturating, as it resulted in maximal or near-maximal phase shifts (see Results). As group differences in photic responsiveness may have been obscured by the use of a saturating light pulse, the second light pulse was of a lower intensity than that which would be expected to produce maximal phase shifts in rats (Honma, 1985; Summer et al., 1984).

Following the 15 days of DD after the 50 lux light-pulse, animals were re-entrained to the 12:12 LD cycle for 10 days and then returned to DD for 10 days. On day 10, animals received a 10 min 100 lux light pulse at CT 22, followed by an additional 15 days of DD. Animals then received a 10 min 50 lux light pulse at CT 22, followed by a final 15 days of DD. CT 22 was chosen because previous studies have shown that the peak of the

advance region of the photic PRC of rats occurs near this phase (Honma, 1985; Summer et al., 1984).

Data Analysis

Circadian drinking and wheel-running rhythms were plotted in actogram format using Dataquest III. Following Piiendrigh and Daan (1976a), eye-fitted lines through consecutive activity onsets were used to determine the desired phase of stimulation, and to calculate phase shift magnitude. The onset of drinking or wheel-running activity (CT 12) in each circadian cycle was used as a marker of circadian phase. On the day of stimulus presentation, Cl 12 was determined by eye-fitted lines drawn through the onsets for the 10 previous days. The desired phase was then calculated relative to CT 12 for that day.

In order to calculate phase shift magnitude, an eye-fitted line through the onsets for the 10 days prior to the stimulus (light pulse, dark pulse, or 8-OH-DPAT injection) was extrapolated to the day following the stimulus, and an eye-fitted line through the onsets for days 4-15 following the stimulus was back-extrapolated to the day following the stimulus (Pittendrigh 8 Daan, 1976a). Phase shifts were defined as the difference (m minutes) between these tines on the first day following the stimulus. Onsets for the first three days following a stimulus, particularly a photic stimulus presented during the advance portion of the photic PRC, often represent transients and were thus exduded. Each phase shift was calculated by two independent raters, and the mean of these values was used as a measure of phase shift magnitude.

Separate analysis of variance (ANOVA) were used to determine effects of neonatal treatment on phase shift magnitude for each phase-shifting stimulus (light pulses at CT14 and CT22, dark pulses and 8-OH-DPAT injections). However, based on the design of the study, only two group comparisons were considered relevant; clomipramine- vs. saline-treated and saline-treated vs. unhandled. Thus, planned comparisons were performed on these pairs for each stimulus.

The amount of activity induced by the dark pulses was defined as the number of wheel-turns recorded during the six hours of darkness. The Pearson product-moment

correlation coefficient (r) was used to assess the relationship between the amount of activity during the dark pulse and the magnitude of the resulting phase shift. The Pearson r was also used to assess any relationship between activity levels following 8-OH-DPAT injections and the magnitude of the resulting phase shift.

Changes in free-running period following each of the stimuli were also examined. Free-running period before and after stimulus exposure was measured with a commonly-used procedure: least-squares cosinor regression using software provided with Dataquest. The cosinor analysis provided estimates of free-running period by iteratively fitting the data with sinusoidal functions across a specified range of periods. The period of the best-fitting cosinor function is taken as an estimate of free-running period. For the cosinor procedures, continuous 10-day data samples (immediately before and after stimulus exposure, excluding the first three post-stimulus days) were examined using test periods within the circadian range, in 0.10 h increments. Estimates of free-running period were included only when significant circadian periodicity ($p<.05$) was detected. For each animal, stimulus-induced changes in period were quantified by subtracting the free-running period following stimulus exposure from the free-running period before stimulus exposure. ANOVA and pairwise comparisons were used to investigate group differences in the extent of period change following each stimulus. Correlations between stimulus-induced phase shifts and period changes were also examined.

In addition to estimates of free-running period, cosinor analysis also provides measures of several other aspects of rhythmicity induding rhythm amplitude, spectral magnitude and average activity level (either drinking or wheel-running activity) over the course of the sample. Rhythm amplitude is defined as the amplitude of the best-fitting cosinor, but because amplitude is strongly correlated with the mean level of activity, ampliie ratios were calculated by dividing each amplitude by the activity mean. This transformation results in a measure of amplitude uncorrelated with the amount of activii. Spectral magnitude (PR) represents the proportion of variance accounted for by the best-fitting cosinor. Although not a primary interest in this study, free-running period, amplitude ratio, spectral magnitude, and average activity level were examined for any group differences

due to neonatal treatment. These measures were taken from the 1 O-day samples preceding each stimulus, thus providing two measures of rhythmicity in LL (before 8-OH-DPAT injections and before dark pulses) and four measures of rhythmicity in DD (before each light pulse). ANOVA and pairwise comparisons were performed on each sample, and correlations between the variables in each sample were examined.

CHAPTER III
RESULTS

General Observations

Across the two squads, a total of 28 timed-pregnant mothers, 14 per squad, participated. Parturition occurred on day 21 of gestation, and litter size ranged from 8-14 pups. As planned, titters were culled and cross-fostered on postnatal day 4, leaving each mother with one female and four males (two for domipramine treatment one for saline treatment and one unhandled), and the neonatal treatment period occurred from day 8 to 21. In squad one, an unhandled pup died between day 4 and day 8, and several pups from another foster litter failed to thrive, so all pups from these two foster litters were excluded from the later experiments. In squad two, all pups survived, so between the two squads, pups from a total of 26 foster titters participated in the photic and non-photic experiments.

In general, the growth and development of the pups did not appear to differ between squads. Although group differences in mass were observed following neonatal treatment {see Mow), no significant differences in mass were observed between squads, and no significant squad x group interactions were found at any time. Therefore, the means reported below represent data pooled across the two squads.

At the start of the neonatal treatment period, the mass of the pups chosen for the domipramine- and saline-treated groups was very similar at 16.9 g and 16.2 g respectively (unhandled animals were not weighed). Day of eye opening, a developmental marker, was the same for pups in all three groups, occurring on day 14. Throughout the treatment period, mass of the domipramine-treated animals was generally fess than #at of the sake-treated animals, such that by the end of treatment on day 21, mean mass of the domipramine-treated pups was 86% of that of the saline-treated pups ($p<.01$). This difference was slightly reduced by day 28, when the animals were weaned, but mean (+/-SEM) mass of the cfomipramine-treated group (82.3+/-1.4g) was still significantly less than that of both saline-treated (92.5+/-2.0g) and unhandled (93.4+/-

1.8g) groups. Of course, the values reported above include many more animafs than actually participated, as fats were not selected for inclusion in the photic and non-photic experiments until later. Therefore, the selected animals were weighed at approximately seven weeks of age, immediatefy before being placed in experimental cages. Although the mean for domipramine-treated rats (205.2 +/-7.7g) was less than the saline-treated (224.5+/-5.6g) and unhandled (221.3 +/-6.8g) rats, these differences were not significant.

Experiment 1: Effects of Non-Photic Stimuli

8-OH-DPAT

Phase Shifting

Figure 1 includes representative actograms from an animal in each group, showing the phase shifts observed in response to 8-OH-DPAT injection at CT 8. Phase shifts were generally small in size, particularly for animals in the clomiiramine-treated group. One-way ANOVA revealed a significant group difference [$F(2,21)=4.4, p<.05$] with the ciomipramine-treated group displaying a mean (+/- SEM) phase advance of 5.75 +/- 12.1 minutes, while phase delays were observed for both saline-treated (-33.4 +/- 5.3 mins) and unhandled (-15.1 +/- 9.4 mins) groups (Figure 2A). Two planned comparisons were performed, in order to examine group differences between relevant pairs. The ciomipramine-and saline-treated groups were significantly different from each other ($p<.01$) but no such difference was found between saline-treated and unhandled groups ($p=.18$). It should be noted that although most of the domipramine-treated rats disptayed relatively little 8-OH-DPAT-induced phase-shifting, one animal showed an 80.5 minute advance. As this value could be considered an outlier, the above statistics were repeated without this data point, but the pattern and statistical significance of the results were unaffected.

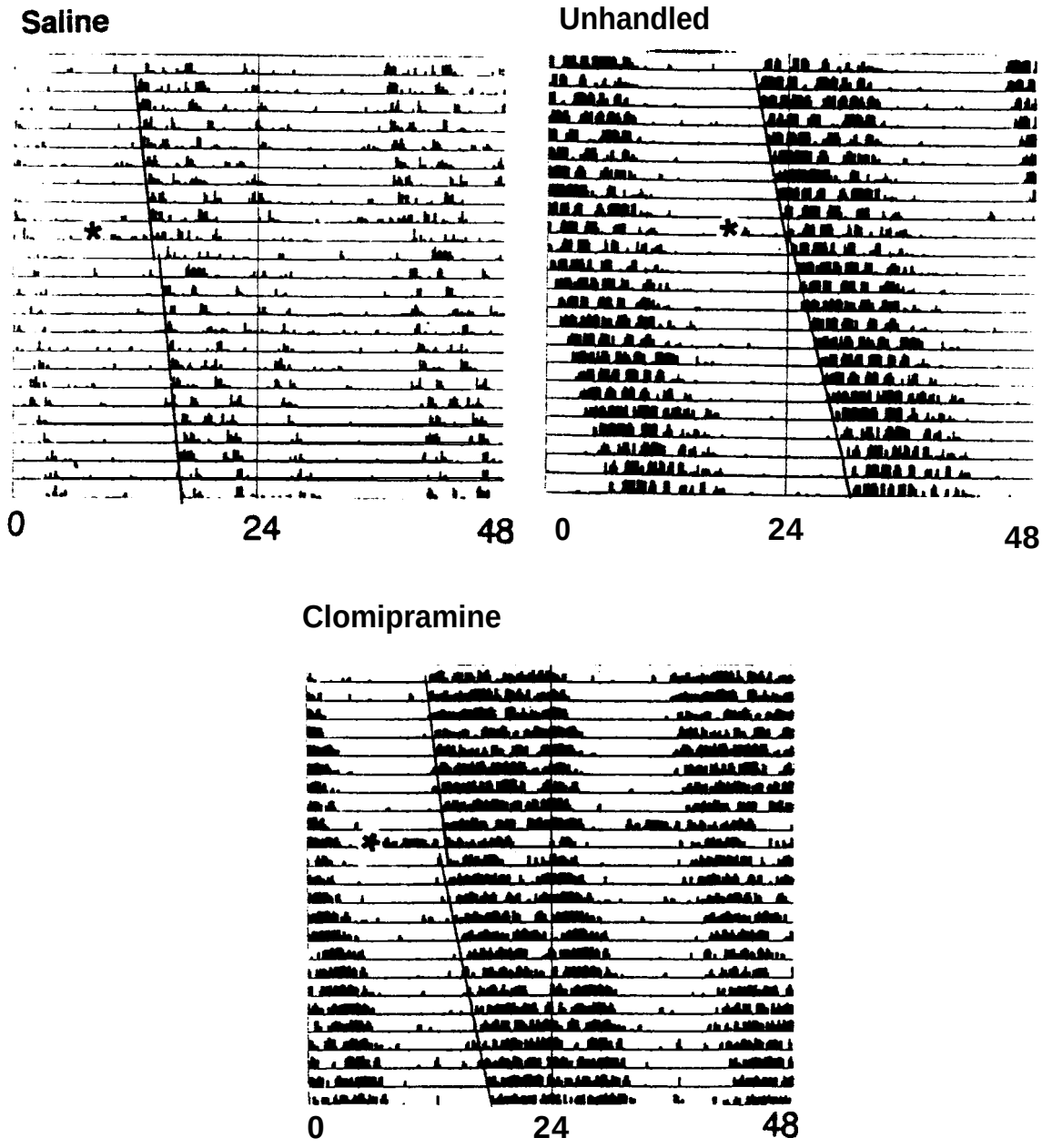

Figure 1. Representative actograms from each group showing phase shifts in circadian wheel-running activii following 8-OH-DPAT injections. Actograms are double-plotted (48 hours) with successive days plotted from top to bottom. Rats were maintained in LL and administered 8-OH-DPAT at CT 8. Asterisk indicates approximate time of injection. Lines drawn through activity onsets illustrate method of phase shift measurement.

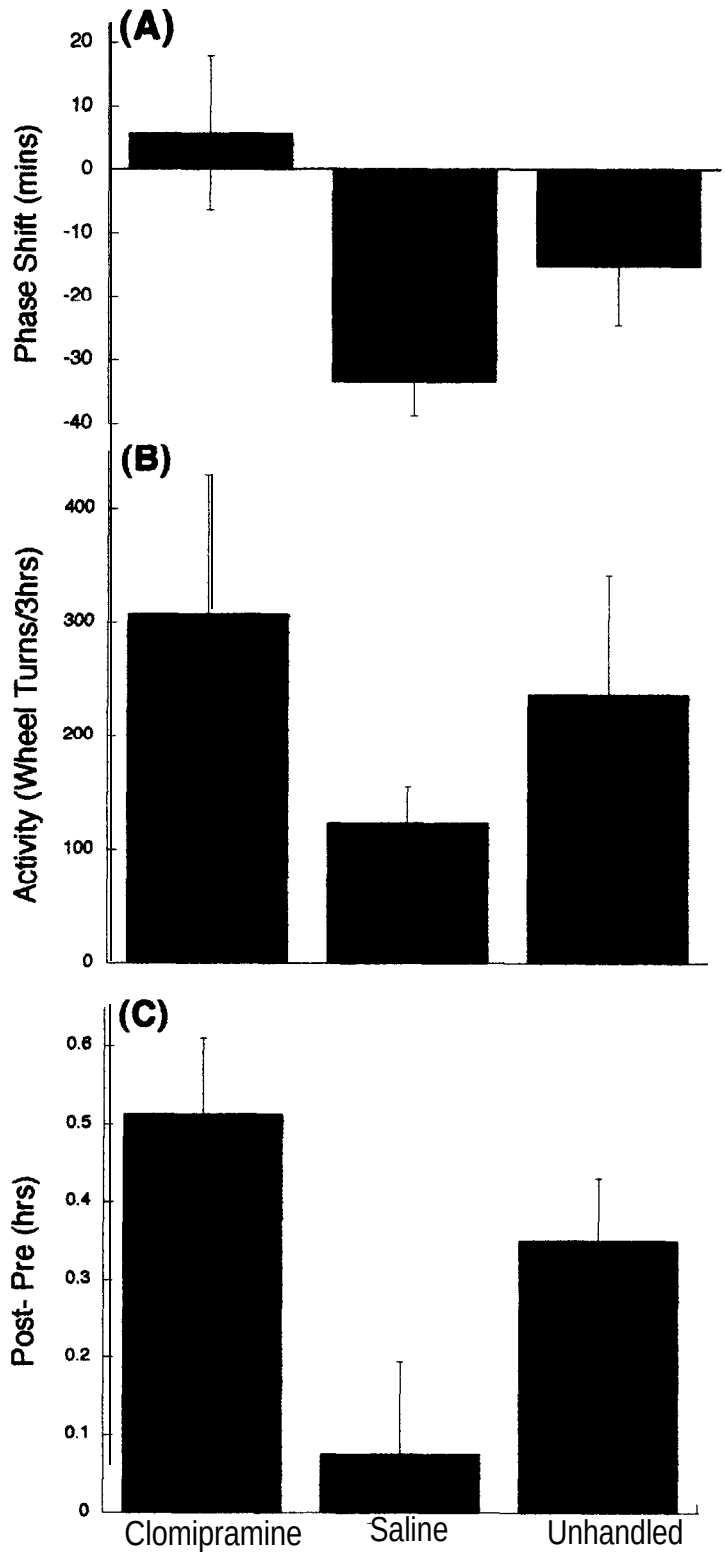

Figure 2. Effects of 8-OH-DPAT injection on phase-shifting (A), activity (B), and free-running period (C) for each group (means +/- SEM).

Activity

In the three hours post-injection, most rats displayed levels of wheel-running activity that would not otherwise be expected at thls circadian phase. Mean (+/-SEM) number of wheel turns was highest for the domipramine-treated rats at 311.8 +/- 121.1, followed by the unhandled rats at 236.9 +/- 105.5, while the lowest activity level was displayed by the saline-treated rats at 119.4 +/- 31.6. (Figure 2B). According to one-way ANOVA, the three groups did not significantly differ [F(2, 21)= 0.95, p=.40], and although clomipramine- and saline-treated groups showed the largest mean difference, it was not statistically significant (p=. 19). In an exploratory effort, data were re-axamined following logarithmic transformation, but again, no significant group diierences were found.

Across the three groups, there was some indication of a positive relationship between observed phase shifts and post-injection activity (Figure 3), as the value of the Pearson correlation coefficient approached significance (Table 1). Interpretation of this result is complicated by the fact that both phase advances and delays were observed following 8-OH-DPAT. Further, when data were re-examined without the outlier mentioned above, no significant correlation was found. Within the clomipramine-treated group, the positive correlation between phase shifts and activity approached significance (Table 2) but only with inclusion of the outlier. Without the 80.5 minute shift, there was no relationship [r=.03, p>.9]. Likewise, within either the saline-treated or unhandled group, no significant correlations were found (Tables 3 and 4). As it may have been possible that size of the phase shift was more important than direction, correlational analyses were repeated using absolute value of the observed phase shifts, ignoring direction. Using this variable, no relationship, either across or within groups, was found between phase shift magnitude and post-injection activity. Finally, since some animals showed relatively little 8-OH-DPAT-induced activity, a correlational analysis was also performed on phase shifts and activity for those animals displaying greater than the median number of wheel turns in the three hours post-injection. Again, no significant relationships were found. Thus, there was little evidence for a relationship between 8-OH-DPAT-induced phase shifts and activity.

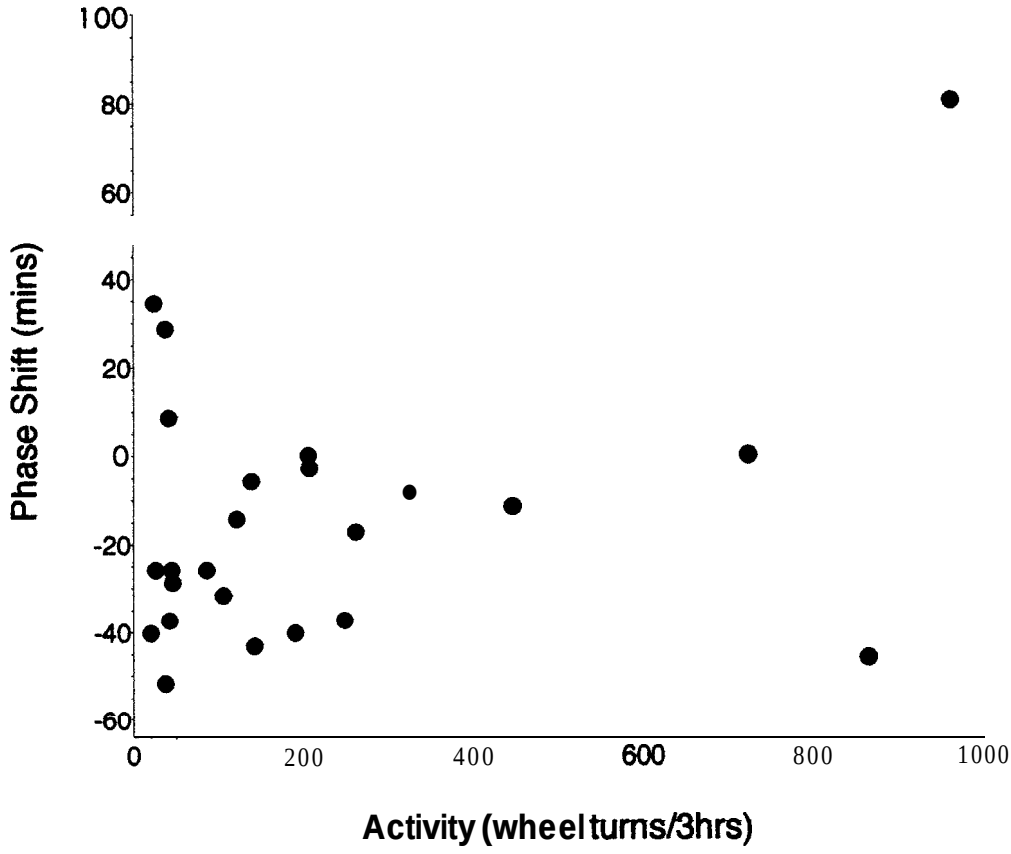

Figure 3. Scatterplot showing 8-OH-DPAT-induced phase shifts as a function of the amount of activity (i.e. wheel turns) observed in the three hours post-injection.

Table 1.

Correlations between phase shifts, wheel-running activity and changes in free-running period induced by 8-OH-DPAT injection.

	Phase shift	Activity (post-injection)
Phase shift		
Activity (post-injection)	.34*	
Change in Period (Post – Pre)	.10	.06

*p<.10

Table 2.

Clomipramine-treated group: Correlations between phase shifts, wheel-running activity and changes in free-running period induced by 8-OH-DPAT injection.

	Phase shift	Activity (post-injection)
Phase shift		
Activity (post-injection)	.68*	
Change in Period (Post – Pre)	-.07	-.42

*p<.10

Table 3.

Saline-treated group: Correlations between phase shifts, wheel-running activity and changes in free-running period induced by 8-OH-DPAT injection.

	Phase shift	Activity (post-injection)
Phase shift		
Activity (post-injection)	.31	
Change in Period (Post – Pre)	-.62*	.15

*p<.10

Table 4.

Unhandled group: Correlations between phase shifts, wheel-running activity and changes in free-running period induced by 8-OH-DPAT injection.

	Phase shift	Activity (post-injection)
Phase shift		
Activity (post-injection)	-.43	
Change in Period (Post – Pre)	-.45	.07

Free-Running Period

Cosinor analysis was used to assess free-funning period immediately before and after injection, and the difference between these values was used as an estimate of the effect of 8-OH-DPAT on circadian period. As seen in Figure 2C, al three groups displayed period-lengthening following 8-OH-DPAT injection, with clomipramine-treated rats showing a mean (+/-SEM) increase of .51+/-.10 h, saline-treated, .08+/-.10 h, and unhandled, .35

+/-.08 h. These differences were significant across the three groups [F(2, 21)=4.88, p<.05], between clomipramine- and saline-treatad groups (p<.01) and approached significance for the saline-treated versus unhandled groups (p=.07). The extent of period lengthening was not significantly related to phase shift magnitude across groups, or within the clomipramine and unhandled groups, but a trend towards a significant negative relationship was found within the saline-treated group (Tables I-4). All saline-treated animals showed phase delays in response to 8-OH-DPAT, so this result suggests that there was a tendency for larger phase delays to be associated with greater period-lengthening in this group.

As discussed previously, values for rhythm amplitude (amplitude ratio), spectral magnitude (PR) and average activii level were also extracted from the cosinor analysis for the sample preceding 8-OH-DPAT injection. However, no signifiint relationships between these variables and phase shift magnitude were found (Table 5).

Table 5.

Correlations between 8-OH-DPAT-induced phase shifts and cosinor parameters for the preceding 10-day sample.

	Phase shift	Period	Amplitude Ratio	PR
Phase shift				
Period	-.18			
Amplitude Ratio	.27	.38		
PR	-. 9	.26	.42	
Activity	-. 9	10	31	86"'

***p<.01

Dark Pulses

Phase Shifting

Figure 4 includes representative actograms from one animal in each group, showing examples of the phase shifts observed in response to six hour dark pulses between CT 6 and CT 12. like the pattern of phase-shifting seen in response to 8-OH-DPAT, minimal shifting was observed in the clomipramine-treated group, and the largest difference was between this group and the saline-treated group. In this condition, three animals (one in each group) showed substantial day-to-day variability in activity onset, such that phase shifts could not be measured accurately. Thus, phase shift values reported below were derived from seven animals per group.

All group means showed phase advances in response to dark pulses (Figure 5A), with clomipramine-treated at 11.1+/- 10.6 mins, satine-treated at 47.2+/- 30.4 mins, and unhandled at 17.25 +/- 11.4 mins. However, mean differences were not significant either across the three groups fF(2, 18)=.96, p=.40] or between reievant pairs (p's>.21). Although most animals showed phase advances, several delays were observed, so data were also examined using absolute values of the phase shifts, but again, no significant group differences were found.

Activity

During the dark pulse, most animals displayed a level of wheel-running activity which would not otherwise be expected at this circadian phase. The domipramine-treated group showed the lowest mean (+/-SEM) number of wheel turns (186.4 +/- 39.4), and the saline-treated group showed the highest (359.6 +/- 91 .5), with the unhandled group (207.1 +/- 28.5) between the others (Figure 5B). These differences approached significance when the three groups were examined together [F(2, 21)=2.5, p=.11], as did the pairwise comparison between the ciomipramine- and saline-treated groups (p=.11).

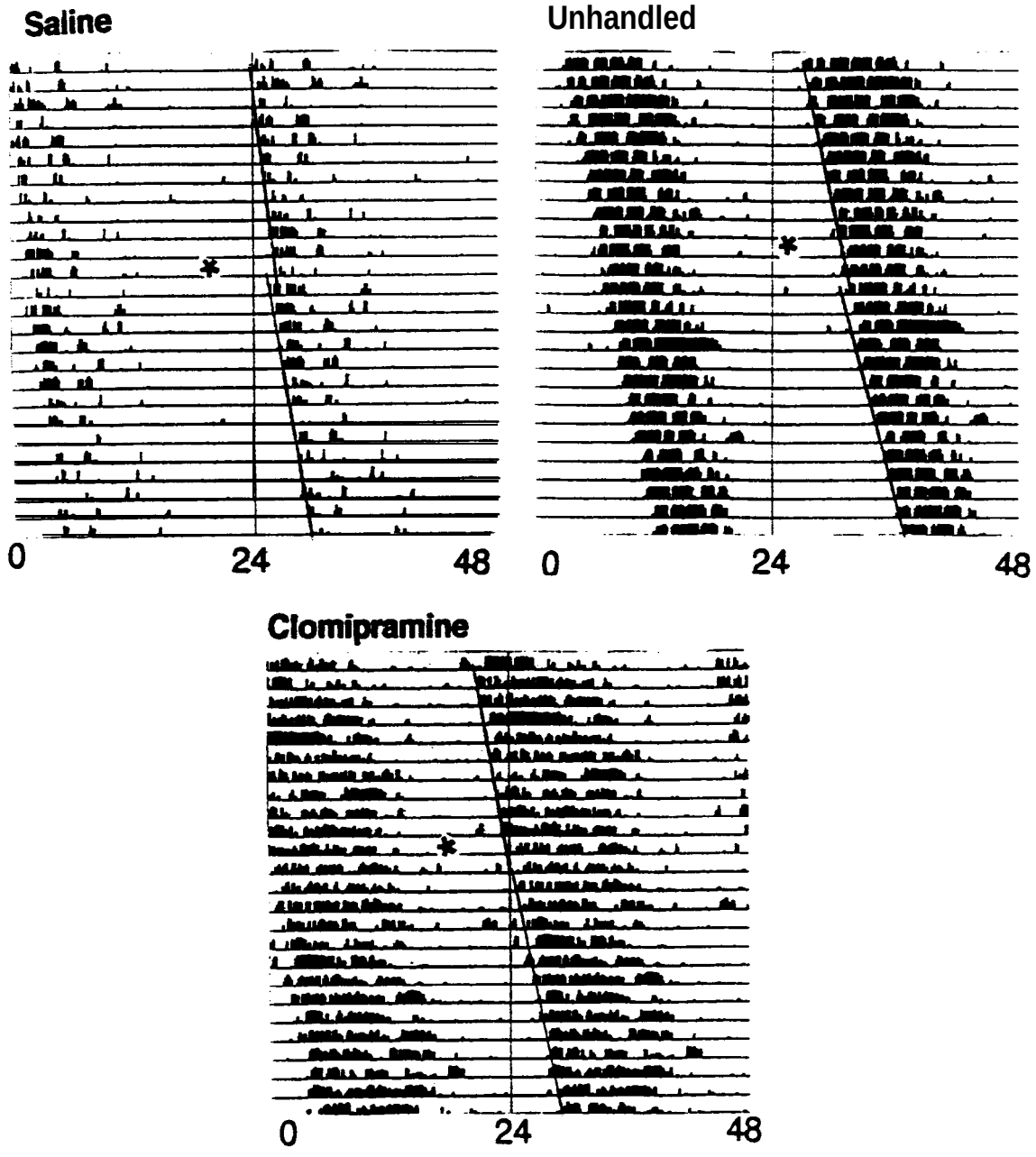

Figure 4. Representative actograms from each group showing dark pulse-induced phase shifts in circadian wheel-running activii. Rats were maintained in LL and presented with six hour dark pulses beginning at CT 6. Asterisk indicates approximate time of dark pulse.

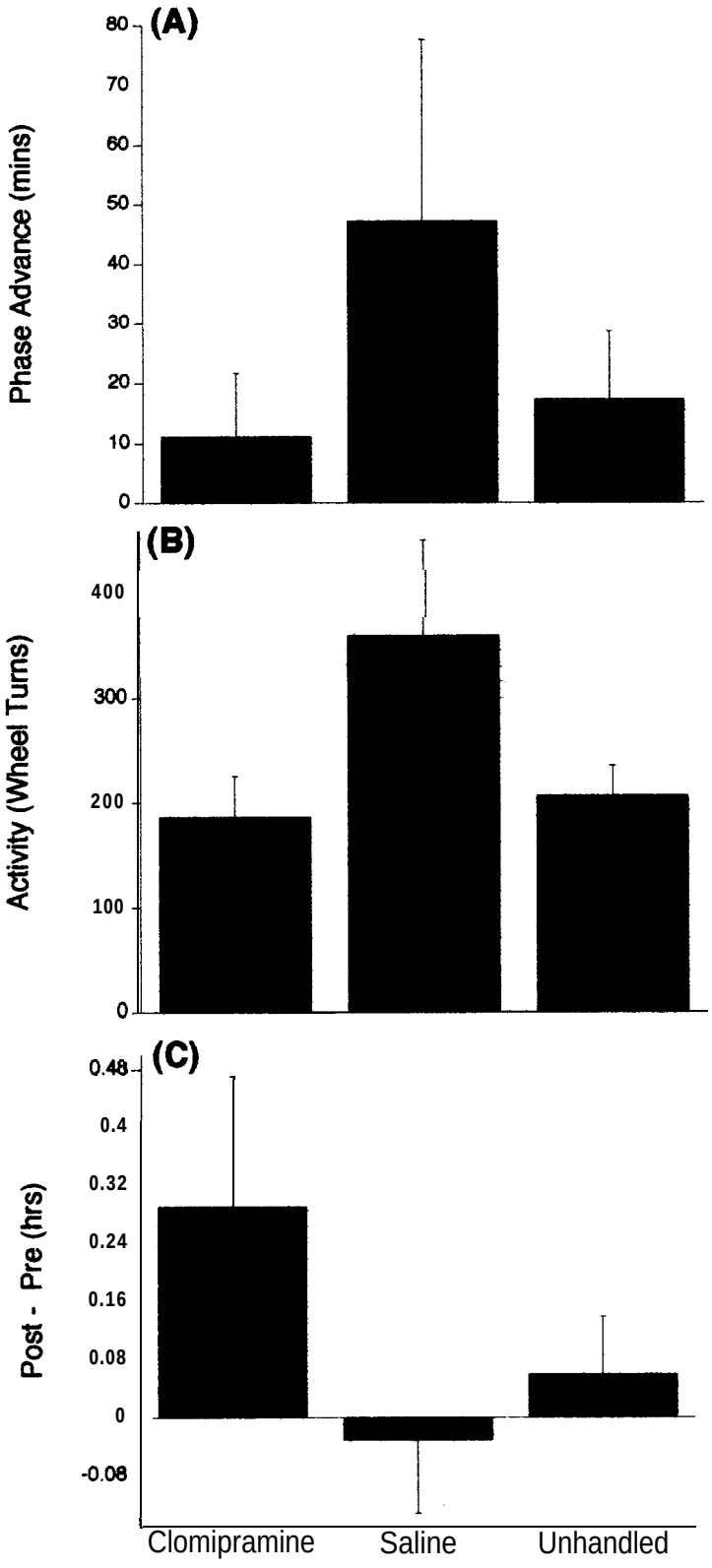

Figure 5. Effects of dark pulses on phase shifting (A), activity (B), and free-running period (C) for each group (means+/-SEM).

As can be seen in Figure 6, the level of activity expressed during the dark pulse was quite similar for most animals. Although group means displayed the same rank order for dark pulse-induced phase shifts and activity, there was no evidence for a significant relationship between these variables across the three groups (Table 6). Using absolute values of the phase shifts, logarithmic transformation of the activity data, or a median-split also did not uncover significant relationships. Within the groups, only the clomipramine-treated rats showed a trend towards a signifiint negative relationship between dark pulse-induced phase shifts and activity (Tables 7-9). This relationship is probably due to the fact that although most of the domipramine-treated animals showed phase advances, two of the more active animals displayed small phase delays.

Table 6.

Correlations between phase shifts, wheel-running activity and changes in free-running period induced by dark pulses.

	Phase shift	Activity (post-injection)
Phase shift		
Activity (post-injection)	-.14	
Change in Period (Post – Pre)	-.21	-.20

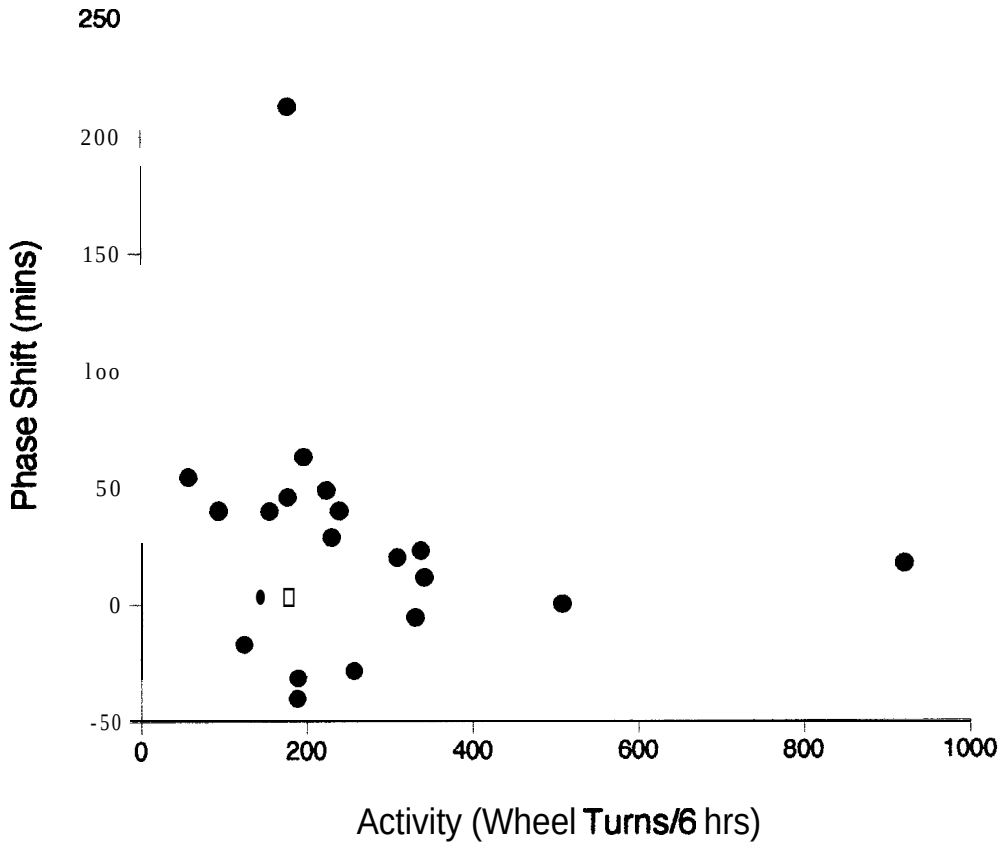

Figure 6. Scatterplot showing dark pulse-induced phase shifts as a function of the amount of activity (i.e. wheel turns) observed during the pulse.

Table 7.

Clomipramine-treated group: Correlations between phase shifts, wheel-running activity and changes in free-running period induced by dark pulses.

	Phase shift	Activity (post-injection)
Phase shift		
Activity (post-injection)	-.69*	
Change in Period (Post – Pre)	.39	-.45

*p<.10

Table 8.

Saline-treated group: Correlations between phase shifts, wheel-running activity and changes in free-running period induced by dark pulses.

	Phase shift	Activity (post-injection)
Phase shift		
Activity (post-injection)	-.30	
Change in Period (Post – Pre)	-.69*	.05

*p<.10

Table 9.

Unhandled group: Correlations between phase shifts, wheel-running and changes in free-running period induced by dark pulses.

	Phase shift	Activity (post-injection)
Phase shift		
Activity (post-injection)	.24	
Change in Period (Post – Pre)	.29	-.05

Free-Running Period

In general, small changes in free-running period were observed following the dark pulses (Figure 5C), and as with 8-OH-DPAT, the clomipramine-treated group showed the largest change in period and the saline-treated group showed the smallest change. Group means (+/-SEM) indicate that period-lengthening was displayed by both clomipramine treated (29 +/-.18h) and unhandled (.06 +/-.08 h) groups, while slight period-shortening was displayed by the saline-treated group (-.03 +/-.10h). These differences were not significant across the three groups [$F(2, 21)=1.6, p=.23$] and only approached significance for the comparison between clomipramine- and saline-treated groups ($p=.10$).

There was no indication of a significant relationship between dark pulse-induced phase shifting and period change across groups (Table 6) or within the clomipramine-treated or unhandled groups (Tables 7 and 9). There was a trend towards a significant negative relationship between these variables in the saline-treated group (Table 8) suggesting that animals showing larger phase advances also tended to show greater period-shortening. However, this relationship was due to the presence of one animal which showed an unusually large advance of 212.75 minutes. When this animal was excluded, the trend was abolished [$r=.49, p=.32$].

Across groups, amplitude ratio and phase shift magnitude showed a significant negative relationship (Table 10),indicating that animals with lower rhythm amplitudes displayed larger phase advances. Free-running period prior to the dark pulse also showed a trend towards a significant positive relationship with phase shift magnitude, indicating that animals with longer periods tended to show larger phase advances. Neither PR nor average activity level over the course of the sample was significantly related to phase shift magnitude.

Table 10.

Correlations between dark pulse-induced phase shifts and cosinor parameters for the preceding 10-day sample.

	Phase shift	Period	Amplitude Ratio	PR
Phase shift				
Period	.38*			
Amplitude Ratio	-.48**	-.03		
PR	-.29	.35	.50**	
Activity	-.15	.25	.35	.87***

*p<.10; **p<.05; ***p<.01

Experiment 2: Effects of Photic Stimuli

Representative actograms from each group, showing phase shifts in response to the 100 lux light pulse at both CT 14 and CT 22 are depicted in Figure 7. A mixed, between (neonatal treatment group) and within (CT, intensity) groups ANOVA did not reveal any significant effects of the neonatal treatments on the magnitude of phase shifts in response to photic stimuli [$F(2, 20) = .97, p = .40$], any significant effects of intensity, nor any significant group interactions (p's>.80). As there was considerable within-group variability

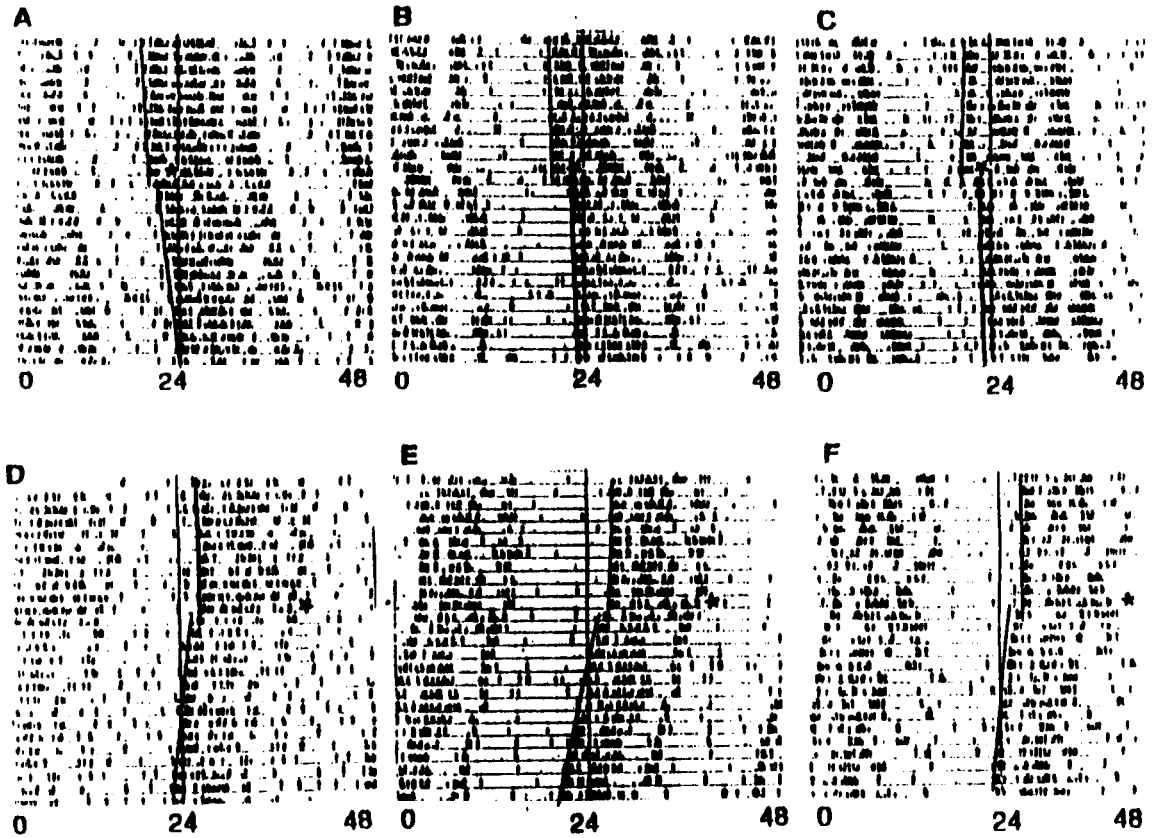

Figure 7. Representative actograms from each group (domipramine: A, **D**; saline: B, E; unhandled: C, F) showing phase delays following light pulse presentation in early subjective night (A, B, C) and phase advances following presentation in late subjective night (D, E, F). Rats were maintained in **DD.** Asterisk indicates approximate time of light pulse presentation.

in phase shift magnitude, data were analyzed following logarithmic transformation but again, no significant group differences were observed. Thus, in purely exploratory efforts, data were compared separately at each CT and at each intensity, and results are reported below.

CT 14

As expected, phase delays were observed following presentation of light pulses at CT 14 (e.g. Figure 7). Group means (+/- SEM) for phase shifts in response to the 100lux light pulse are presented in Figure 8. The unhandled group showed the largest shifts (mean +/- SEM:1 37.5 +/-12.6 mins), and slightly smaller shifts were seen in the clomipramine-(129.7 +/- 11.6 mins) and saline-treated treated (116.4 +/- 13.0 mins) groups. These means were not significantly different either across the three groups [F(2,21)=.73, p=.49] or between comparison groups (p's>.24). At 50 lux, group means were reduced in magnitude compared to those seen for the 100 lux pulse, but the same rank order was maintained (Figure 8), as the unhandled group showed a mean phase delay of 125.5 +/ 18.4 mins, clomipramine-treated, 113.9 +/- 19.4 mins and saline-treated, 100.6 +/- 16.0 mins. However, as with the 100 lux shifts, these differences were not significant [F(2,21)=.48, p=.63] across groups or between pairs (p's>.34). Examination of the pooled data for the100 and 50 lux pulses and the use of non-parametric tests also did not uncover significant group differences.

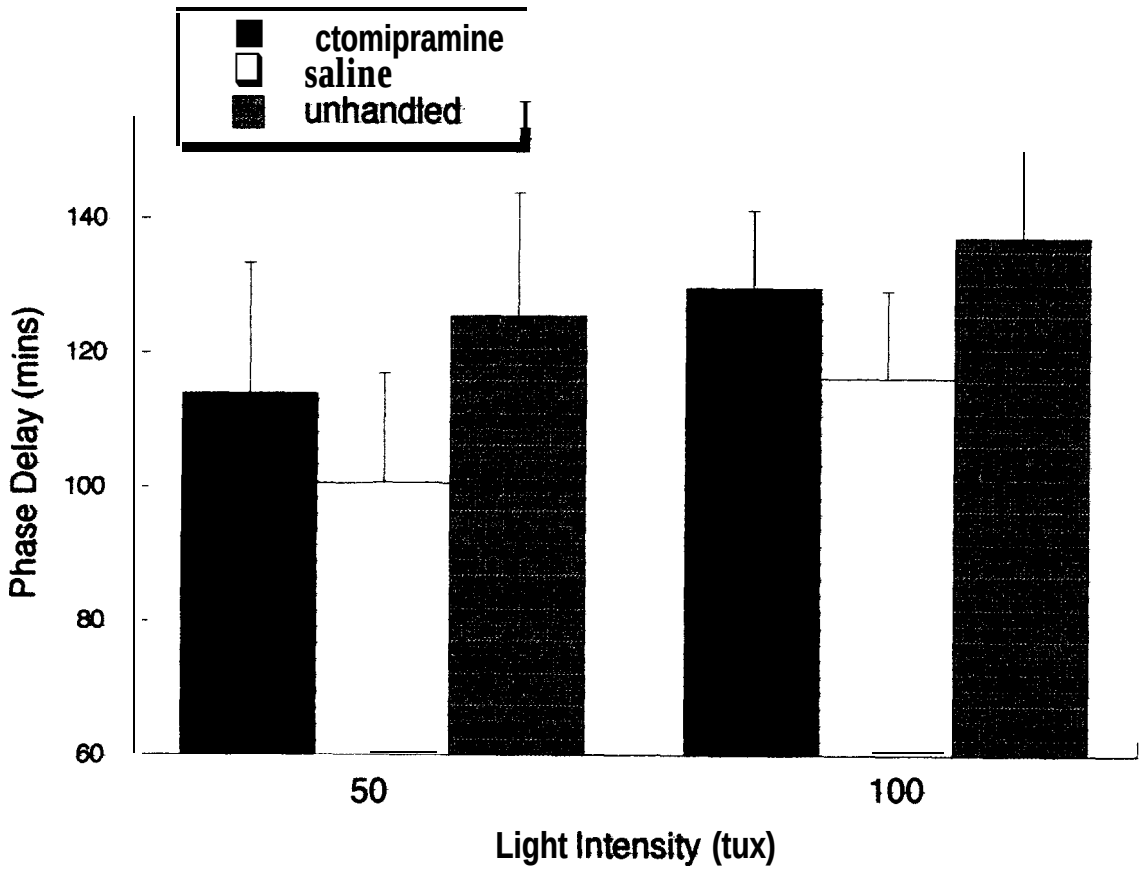

Figure 8. Mean (+/-SEM) phase delay for each of the three neonatal treatment groups following the 50 lux (left) and 100 lux (right) tight pulse at CT14

As period-lengthening is often observed following photic phase delays, cosinor analysis was used to quantify changes in free-running period following the 100 lux and 50 lux light pulses. Only very small changes in period were found for each of the three groups following either light pulse (all means <.10 h; Figure 9), none of which were significantly different from each other, or significantly related to phase shii magnitude for the corresponding light pulse. Further, other cosinor parameters for the samples prior to the light pulses also did not significantly predict phase shift magnitude (Tables 11 and 12).

Table 11

Correlations between phase shifts and cosinor parameters immediately preceding the 100 lux light pulse at CT 14.

	Phase shift	Period	Amplitude Ratio	PR
Phase shift				
Period	.17			
Amplitude Ratio	-.05	-.11		
PR	.29	0.0	.60**	
Activity	.24	-.16	.17	.43

**p<.05

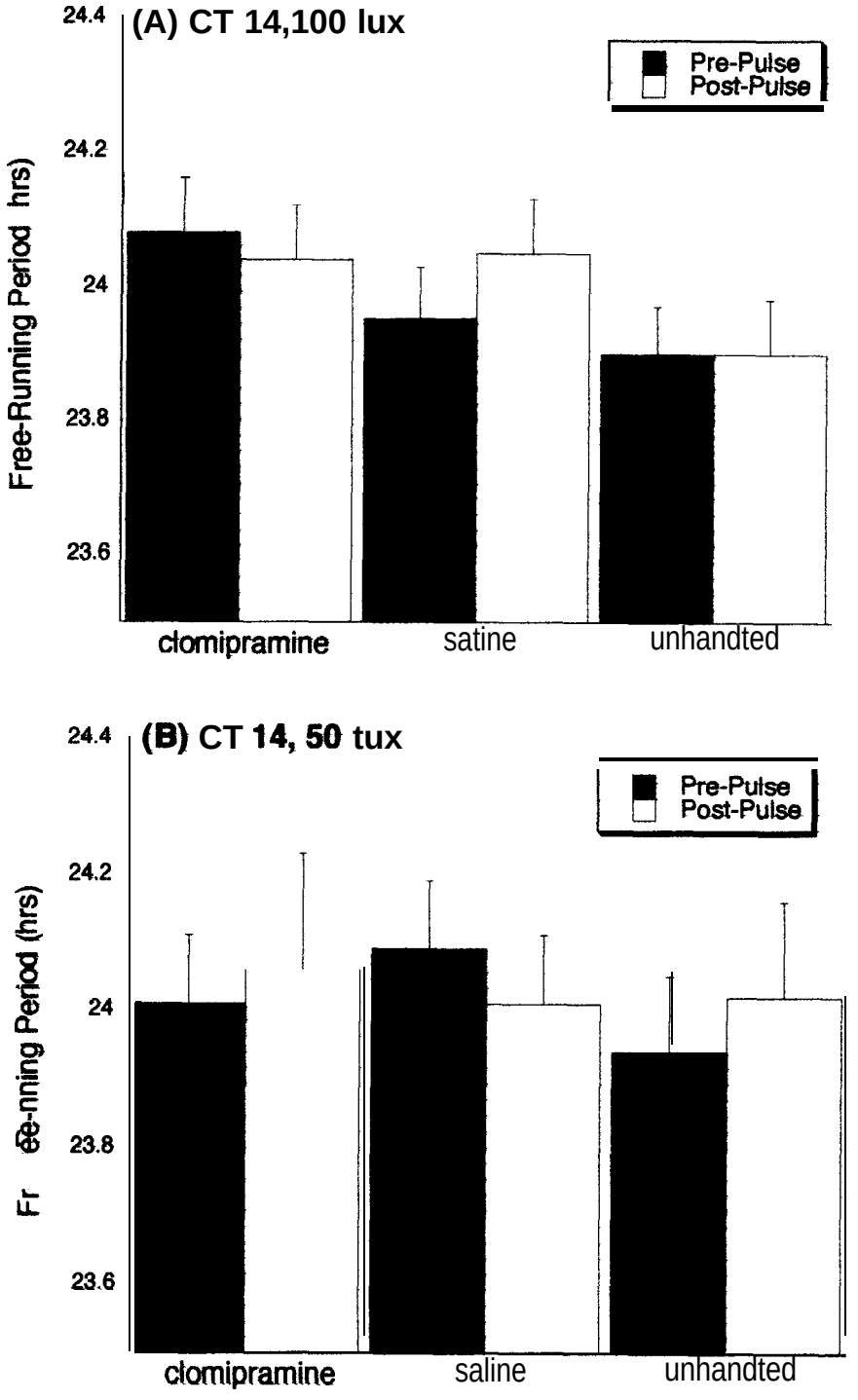

Figure 9. Group means (+/-SEM) for free-running period before and after the 100 lux pulse (A) and 50 lux putse (B) at CT14.

Table 12

Correlations between phase shifts and cosinor parameters immediately preceding the 50 lux light pulse at CT 14.

	Phase shift	Period	Amplitude Ratio	PR
Phase shift				
Period	-.02			
Amplitude Ratio	-.09	-.33		
PR	-. 2	-.2	82"	
Activity	-.	-.	25	34

**p<.05

CT 22

As expected, phase advances were observed in response to light pulses presented at CT 22 (e.g. Figure 7). Due to variable daily activity onsets, phase shifts in response to either the 100 lux or 50 lux light pulse at CT 22 could not be measured for one unhandled animal, so the means for this group were derived from seven animals. As shown in Figure 10, group means for the 100 lux liiht pulse were very similar [mean +/-SEM: unhandled = 84.2 +/-12.5 mins; clomipramine-treated = 78.7 +/- 9.0 mins; saline-treated = 75.5 +/- 5.0 mins] and no significant differences were found across groups [F(2, 21)=.23, p=.80] or between pairs (p's >.50). Although not significant, group means were actually higher for the 50 lux pulse as compared to the 100 lux pulse, with unhandled rats showing a mean of 101.5 +/- 21.1 mins, clomipramine-treated 80.1 +/- 13.5 mins, and saline-treated 83.7 +/- 11.9 mins (Figure 10). Again, these group differences were neither significant overall [F(2, 21)=.52, p=.60] nor between pairs (p's>.43). Logarithmic transformation, pooling the data across intensities or the use of non-parametric tests also did not result in significant findings.

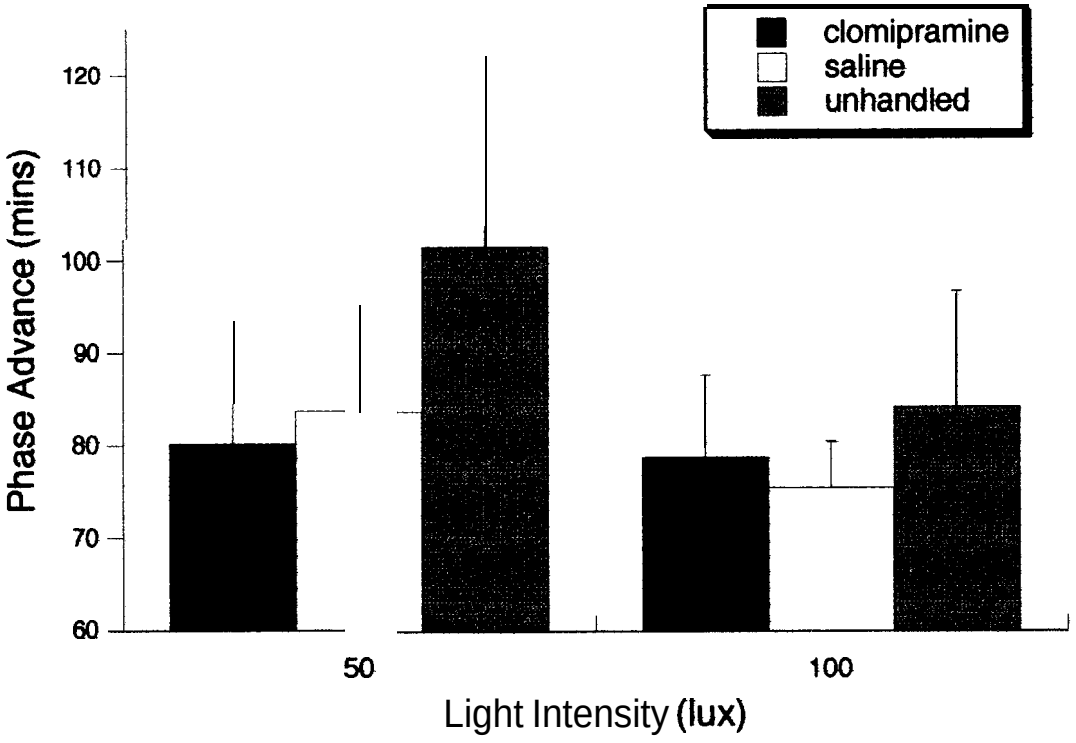

Figure10. Mean (+/-SEM) phase advance for each of the three neonatal treatment groups following the 50 lux (left) and 100 lux (right) light pulses at CT22.

Period-shortening is often observed following light puke-induced phase advances, and after the 100 lux puke, period-shortening was seen in the unhandled group, [mean change =.21h] and to a lesser extent in the clomipramine-treated group [.04 h], but no change was seen in the saline-treated group (Figure 11). After the 50 lux puke, almost no change in period was found in either the clomipramine or saline-treated groups[<.001h] and only slight period-lengthening was seen in the unhandted group[.06h]. For either pulse, group differences in the amount of period changes were not statistically significant and no significant relationship was found between period change and phase shift magnitude. Other cosinor parameters ako were not significantly related to phase shift magnitude (Tables 13 and 14).

Table 13

Correlations between phase shifts and cosinor parameters immediately preceding the 100 lux light pulse at CT 22.

	Phase shift	Period	Amplitude Ratio	PR
Phase shift				
Period	.08			
Ampl Ratiplitude	-.26	0.0		
PR	-.2	12	9 2 "	
Activity	-.2	33	32	35

'p<.01

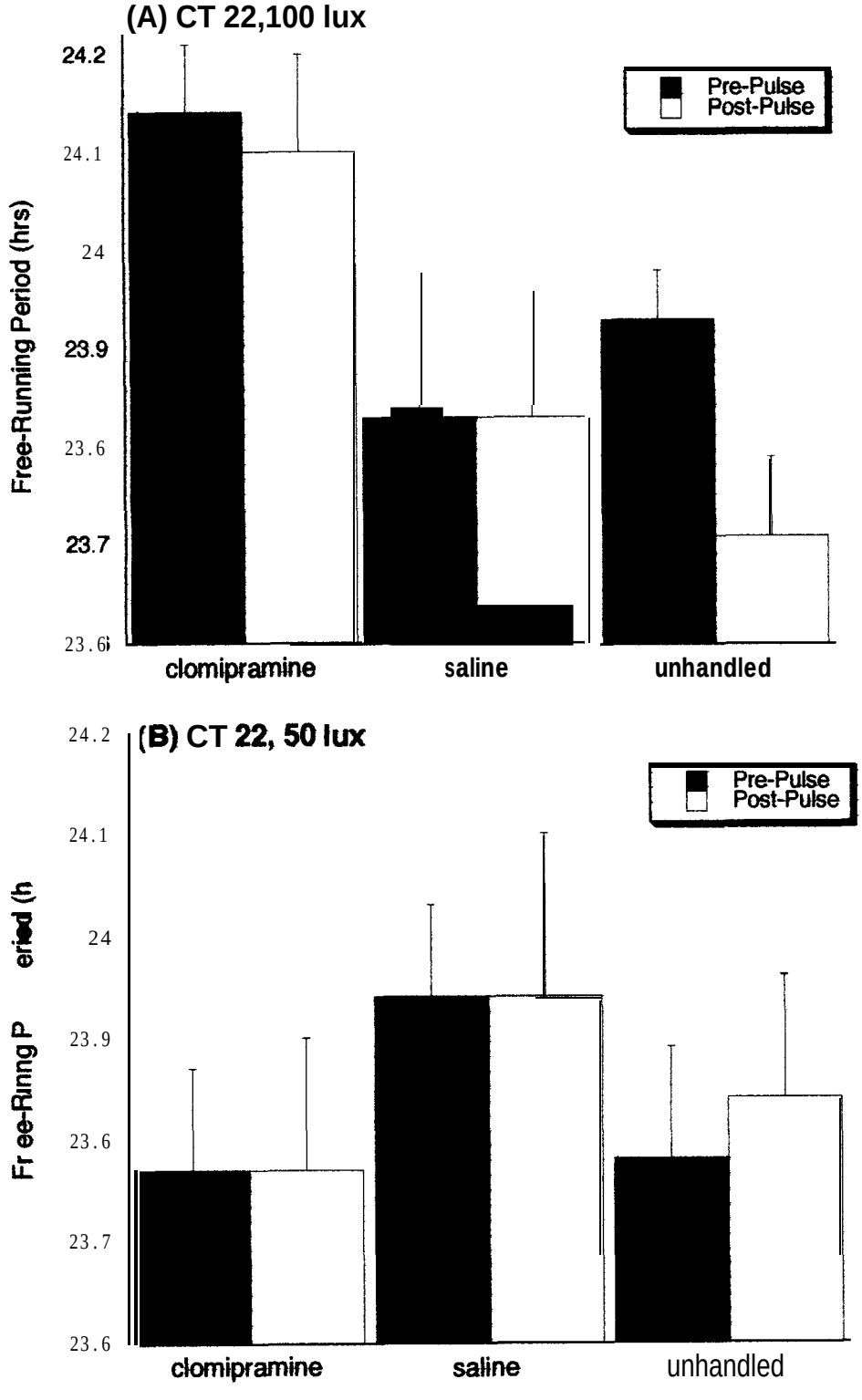

Figure 11. Group means (+I-SEM) for free-running period before and after the 100 tux pulse (A) and 50 tux pulse (B) at CT 22.

Table 14

Correlations between phase shifts and cosinor parameters immediately preceding the 50 lux light pulse at CT 22.

	Phase shift	Period	Amplitude Ratio	PR
Phase shift				
Period	.05			
Amplitude Ratio	.15	.12		
PR	.23	.21	.92***	
Activity	.39	.29	-.34	.45

***p<.01

Cosinor parameters

LL

Prior to 8-OH-DPAT, when animals were maintained under LL of 5 lux, free-running period was very close to 24 hours in all groups, and no significant group differences were observed (Table 15). As expected, when animals were maintained under the higher intensity LL prior to the dark pulse (i.e. 40 lux) free-running period was longer for all groups, but again, no significant group differences were observed (Table 16). Amplitude ratio, PR, and average level of wheel-running activity also did not differ by group under either intensity, although rank order on these three variables was maintained; the saline-treated group consistently showed the lowest amplitude, PR and activity level and the unhandled group consistently showed the highest values for these variables,

Table 15.

Means (+/- SEM) by group for cosinor parameters on the 10-day sample preceding the 8-OH-DPAT injection.

Group	Period	Amplitude Ratio	PR	Activity
Clomipramine	23.93 (.11)	1.36 (.07)	26.16 (3.99)	21.96 (4.72)
Saline	24.15 (.03)	1.33 (.04)	23.31 (4.11)	14.12 (5.84)
Unhandled	24.13 (.09)	1.38 (.07)	29.39 (5.85)	28.54 (10.44)
p_{GROUP}	.15	84	67	40

Table 16.

Means (+/- SEM) by group for cosinor parameters on the 10-day sample preceding the dark pulse.

Group	Period	Amplitude Ratio	PR	Activity
Clomipramine	24.34 (.09)	1.35 (.08)	26.36 (6.1 *f*)	14.31 (3.48)
Saline	24.33 (.10)	1.24 (.11)	22.65 (4.35)	11.43 (4.65)
Unhandled	24.36 (.07)	1.45 (.06)	28.84 (5.95)	21.86 (9.12)
p_{GROUP}	.96	22	73	53

DD

Free-running period, amplitude ratio and PR measures did not display significant group differences for any of the four cosinor samples (Tables 17-20). However, significant group differences, or trends toward significance, were observed in the average level of drinking activity observed in DD. In each sample, rank order was maintained, and was the same as that observed for wheel-running activity in the LL conditions; the saline-treated group displayed the lowest level of activity and the unhandled group displayed the highest. Pairwise comparisons revealed that these two groups were significantly different in each sample (p's<.05), while the clomipramine- and saline-treated groups did not significantly differ.

Table 17.

Means (+/- SEM) by group for cosinor parameters on the 10-day sample preceding the 100lux light pulse at CT14.

Group	Period	Amplitude Ratio	PR	Activity
Clomipramine	24.08 (.08)	.58 (.04)	11.65 (1.41)	31.24 (4.64)
Saline	23.95 (.07)	68 (.03)	13.95 (1.49)	24.68 (2.62)
Unhandled	23.90 (.08)	68 (.04)	15.21 (2.03)	39.98 (3.17)
p_{GROUP}	.23	13	33	02

Table 18.

Means (+/- SEM) by group for cosinor parameters on the 10-day sample preceding the 50 lux light pulse at CT14.

Group	Period	Amplitude Ratio	PR	Activity
Clomipramine	24.01 (.10)	60 (.06)	10.93 (2.06)	29.06 (4.44)
Saline	24.09 (.10)	62 (.05)	11.64 (1.81)	21.40 (4.12)
Unhandled	23.94 (.11)	68 (.04)	12.81 (1.97)	35.64 (4.54)
p_{GROUP}	.61	51	80	09

Table 19.

Means (+/- SEM) by group for cosinor parameters on the 10-day sample preceding the 100 lux light pulse at CT22.

Group	Period	Amplitude Ratio	PR	Activity
Clomipramine	24.06 (.06)	64 (.06)	10.80 (1.77)	22.52 (3.20)
Saline	23.84 (.13)	.61 (.08)	10.10 (3.14)	15.50 (3.65)
Unhandled	23.93 (.05)	77 (.07)	12.23 (1.92)	26.91 (4.75)
P_{GROUP}	.20	[illegible]	[illegible]	.16

Table 20.

Means (+/-SEM) by group for cosinor parameters on the 10-day sample preceding the 50 lux light pulse at CT22.

Group	Period	Amplitude Ratio	PH	Activity
Clomipramine	23.77 (.10)	65 (.08)	9.47 (2.10)	18.76 (3.01)
Saline	23.94 (.09)	66 (.03)	10.61 (1.16)	16.53 (3.02)
Unhandled	23.78 (.11)	75 (.06)	12.29 (1.88)	28.42 (4.47)
p_{GROUP}	.40	43	53	06

CHAPTER IV
DISCUSSION

Alterations in circadian regulation are associated with clinical depression and have been observed in several animal models of depression (Duncan, 1996; Rosenwasser & Wirz-Justice, 1997). Administration of monoaminergic antidepressants to rats during early postnatal life produces numerous behavioral and physiological alterations in adulthood that parallel those observed in depressed patients. Thus, neonatal antidepressant treatment has been put forth as an animal model of depression (Vogel & Vogel, 1982). As yet, few studies have examined circadian parameters following neonatal antidepressant treatment, so the purpose of the present study was to explore circadian phase shifting in rats following neonatal treatment with clomipramine, a 5-HT re-uptake inhibitor. Evidence suggests that the behavioral alterations observed in rats following neonatal clomipramine treatment arise from a permanent down-regulation in serotonergic systems (e.g. Feenstra et al., 1996; Maudhuit et al., 1996). As 5-HT pathways are important in the regulation of both circadian rhythmidty and affective behavior, it was expected that alterations in both non-photic and photic phase shifting would be uncovered in this model.

Non-photic phase-shifting stimuli included dark pulses and injections of 8-OH-DPAT, a 5-HT agonist, administered during subjective day, while photic phase shifting was examined through the presentation of light pulses in early and late subjective night. The phase shifts displayed by clomipramine-treated rats were of primary interest, but in an effort to parse the effects of the neonatal handling procedure from the pharmacological effects of clomipramine, three groups were included in this study; domipramine-treated, saline-treated, and unhandled groups. As discussed below, the results of this study provide support for alterations in non-photic but not photic phase shifting. Comparison of the results of this study with previous work in hamsters provides support for species differences in the role of 5-HT on circadian parameters.

Non-Photic Experiment

8-OH-DPAT

The domipramine- and saline-treated groups showed a significant difference in the phase-shifting response to 8-OH-DPAT administration. Group means differed in both size and direction, as the domipramine-treated group showed a small mean phase advance while a larger mean phase delay was displayed by the saline-treated group. These results are difficult to interpret within the context of neonatal domipramine studies as the one published study examining circadian phase shifting following this treatment employed hamsters (Yannieiii, Cutrera et al., 1998). That study reported phase advances in response to 8-OH-DPAT in both clomipramine and saline-treated groups, with a significant increase in phase shift magnitude due to domipramine treatment. This result was thought to reflect hypersensitivity of serotonergic receptors to acute activation, and complements other studies in which altered 5-HT function follows neonatal domipramine treatment. The present study did not replicate that result in rats, but this discrepancy may reflect species differences in the response to either neonatal antidepressant treatment or to 8-OH-DPAT. At present, there are too few neonatal antidepressant studies examining circadian parameters to adequately address species differences in response to this treatment. However, in regard to the latter, interpretation of the present data may be facilitated through comparison of the phase-shifting responses to 8-OH-DPAT across the three neonatal groups.

in general, phase shifts tended to be small in magnitude with the largest mean difference observed between domipramine- and saline-treated groups. The unhandled group showed a mean phase delay smaller than, although not significantly different from, that of the saline-treated group. This pattern of results suggests that exposure to nonspecific stressors associated with neonatal saline treatment may increase the size of phase delays in response to 8-OH-DPAT, an effect which is counter-acted by domipramine. Although not related to phase shift magnitude, opposing effects of the neonatal domipramine and saline treatments were also evident for both 8-OH-DPAT-induced activii and period changes. Indeed, although statistical significance was often

lacking, clomipramine and saline treatment appeared to exert effects in opposite directions for many of the variables in this study. Possibly, this indicates a reliable differential effect of these two treatments which may have been confirmed had this study had greater statistical power (see below).

Opposite effects of neonatal antidepressants and neonatal saline-treatment (or similar non-pharmacological neonatal handling procedures) relative to unhandled rats have not been explored explicitly within previous studies. Neonatal handling is an embedded feature of all neonatal antidepressant protocols and in studies employing these protocols, the comparison group generally consists of saline-treated controls, rather than unhandled animals. However, several studies have examined the physiological and behavioral effects of neonatal handling in direct comparison to unhandled animals. This treatment has been put forth as a putative animal model of a low anxiety or stress-resistant state, as neonatally handled animals consistently show enhanced adaption to environmental stressors in adulthood (e.g. Maccari et al., 1997; Ogawa et al., 1994; Wakshlak & Weinstock, 1990).

Comparisons between studies in which the experimental group was exposed to either neonatal antidepressants or handling support the opposing effects of these two treatments suggested by the present results. For example, neonatally handled rats show increased exploratory behavior in an open field (Nunez et al., 1996), decreased immobilii in the forced-swim test and decreased voluntary alcohol intake (Hilakivi-Clarke et al., 1991). Neonatal handling has also been shown to decrease the amplitude of the circadian temperature rhythm (Amir & Schiavetto, 1990). As discussed previously, neonatal clomipramine treatment has the opposite effect on each of these behaviors. A common site of action for these two treatments is as yet unclear, but the effects of neonatal handling are believed to be mediated through permanent changes in responsiveness of the HPA axis, which may have widespread implications for developing neurotransmitter systems (Nunez et al., 1996; Wakshlak & Weinstock, 1990).

Although differential effects of neonatal clomipramine and saline treatment can be supported by existing behavioral literature, the direction of phase shifting in both the

saline-treated and unhandled groups was unexpected. In particular, as the unhandled rats were undisturbed during the neonatal period, it was expected that these rats would display 8-OH-DPAT–induced phase shifts comparable to those reported in other studies in which neonatal treatments were not employed. As this study was planned, 8-OH-DPAT administration had been shown to induce relatively large phase advances during subjective day in several hamster behavioral studies (e.g. Tominaga et al., 1992), in *vitro* rat studies (e.g. Prosser et al., 1993) and in a single previous in *vivo* rat study (Edgar et al., 1993). Therefore, it was expected that the unhandled rats would also display relatively large phase advances in response to 8-OH-DPAT. In contrast, phase shifts in this group were relatively small and observed in either direction. As discussed previously, differences in SCN organization may preclude direct comparisons across species, and methodological differences may explain discrepancies between in *vitro* and *in vivo* results, but it is more difficult to account for a lack of agreement between the present data and the previous *in vivo* study. However, several more recent studies have also examined the phase-shifting effects of 5-HT agonists in rats during subjective day, and results are also contradictory. For example, Edgar et al. (1993) repotted phase advances in wheel-running activity in response to either quipazine or 8-OH-DPAT during subjective day in rats, but Kohler, Kalkowski and Wollnik (2000) found no significant effects of quipazine at this same phase. In addition, small phase delays in the circadian rhythm of melatonin production have been found in response to quipazine, although not to 8-OH-DPAT, during subjective day (Kennaway et al., 1996). Thus, at present, the phase-shifting effects of 5-HT agonists in rats during subjective day remain to be clarified. The results of the present study indicate that although neonatal clomipramine and saline treatment significantly alter 8-OH-DPAT-induced phase shifts during subjective day, the effect of this stimulus on unhandled rats is minimal.

Dark Pulses

All published studies of the effects of dark pulses on circadian phase shifting have been conducted with hamsters (e.g. Boulos & Rusak, 1982a; Ellis, McKlveen & Turek, 1982; Reebs et al., 1989). In agreement with these studies, most rats in the present study displayed phase advances in response to six hour dark pulses beginning at CT 6. Neonatal treatment did not significantly predict the magnitude of these shifts, but as with 8-OH-DPAT-induced phase shifts, clomipramine and saline treatment appeared to exert opposite effects, in that the saline-treated group showed the largest mean phase advance compared to either of the other groups. As there was considerable within-group variability, the lack of a significant group difference may be a function of the relatively small group size, so increasing the number of animals per group may have helped to uncover potential group differences. Alternatively, it is possible that the use of dark pulses as a non-photic stimulus may have been inappropriate for this species. Dark pulses are particularly effective phase-shifting stimuli for hamsters, but the magnitude of the shifts displayed by the unhandled animals suggests that this stimulus may be less effective in rats.

Small phase shifts were observed in the unhandled group, while much larger dark pulse-induced phase advances have been reported for hamsters. Further, average level of dark pulse-induced activity was much less than that reported typically for hamsters; although many of the unhandled rats did display substantial wheel-running activity during dark pulses, levels of induced activity predicted neither presence of, nor size of, significant phase shifts. As discussed previously, stimulus-induced activity in hamsters has been taken as a marker for increased arousal, and it is the arousal, or some biochemical correlate, which is thought to be responsible for the phase shifts in response to behaviorally activating stimuli. Based on data from the unhandled group, it is unclear whether dark pulses are less arousing to rats or whether their circadian system is less sensitive to behavioral feedback. Although there are no other published studies explicitly examining dark pulse-induced phase shifting in rats, studies of other environmental stimuli generally suggest that rats may be less sensitive to arousal-induced phase shifting as

compared to hamsters. For example, episodes of social interaction during subjective day can be effective phase-shifting stimuli in hamsters (Mrosovsky, 1988), but not rats (e.g. Meerlo & Daan, 1998). On restricted feeding schedules, rats display intense bouts of activity in anticipation of food availability, but this activity does not alter circadian phase (Stephan, 1984). Finally, scheduled daily activity is an effective entraining agent in hamsters (Reebs & Mrosovsky, 1989) but rats display only weak entrainment under severely constrained conditions of enforced activity (Mistlberger, 1991).

Although there was considerable within-group variability, the saline-treated group in this study showed the largest mean advance, and the greatest amount of dark-pulse-induced wheel-running, suggesting that dark pulses were a particularly salient stimulus for these rats. Perhaps the physiological changes induced by neonatal handling increase sensitivity to a behavioral stimulus like a dark pulse, or reduce behavioral inhibition in this novel situation. Clomipramine-treated rats generally displayed smaller phase advances suggesting that any effects of neonatal handling on dark pulse-induced phase shifting are negated by clomipramine administration. However, as significant differences were not found, these conclusions are speculative. The present study does provide some evidence for dark pulse-induced phase shifting during subjective day, but the relatively small phase shifts observed for the unhandled rats support the idea that compared to hamsters, the rat circadian system is not particularly sensitive to behavioral feedback.

Photic Experiment

Considerable research has been devoted to the role of serotonin in photic phase-shifting. Most of these studies have been conducted with hamsters and generally support the idea that serotonergic pathways to the circadian system have an inhibitory influence on photic phase-shifting. In the present study, no significant group effects were observed in response to photic stimuli in either early or late subjective night. Thus, either neonatal clomipramine treatment does not influence photic phase-shifting, or alterations do occur, but were not detected in the present study. As with dark pulses, within-group variability in the size of the photic phase shifts was considerable, so increased statistical power

through increases in the number of animals per group may have provided more conclusive mutts. This may be particularly true for photic phase delays. Rank order was maintained across the 100 and 50 lux pulses at CT 14, suggesting a possible trend towards increased photic phase delays in the domipramine-treated groups. Significant results in this direction would have supported the idea that reduced 5-HT function -in thii animal model reduces inhiii of photii responsiveness. However, there -is some evidence to indicate that neonatal ctomiiamine treatment may not have consistent effects on photic phase shifting, and that, lie non-photic effects, the influence of 5-HT on photic responsiveness may differ between hamsters and rats

The previous study of neonatal clomipramine treatment in hamsters also examined photic phase-shifting in both early and late subjective night, and like the present study, neonatal clomipramine treatment did not significantly affect the magnitude of photic phase delays. However, treated hamsters did show signifiiy reduced phase advances at CT 22 relative to controls. Given the many studies supporting an inhibiiry role of 5-HT on photic responsiveness in hamsters, neonatal clomipramine treatment would have been expected to result in increased rather than decreased photic phase shifts. No further studies ofthistreatment in hamsters have as yet been reported, so the implication of this effect is unclear.

The idea that reduced 5-HT function leads to increased photic phase shifting is largely based on work with hamsters (Morin, 1999). Although the present study dii not find significant effects on photic phase shift magnitude in either direction following the neonatal treatments, recent evidence suggests that 5-HT may not have the inhibitory influence on photic phase-shifting in rats that it does in hamsters. For example, although one study did not find phase shifts in rats following quipazine administration during subjective night (Edgar et al., 1993), several more recent studies have reported that quipazine mimics the phase-shifting effects of photic stimuli during subjective night on both circadian wheel-running activity (Kohler et al., 2000) and melatonin production (Kennaway et al., 1996; 1997). Further, quipazine administration has been shown to induce c-fos expression in the SCN in a pattern similar to that observed following light exposure (Kennaway & Moyer,

1998). Thus, at this point, the available literature on the contribution of 5-HT to photic phase shifting is contradictory, but the most recent evidence suggests that 5HT may mediate, rather than inhibit, photic input to the rat circadian system.

Finally, in addition to group size, another methodological problem evident in the photic experiment concerns the choice of light intensities for the light pulses. Based on published studies, the 10 min 100 lux pulses were selected so as to result in sub-maximal phase shifts. However, it can be difficult to compare dynamics of light pulse presentation across laboratories, and indeed, the 100 lux pulses did appear to be saturating. Thus, a more effective photic stimulus may have been a light pulse of a lesser intensity, or shorter duration. As mentioned in the Method section, additional pulses at 50 lux were presented in an effort to reduce phase shift magnitude, however, mean shift size was unaffected (CT 22) or only slightly reduced (CT 14). It is unclear whether the 50 lux pulse was also saturating, or whether the additional time in DD increased photic phase shift magnitude to a less than saturating stimulus. Photic responsiveness can be increased by increased time in DD (Shimomura & Menaker, 1994) and in the present study, rats were not re-entrained to an LD cycle between the 100 and 50 lux pulses at either circadian phase. Thus, although the present study suggests that neonatal clomipramine treatment does not alter the upper asymptote for photic phase shift magnitude, definitive conclusions regarding the effects of this treatment on photic phase-shifting await further study

Conclusions

The results of thii study provide some support for alterations in non-photic, but not photic, phase-shifting in the neonatal antidepressant model of depression. Neonatal clomipramine treatment alters the phase-shifting response of rats to a 5-HT agonist during subjective day, but significant effectsvvere notobserved in response to either dark pulses during subjective day or light pulses during subjective night. In general, evidence in this study also provides support for opposing effects of neonatal clomipramlne and saline treatments, particularly in regard to non-photic phase shifting. Finally, comparing the present results to those of priorstudiiin hamsterssupportsdistinctionsbetween

species in the role of 5-HT in circadian phase shifting.

At present, although alterations in circadian regulation are a common feature of clinical depression and have been observed in several animal depression models, no clear pattern in the direction of these alterations has emerged. Some evidence now shows that rhythmicity can be altered by neonatal domipramine treatment, but further work will be required to determine the extent of these alterations, and the mechanisms through which 5-HT mediates circadian and affective behaviors.

REFERENCES

Albers, H.E., & Ferris, C.F. (1984). Neuropeptiie Y:Role in the light-dark cycle entrainment of hamster rhythms. Neuroscience Letters, 50,162-l 68.

Amir, S., & Edelstein, K. (1997). A blocker of nitric oxide synthase, NG-nitro-L-arginine methyl ester, attenuates light-induced Fos protein expression in the rat suprachiasmatic nucleus. Neuroscience Letters, 224, 29-32.

Amir S., & Schiavetto, A. (1990). Neonatal handling reduces the amplitude of the daily temperature rhythm in the rat. Society for Neuroscience Abstract, 16, 1334.

Aschoff, J. (1981). Free-running and entrained circadian rhythms. In J. Aschoff (Ed.), Handbook of Behavioral Neurology, Volume 4. R˙ l -˙(pp. 81-93). New York:Plenum.

Biello, SM. (1995). Enhanced photic phase shifting after pretreatment with antiserum to neuropeptide Y. Brain Research, 673, 25-29.

Biello, S.M., Golombek, D.A., & Harrington, ME. (1997). Neuropeptide Y and glutamate block each other's phase shifts in the suprachiasmatic nucleus in vitro. Neuroscience 77,1049-l 057.

Biello, S.M., Golombek, D.A., Schak, K.M., & Harrington, M.E. (1997). Circadian phase shifts to neuropeptide Y in vitro:cellular communication and signal transduction. Journal of Neuroscience, 17, 8468-8475.

Biello, S.M., Harrington, M.E., & Mason, R. (1991). Geniculo-hypothalamic tract lesions block chlordiazepoxide-induced phase advances in Syrian hamsters. Brain Research, 552, 47-52.

Biello, S.M., Janik, D., & Mrosovsky, N. (1994). Neuropeptide Y and behaviorally induced phase shifts. Neuroscience, 62, 273-279.

Biello, SM., & Mrosovsky, N. (1993). Circadian phase-shifts induced by chlordiazepoxide without increased locomotor activity. Brain Research, 622, 58-62.

Biello, S.M., & Mrosovsky, N. (1995). Blocking the phase-shifting effect of neuropeptide Y. Proceedings of the Royal Society of London, 259, 179-187.

Bina, K.G., & Rusak, B. (1996). Muscarinic receptors mediate carbachol-induced phase shifts of circadian activity rhythms in Syrian hamsters. Brain Research, 743, 202-211.

Bina, K.G., Rusak, B., & Semba, K. (1993). Localization of cholinergic neurons in the forebrain and brainstem that project to the suprachiasmatic nucleus of the hypothalamus in rat. Journal of Comparative Neurology, 335, 295-307.

Bobrzynska, K.J., Godfrey, M.H., & Mrosovsky, N. (1996). Serotonergic stimulation and non-photic phase-shifting in hamsters. Physiology & Rehavior. 59, 221-230.

Bobrzynska, K.J., Vrang, N., & Mrosovsky, N. (1996). Persistence of non-photic phase shifts in hamsters after serotonin depletion in the suprachiasmatic nucleus. Brain Research 741,205214.

Borella, A., Bindra, M., & Whitaker-Azmitia, P. (1997). Role of the 5-HT$_{1A}$ receptor in development of the neonatal rat brain:Preliminary behavioral studies. Neuropharmacology, 36, 445-450.

Boulos, Z., & Rusak, B. (1982a). Circadian phase response curves for dark pulses in the hamster. Journal of Comparative Physiology, A.146, 41l-41 7.

Boulos, Z., & Rusak, B. (1982b). Phase-response curves and the dual-oscillator model of circadian pacemakers. In J. Aschoff, S. Daan and G.A. Groos (Eds.), Vertebrate Circadian Systems:Structure and Physiology (pp.215-223). Berlin:Springer-Verlag.

Card, J.P., & Moore, R.Y. (1989). Organization of lateral geniculate-hypothalamic connections in the rat. Journal of Comparative Neurology, 284, 135-147.

Colwell, C.S., & Foster, R.G. (1992). Photic regulation of Fos-like immunoreactivity in the suprachiasmatic nuclei of the mouse. Journal of Comparative Neurology, 324, 135-142.

Colwell, C.S., Foster, R.G., & Menaker, M. (1991). NMDA receptor antagonists block the effects of light on circadian behavior in the mouse. Brain Research, 554, 105-l 10.

Colwell, C.S., Kaufman, C.M., & Menaker, M. (1993). Phase-shifting mechanisms in the mammalian circadian system:New light on the carbachol paradox. Journal of Neuroscience, 13, 1454-l459.

Cutrera, R.A., Kalsbeek, A., & Pevet, P. (1993). No triazolam-induced expression of Fos protein in the raphe nuclei of the male Syrian hamster. Brain Research, 602, 14-20.

Cutrera, R.A., Kalsbeek, A., & Pevet, P. (1994). Specific destruction of the serotonergic afferents to the suprachiasmatic nuclei prevents triazolam-induced phase advances of hamster activity rhythms. Behavioral Rrain Research, 62, 21-28.

Cutrera, R.A., Ouarour, A., & Pevet, P. (1994). Effects of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT and other non-photic stimuli on the circadian rhythm of wheel-running activity in hamsters under different constant conditions. Neuroscience Letters, 172, 27-30.

Cutrera, R.A., Saboureau, M., & Pevet, P. (1996). Phase-shifting effect of 8-OH-DPAT, a 5-HT$_{1A}$/5-HT$_{7}$ receptor agonist, on locomotor activity in golden hamster in constant darkness. Neuroscience Letters, 210, l-4.

DeVries, M.J., Cardoza, B.N., Van Der Want, J., DeWolf, A., & Meijer, J.H. (1993). Glutamate immunoreactivity in terminals of the retinohypothalamic tract of the brown Norwegian rat. Brain Research, 612, 231-237.

Ding, J.M., Chen, D., Weber, E.T., Faiman, L.E., Rea, M.A., & Gillette, M.U. (1994). Resetting the biological clock:Mediation of nocturnal circadian shifts by glutamate and NO. Science, 266, 1713-1717.

Duncan, W.C. (1996). Circadian rhythms and the pharmacology of affective illness, Pharmacological Therapy, 71, 253-312.

Duncan, W.C., Johnson, K.A., Sutin, E., & Wehr, T.A. (1998). Disruption of the activity-rest cycle by MAOI treatment:Dependence on light and a secondary visual pathway to the circadian pacemaker. Brain Research Bulletin, 45, 457-465.

Duncan, W.C., Tamarkin, L., Sokolove, P.G., & Wehr, T. A. (1988). Chronic dorgyline treatment of Syrian hamsters:An analysis of effects on the circadian pacemaker. Journal of Biological Rhythms, 3, 305-322.

Dwyer, S.M., & Rosenwasser, A.M. (1998a). Effects of chronic donidine administration on free-running period, photic phase-shifting and entrainment of hamsters. Society or Research on Biological Rhythms Abstract. 296.

Dwyer, S.M., & Rosenwasser, A.M. (1998b). Neonatal clomipramine treatment, alcohol intake, and circadian rhythms in rats. Psychopharmacology, 138, 176-183.

Earnest, D.J., & Turek, F.W. (1985). Neurochemical basis for the photic control of circadian rhythms and seasonal reproductive cycles: Role for acetylcholine. Proceedings of the National Academy of Sciences, USA, 82, 4277-4281.

Edelstein, K., & Amir, S. (1995). Non-photic manipulations induce expression of Fos protein in the suprachiasmatic nucleus and intergeniculate leaflet in the rat. Brain Research, 690, 254-258.

Edgar, D.M., & Dement, W.C. (1991). Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. American Journal of Physiology, 261, R928-R933.

Edgar, D.M., Martin, C.E., & Dement, W.C. (1991). Activity feedback to the mammalian circadian pacemaker: Influence on observed measures of period length. Journal of Biological Rhythms, 6, 185-l 99.

Edgar, D.M., Miller, J.D., Prosser, R.A., Dean, R.R., & Dement, W.C. (1993). Serotonin and the mammalian circadian system: II. Phase-shifting rat behavioral rhythms with serotonergic agonists. Journal of Biological Rhythms, 8, 17-31.

Ellis, G.B., McKlveen, R.E., Turek, F.W. (1982). Dark pulses affect the circadian rhythm of activity in hamsters kept in constant light. American Journal of Physiology, 242, R44-50.

Feenstra, M.G.P., VanGalen, H., Te Riele, P.J.M., Botterblom, M.H.A. & Mirmiran, M. (1994). Decreased hypothalamic serotonin levels in adult rats treated neonatally with domipramine. Pharmacology, Biochemistry & Behavior, 55, 647-652.

Femandez-Pardal, J. & Hilakivi, L.A. (1989). Effect of low alcohol dose on behavioral "despair" in rats neonatally treated with antidepressant drugs. Alcohol, 6, 93-95.

Frank, M.G., Page, J., & Heller, H.C. (1997). The effects of REM sleep-inhibiting drugs in neonatal rats: Evidence for a distinction between neonatal active sleep and REM sleep. Brain Research, 778, 64-72.

Fride, E., Dan, Y., Feldon, J., Halevy, G., & Weinstock, M. (1988). Effects of prenatal stress in prepubertal and adult rats. Physiology & Behavior, 37, 681-687.

Furukawa, T., Murakami, N., Takahashi, K., & Etoh, T. (1987). Effect of implantation of carbachol pellet near the suprachiasmatfc nucleus on the free-running period of rat locomotor activity rhythm. Japanese Journal of Physiology, 37, 321-326.

Gannon, R.L., & Rea, M.A. (1995). Twelve-hour phase shifts of hamster circadian rhythms elicited by voluntary wheel-running. Journal of Biological Rhythms, 10, 196-210.

Giardina, W.J., & Radek, R.J. (1991). Effects of imipramine on the nocturnal behavior of bilateral olfactory bulbectomized rats. Biological Psychiatry, 29, 1200-l 208.

Gillette, M.U. (1996). Regulation of entrainment pathways by the suprachiasmatic circadian clock: Sensitivities to second messengers. In: R.M. Buijs, A. Kalsbeek, H.J. Romijn, C.M.A. Pennartz, and M. Mirmiran (Eds.), Progress in Brain Research, Volume 11, Hypothalamic Integration of Circadian Rhythms, (pp. 147-l 74). Amsterdam: Elsevier.

Glass, J.D., Selim, M., & Rea, M.A. (1994). Modulation of light-induced c-fos expression in the suprachiasmatic nuclei by 5-HT_1A receptor agonists. Brain Research, 638, 235-242.

Golden, R.N., & Janowsky, D.S. (1990). Biological theories of depression. In B.B. Wolman and G. Stricker (Eds.), Depressive Disorders: Facts, Theories and Treatment Methods, (pp. 3-21). New York: Wiley.

Goodwin, F.K. & Jamison, K.R. (1990). Manic Depressive Illness, (pp. 541-574). New York: Oxford.

Harrington, M.E. (1997). The ventral lateral geniculate nucleus and the intergeniculate leaflet: Interrelated structures in the visual and circadian systems. Neuroscience and Biobehavioral Reviews, 21, 705-727.

Harrington, M.E., & Rusak, B. (1986). Lesions of the thalamic intergeniculate leaflet alter hamster circadian rhythms. Journal of Biological Rhythms, 1, 309-325.

Harrington, M.E., & Rusak, B. (1989). Photic responses of geniculohypothalamic tract neurons in the Syrian hamster. Visual Neuroscience, 2, 367-375.

Hastings, M.H., Best, J.D., Ebling, F.J.P., Maywood, E.S., McNulty, S., Schurov, I., Selvage, D., Sloper, P., & Smith, K.L. (1996). Entrainment of the circadian clock. In R.M. Buijs, A. Kalsbeek, H.J. Romijn, C.M.A. Pennartz, and M. Mirmiran (Eds.), Progress in Brain Research. Volume 11. Hypothalamic Integration of Circadian Rhythms. (pp. 147-174). Amsterdam:Elsevier.

Hastings, M.H., Mead, S.M., Vidlacheruvu, RR., Ebling, F.J.P., Maywood, E.S., & Groose, J. (1992). Non-photic phase-shifting of the circadian activity rhythm of Syrian hamsters:The relative potency of arousal and melatonin. Brain Research. 591, 20-26.

Henry, C., Kabbaj, M., Simon, H., LeMoal, M., & Maccari, S. (1994). Prenatal stress increases the hypothalamic-pituitary-adrenal axis response in young and adult rats. Neuroendocrinology. 6. 341-345.

Hilakivi, L.A. & Hilakivi, I. (1987). Increased adult behavioral 'despair' in rats neonatally exposed to desipramine or zimeldine:An animal model of depression? Pharmacology. Biochemistry&Behavior. 28:367-369.

Hilakivi, L.A., Sinclair, J.D. & Hilakivi, I. (1984). Effects of neonatal treatment with clomipramine on adult ethanol related behavior in the rat. Developmental BrainResearch. 15:129-132.

Hilakivi, L.A., Stenberg, D., Sinclair, J.D. & Kiianmaa, K. (1987) Neonatal desipramine or zimeldine treatment causes long-lasting changes in brain monoaminergic systems and alcohol related behavior in rats. Psychopharmacology. 91: 403-409.

Hilakivi-Clarke, L.A., Turkka, J., Lister, R.G., & Linnoila, M. (1991). Effects of early postnatal handling on brain beta-adrenoceptors and behavior in tests related to stress. BrainResearch. 542. 286-92.

Holson, R.R., & Pierce, B. (1992). Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. Neurotoxicology & Teratology. 14. 221-228.

Honma, K. (1985). Phase-response curves of the circadian locomotor rhythm in rats:Analysis of the pacemaker structure. In T. Hiroshige and K. Honma (Eds.), Circadian Clocks and Zeitgebers. (pp. 106-117). Japan:Hokkaido University.

Honmann, W., Michel, S., & Fleissner, G. (1990). Locomotor activity and light pulses as competing zeitgeber stimuli in the scorpion circadian system. Society for Neuroscience Abstract, 2, 52.

Huhman, K.L., & Albers, H.E. (1994). Neuropeptide Y microinjected into the suprachiasmatic region phase shifts circadian rhythms in constant darkness. Peptides, 15, 1475-1478.

Huhman, K.L., Babagbemi, T.O., & Albers, H.E. (1995). Bicuculline blocks neuropeptide Y induced phase advances when microinjected in the suprachiasmatic nucleus of Syrian hamsters. Brain Research, 675, 333-336.

Huhman, K.L., Marvel, C.L., Gillespie, C.F., Mintz, E.M., & Albers, H.E. (1997). Tetrodotoxin blocks NPY-induced but not muscimol-induced phase advances of wheel-running activity in Syrian hamsters. Brain Research, 773 176-180.

Janik, D. & Mrosovsky, N. (1992). Gene expression in the geniculate induced by a nonphotic circadian phase-shifting stimulus. NeuroReport, 3, 575-578.

Janik, D., & Mrosovsky, N. (1993). Nonphotically induced phase shifts of circadian rhythms in the Golden Hamster: Activity-response curves at different ambient temperatures. Physiology & Behavior, 53, 431-436.

Janik, D., & Mrosovsky, N. (1994). Intergeniculate leaflet lesions and behaviorally-induced shifts of circadian rhythms. Brain Research, 651, 174-182.

Janowsky, D.S., El-Yousef, M.K., Davis, J.M., & Sererke, H.J. (1972). A cholinergic-adrenergic hypothesis of mania and depression. Lancet, 2, 6732-6735.

Jesberger, J.A., & Richardson, J.S. (1988). Brain output dysregulation induced by olfactory bulbectomy: An approximation in the rat of major depressive disorder in humans? International Journal of Neuroscience, 38, 241-265.

Johnson, RF., Moore, R.Y., & Morin, L.P. (1988). Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. Brain Research, 460, 297-313.

Johnson, R.F., Moore, R.Y., & Morin, L.P. (1989). Lateral geniculate lesions alter circadian activity rhythms in the hamster. Brain Research Bulletin, 22, 411-422.

Johnson, RF., Smale, L., Moore, R.Y., & Morin, L.P. (1988). Lateral geniculate lesions block circadian phase-shift responses to a benzodiazepine. Proceedings of the National Academy of Sciences. USA. 85. 5301-5304.

Jones, B.E. (1991). The role of noradrenergic locus coeruleus neurons and neighboring cholinergic neurons of the pontomesencephalic tegmentum in sleep-wake states. Progress in Brain Research. 88. 533-543.

Kawai, K., Yokota, N., & Yamawaki, S. (1994). Effects of chronic tryptophan depletion on the circadian rhythm of wheel-running activity in rats. Physiology & Rehavior. 55. 1005-1013.

Keefe, D.L., Earnest, D. J., Nelson, D., Takahashi, J.S. & Turek, F. W. (1987). A cholinergic antagonist, mecamylamine, blocks the phase-shifting effects of light on the circadian rhythm of locomotor activity in the golden hamster. Brain Research. 403. 308-312.

Kennaway, D.J. (1997). Liiht, neurotransmitters and the suprachiasmatic nucleus control of pineal melatonin production in the rat. Biological Signals. 6, 247-254.

Kennaway, D.J., & Moyer, R.W. (1998). Serotonin 5-HT2c agonists mimic the effect of light pulses on circadian rhythms. Brain Research. 806, 257-270.

Kennaway, D.J., Rowe, S.A., & Ferguson, S.A. (1996). Serotonin agonists mimic the phase shifting effects of light on the melatonin rhythm in rats. Brain Research. 737, 301-307.

Klein, D.C., Moore, R.Y., & Reppert, S.M. (1991). The Suprachiasmatic Nucleus: The Mind's Clock. New York: Oxford.

Koehl, M., Barbazanges, A., LeMoal, M., & Maccari, S. (1997). Prenatal stress induces a phase advance of circadian corticosterone rhythm in adult rats which is prevented by postnatal stress. Brain Research. 759. 317-320.

Kohler, M., Kalkowski, A., & Wollnik, F. (2000). Species differences between hamsters and rats with regard to the putative role of serotonin in the circadian system. Biological Rhythms Research. 31, 340-354.

Komhauser, J.M., Ginty, D.D., Greenberg, M.E., Mayo, K.E., & Takahashi, J.S. (1996). Light entrainment and activation of signal transduction pathways in the SCN. In R.M. Buijs, A. Kalsbeek, H.J. Romijn, C.M.A. Pennartz, and M. Mirmiran (Eds.), Progress in Rrain Research. Volume 11. Hypothalamic Integration of Circadian Rhythms. (pp. 133-146). Amsterdam:Elsevier.

Kornhauser, J.M., Nelson, D.E., Mayo, K.E., & Takahashi, J.S. (1990). Photic and circadian regulation of *c-fos* gene expression in the hamster suprachiasmatic nucleus. Neuron. 5. 127-134.

Kuroda, H., Fukushima, M., Nakai, M., Katayama, T., & Murakami, N. (1997). Daily wheel running activity modifies the period of free-running rhythm in rats via intergeniculate leaflet. (1997). Physioloov & Behavior. 61. 633-637.

Lehman, M.N., Silver, R., Gladstone, W.R., Kahn, R.M., Gibson, M., & Bittrnan, E.L. (1987). Circadian rhythmicity restored by neural transplant: Immunocytochemical characterization of the graft and its integration with the host brain. Journal of Neuroscience. 7. 1626-l 638.

LeMarquand, D., Pihl, R.O., & Benkelfat, C. (1994). Serotonin and alcohol intake, abuse, and dependence: Findings of animal studies. Biological Psychiatry. 36. 396-421.

Levine, J.D., Rosenwasser, A.M., Yanovski, J.A., & Adler, N.T. (1986). Circadian activity rhythms in rats with midbrain raphe lesions. Brain Research. 384. 240-249.

Levine, S. (1970). The pituitary-adrenal system and the developing brain. Progress in Brain Research. 32. 79-85.

Liou, S.Y., Shibata, S., Iwasaki, K., & Ueki, S. (1986). Optic nerve stimulation-induced increase of release of ^{3}H-glutamate and ^{3}H-aspartate but not ^{3}H-GABA from the suprachiasmatic nucleus in slices of rat hypothalamus. Brain Research Bulletin. 16. 527-531.

Liu, C., & Gillette, M.U. (1996). Cholinergic regulation of the suprachiasmatic nucleus circadian rhythm via a muscarinic mechanism at night. Journal of Neuroscience. 16. 744-751.

Lovenberg, T.W., Baron, B.M., de Lecea, L., Miller, J.D., Prosser, R.A., Rea, M.A., Foye, P.E., Racke, M., Alone, A.L., Siegel, B.W., Danielson, P.E., Sutcliffe, J.G., & Erlander, M.G. (1993). A novel cyclase-activating serotonin receptor (5-HT7) implicated in the regulation of mammalian drcadian rhythms. Neuron, 11, 449-458.

Lumia, A.R., Teicher, M.H., Salchli, F., Ayers, E., & Possidente, B. (1992). Olfactory bulbectomy as a model for agitated hyposerotonergic depression. Brain Research, 587, 181-l 85.

Maccan, S., Koehl, M., LeMoal, M., Dulluc, J., Olivares, E., Turek, F.W., & Van Reeth, 0. (1997). Prenatal stress induces an advance of both corticosterone and locomotor activity rhythms in adult female rats. Society for Neuroscience Abstract, 23, 1327.

Marchant, E.G. & Mistlberger, R.E. (1996). Entrainment and phase shifting of circadian rhythms in mice by forced treadmill running. Physiology & Behavior, 60, 657-663.

Marchant, E.G. Watson, N.V., & Mistlberger, R.E. (1997). Both neuropeptide Y and serotonin are necessary for entrainment of circadian rhythms in mice by daily treadmill running schedules. The Journal of Neuroscience, 17, 7974-7987.

Maudhuit, C., Hamon, M. & Adrien, J. (1996). Effects of chronic treatment with zimeldine and REM sleep deprivation on the regulation of raphe neuronal activity in a rat model of depression. Psychopharmacology, 174: 267-274.

Maudhuit, C., Hamon, M., & Adrien, J. (1995). Electrophysiological activity of raphe dorsalis serotonergic neurones in a possible model of endogenous depression. NeuroReport, 6, 681-684.

Mazer, C., Muneyyirci, J., Taheny, K., Raio, N., Borella, A., & Whitaker-Azmitia, P. (1997). Serotonin depletion during synaptogenesis leads to decreased synaptic density and learning deficits in the adult rat: A possible model of neurodevelopmental disorders with cognitive deficits. Brain Research, 760, 68-73.

Mead, S., Ebling, F.J.P., Maywood, E.S., Humby, T., Herbert, J., & Hastings, M.H. (1992). A nonphotic stimulus causes instantaneous phase advances of the light-entrainable circadian oscillator of the Syrian hamster but does not induce the expression of c-fos in the suprachiasmatic nuclei. Journal of Neuroscience, 13 25162522.

Medanic, M., & Gillette, M.U. (1992). Serotonin regulates the phase of the rat suprachiasmatic circadian pacemaker in vitro only during the subjective day. Journal of Physiology, 450, 629-642.

Meerlo, P., & Daan, S. (1998). Aggressive and sexual social stimuli do not phase shift the circadian temperature rhythm in rats. Chronobiology Intern- 231-40.

Meijer, J.H. (1991). Integration of visual information by the suprachiasmatic nucleus. In: D.C. Klein, R.Y. Moore, and S.M. Reppert. (Eds.), The Suprachiasmatic Nucleus: The Mind's Clock. (pp.1 07-l 19). New York: Oxford.

Meijer, J.H., Van Der Zee, E., & Dietz, M. (1988). The effects of intraventricular carbachol injections on the free-running activity rhythm of the hamster. Journal of Rhythms, 3, 333-348.

Meijer, J.H., Watanabe, K., Schaap, J., Albus, H., & Detari, L. (1998). Light responsiveness of the suprachiasmatic nucleus:Long-term multiunit and single-unit recordings in freely moving rats. The Journal of Neuroscience, 18, 9078-9087.

Meyer, E.L., Harrington, M.E., & Rahmani, T. (1993). A phase-response curve to the benzodiazepine chlordiazepoxide and the effect of geniculo-hypothalamic tract ablation. Physiology & Behavior, 53, 237-243.

Meyer-Bernstein, E.L., & Morin, L.P. (1996). Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation. Journal of Neuroscience, 16, 2097-2111.

Miller, J.D., & Fuller, C. (1990). The response of suprachiasmatic neurons of the rat hypothalamus to photic and serotonergic stimulation. Brain Research, 515, 155-162.

Miller, J.D., Morin, L.P., Schwartz, W.J., & Moore, R.Y. (1996). New insights into the mammalian circadii clock. Sleep, 19, 641-667.

Miller, J.D., Murakami, D.M., & Fuller, C.A. (1987). The response of suprachiasmatic neurons of the rat hypothalamus to photic and nicotinic stimuli. Journal of Neuroscience, 7, 978-986.

Mintz, E.M., & Albers, H.E. (1997). Microinjection of NMDA into the SCN region mimics the phase shifting effect of light in hamsters. Brain Research, 758,245249.

Mirmiran, M. (1986). The role of the central monoaminergic system and rapid eye movement sleep in development. Brain & Development, 8, 382-389.

Mirmiran, M., Scholtens, J., Van de Poll, N.E., Uylings, H.B., Van Der Gugten, J., & Boer, G.J. (1983). Effects of experimental suppression of active (REM) sleep during early development upon adult brain and behavior in the rat. Brain Research, 283, 277-286.

Mirmiran, M. & Van Someren, E. (1993). The importance of REM sleep for brain maturation. Journal of Sleep Research, 2:188-192.

Mistlberger R.E. (1991). Effects of daily schedules of forced activity on free-running rhythms in the rat. Journal of Biological Rhythms, 6, 71-80.

Mistlberger, R.E., & Antle, M.C. (1997). Behavioral inhibition of light-induced circadian phase resetting is phase and serotonin dependent. Brain Research, 786, 31-38.

Mistlberger, R.E., Houpt, T.A., & Moore-Ede, M.C. (1991). The benzodiazepine triazolam phase-shifts circadian activity rhythms in a diurnal primate, the squirrel monkey (*Saimiri sciureus*). Neuroscience Letters, 124, 27-30.

Mistlberger, R.E., Marchant, E.G., & Sinclair, S.V. (1996). Nonphotic phase shifting and the motivation to run: Cold exposure reexamined. Journal of Biological Rhythms, 11, 208-215.

Moga, M.M. & Moore, R.Y. (1997). Organization of neural inputs to the suprachiasmatic nucleus in the rat. Journal of Comparative Neurology, 389, 508534.

Moga, M.M., Weis, R.P., & Moore, R.Y. (1996). Paraventricular thalamic nucleus projections in the rat. Journal of Comparative Neurology, 359, 221-238.

Moore, R.Y. (1992). The enigma of the geniculohypothalamic tract: Why two visual entraining pathways? Journal of Interdisciplinary Cycle Research, 23, 144-152.

Moore, R.Y. (1995). Organization of the mammalian circadian system. In D.J. Chadwick and K. Ackrill (Eds.), Circadian clocks and their adjustment, Ciba Foundation Symposium, 183, (pp. 88-106). Chichester: Wiley.

Moore, R.Y. (1996). Entrainment pathways and the functional organization of the circadian system. in: R.M. Buijs, A. Kalsbeek, H.J. Romijn, C.M.A. Pennartz, and M. Mirmiran (Eds.), Progress in Brain Research, Volume 11 Hypothalamic Integration of Circadian Rhythms. (pp. 103-119). Amsterdam: Elsevier.

Moore, R.Y., & Card, J.P. (1994). Intergeniculate leaflet: An anatomically and functionally distinct subdivision of the lateral geniculate complex. The Journal of Comparative Neurology, 344, 403-430.

Moore, R.Y., Halaris, A.E., & Jones, B.A. (1978). Serotonin neurons from the midbrain raphe: Ascending projections. Journal of Comparative Neurology, 180, 417-438.

Moore, R.Y., & Speh, J.C. (1993). GABA is the principal neurotransmitter of the circadian system. Neuroscience Letters, 150, 112-116.

Moore, R.Y., Speh, J.C., & Card, J.P. (1995). The retinohypothalamic tract originates from a distinct subset of retinal ganglion cells. Journal of Comparative Neurology, 352, 351-366.

Moore-Ede, M.C., Sulzman, F.M., & Fuller, C.A. (1982). The Clocks That Time Us. Cambridge: Harvard University Press.

Morin, L.P. (1991). Neural control of circadian rhythms as revealed through the use of benzodiazepines. In: D.C. Klein, R.Y. Moore, and SM. Reppert. (Eds.), The Suprachiasmatic Nucleus: The Mind's Clock. (pp. 324-338). New York: Oxford.

Morin, L.P. (1994). The circadian visual system. Brain Research Review- 102-127.

Morin, L.P. (1999). Serotonin and the regulation of mammalian circadian rhythmiclty. Annals of Medicine, 31, 12-33.

Morin, L.P. & Blanchard, J, (1991), Depletion of brain serotonin by 5,7-DHT modifies hamster circadian response to light. Brain Research, 566, 173-185.

Moyer, R. W., Kennaway, D. J., Ferguson, S.A., & Dijstelbloem, Y.P. (1997). Quipazine and light have similar effects on c-fos induction in the rat suprachiasmatic nucleus. Brain Research, 765, 337-342.

Mrosovsky N. (1988). Phase response curves for social entrainment. Journal of Comparative Physiology A, 162, 35-46.

Mrosovsky, N. (1995). A non-photic gateway to the circadian clock of hamsters. In: D.J. Chadwick and K. Ackrill (Eds.), Circadian clocks and their adjustment. Ciba Foundation Symposium, 183, (pp. 154-174). Chichester: Wiley.

Mrosovsky, N. (1996). Locomotor activity and non-photic influences on circadian clocks. Biological Reviews, 71, 343-372.

Mrosovsky, N. & Biello, S.M. (1994). Nonphotic phase-shifting in the old and the cold. Chronobiology International, 11, 232-252.

Mrosovsky, N., Reebs, S.G., Honrado, G.I., & Salmon, P.A. (1989). Behavioral entrainment of circadian rhythms. Experientia, 45, 696702.

Mrosovsky, N., & Salmon, P.A. (1990). Triazolam and phase-shifting acceleration re-evaluated. Chronobiology International, 7, 35-41.

Murakami, N., Takahashi, K., & Kawashima, K. (1984). Effect of light on the acetylcholine concentrations of the suprachiasmatic nucleus in the rat. Brain Research, 311, 358-360.

Neill, D., Vogel, G.W., Hagler, M., Kors, D., & Hennessey, A. (1990). Diminished sexual activity in a new animal model of endogenous depression. Neuroscience & Biobehavioral Reviews, 14: 73-76.

Nelson, D.E., & Takahashi, J.S. (1991). Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (*Mesocricetus auratus*). Journal of Physiology, 439115-145.

Nunez, J.F., Ferre, P., Escorihuela, R.M., Tobena, A., Fernandez-Teruel, A. (1996). Effects of postnatal handling of rats on emotional, HPA-axis, and prolactin reactivity to novelty and conflict. Physiology & Rehavior. 60. 1355-9.

Ogawa, T., Mulini, M., Kuroda, Y., Muneoka, K., Mori, K., & Takahashi, K. (1994). Periodic maternal deprivation alters stress responses in adult offspring:Potentiates the negative feedback regulation of restraint stress-induced adrenocortical response and reduced the frequencies of open field-induced behaviors. Pharmacology. Biochemistry & Behavior. 49. 961-967.

O'Hara, B.F., Edgar, D.M., Cao, V.H., Wiler, S.W., Heller, H.C., Kilduff, T.S., & Miller, J.D. (1998). Nicotine and nicotinic receptors in the circadian system. Psychoneuroendocrinology. 23. 161-l 73.

Overstreet, D.H. (1986). Selective breeding for increased cholinergic function:Development of a new animal model for depression. Biological Psychiatry. 21. 49-58.

Overstreet, D.H., Russell, R.W., Crocker, A.D., & Schiller, G.D. (1984). Selective breeding for differences in cholinergic function:Pre- and postsynaptic mechanisms involved in sensitivity to the anticholinesterase, DFP. Brain Research. 294. 327-332.

Pauly, J.R., & Horseman, N.D. (1985). Anticholl nergic agents do not block light-induced circadian phase shifts. . Brain Research. 348. 163-167.

Penev, P.D., Turek, F.W. & Zee, P.C. (1993). Monoamine depletion alters the entrainment and the response to light of the circadian activity rhythm in hamsters. Brain Research. 612,156-l 64.

Penev, P.D., Turek, F.W. & Zee, P.C. (1995). A serotonin neurotoxin attenuates the phase-shifting effects of triazolam on the circadian dock in hamsters. Brain Research. 669, 207-216.

Penev, P.D., Zee, P.C., & Turek, F.W. (1994). Monoamine depletion blocks triazolam-induced phase advances of the circadian clock in hamsters. Brain Research, 637, 255-261.

Penev, P.D., Zee, P.C., & Turek, F.W. (1997). Serotonin in the spotlight. Nature. 385, 123.

Peters, D.A.V. (1984). Prenatal stress:Effects on development of rat brain adrenergic receptors. Pharmacology, Biochemistry & Behavior, 21, 417-422.

Peters, D.A.V. (1986). Prenatal stress:Effect on development of rat brain serotonergic neurons. Pharmacology, Biochemistry & Behavior, 74, 1377-l 382.

Peters, D.A.V. (1990). Maternal stress increases fetal brain and neonatal cerebral cortex 5-hydroxytryptamine synthesis in rats:A possible mechanism by which stress influences brain development. Pharmacology, Biochemistry & Behavior, 35, 943-947.

Peters, R.V., Aronin, N., & Schwartz, W.J. (1996). C-Fos expression in the rat intergeniculate leaflet:Photic regulation, co-localization with Fos-B, and cellular identification. Brain Research, 728, 231-241.

Pickard, G.E. (1985). Bifurcating axons of retinal ganglion cells terminate in the suprachiasmatlc nucleus and the intergeniculate leaflet of the thalamus. Neuroscience Letters, 55, 21 l-217.

Pickard, G.E., Ralph, MR., & Menaker, M. (1987). The intergeniculate leaflet partially mediates the effects of light on circadian rhythms. Journal of Biological Rhythms, 2, 35-56.

Pickard, G.E., Weber, E.T., Scott P.A., Riberdy, A.F., & Rea, M.A. (1996). 5-HT_1B receptor agonists inhibit light-induced phase shifts of behavioral circadian rhythms and expression of immediate-early gene c-fos in the suprachiasmatic nucleus. Journal of Neuroscience, 16, 8208-8220.

Pieper, D.R., & Lobocki, C.A. (1991). Olfactory bulbectomy lengthens circadian period of locomotor activity in golden hamsters. American Journal of Physiology, 261, R973-R978.

Piggins, H.D., & Rusak, B. (1997). Effects of microinjections of substance P into the suprachiasmatic nucleus region on hamster wheel-running rhythms. Brain Research Bulletin, 42, 451-455.

Pittendrigh, C. (1981). Circadian systems:Entrainment. In: J. Aschoff (Ed.) Handbook of Behavioral Neurology, Volume 4, Biological Rhythms. New York:Plenum, pp. 95-124.

Pittendrigh, C., & Daan, S. (1976a). A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase-response curves. Journal of Comparative Physiology, 106, 253-266.

Pittendriih, C., & Daan, S. (1976b). A functional analysis of circadian pacemakers in nocturnal rodents. IV. Entrainment:Pacemaker as clock. Journal of Comparative Physiology, 106, 291-331.

Pittendrigh, C., & Daan, S. (1976c). A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker structure:A clock for all seasons. Journal of Comparative Physiology, 106, 333-355.

Possidente, B., Lumia, A.R., McEldowney, S. & Rapp, M. (1992). Fluoxetine shortens circadian period for wheel-running activity in mice. Brain Research Bulletin, 28: 629631.

Possidente, B., Lumia, A.R., McGinnis, M.Y., Rapp, M., & McEldowney, S. (1996). Effects of fluoxetine and olfactory bulbectomy on mouse circadian activity rhythms. Brain Research, 713, 108-113.

Possidente, B., Lumia, A.R., McGinnis, M.Y., Teicher, M.H., deLemos, E., Sterner, L., & Deros, L. (1990). Olfactory bulb control of circadian activity rhythm in mice. Brain Research. 513.325328.

Prosser., R.A., Dean, R.R., Edgar, D.M., Heller, H.C. & Miller, J.D. (1993). Serotonin and the mammalian circadian system:I. In vitro phase shifts by serotonergic agonists and antagonists. Journal of Biological Rhythms, 8, 1-16.

Prosser, R.A., & Gillette, M.U. (1989). The mammalian circadian clock in the suprachiasmatic nuclei is reset in vitro by cAMP. Journal of Neuroscience, 9, 1073-1081.

Prosser, R.A., Heller, H.C. & Miller, J.D. (1992). Serotonergic phase shifts of the mammalian circadian clock: Effects of tetrodotoxin and high Mg^{2+}. Brain Research, 573, 336-340.

Prosser, R.A., Miller, J.D., & Heller, H.C. (1990). A serotonin agonist phase-shifts the circadian clock in the suprachiasmatic nuclei in vitro. Brain Research, 534 336-339.

Ralph, M.R. (1991). Suprachiasmatic nucleus transplant studies using the *Tau* mutation in golden hamsters. In: D.C. Klein, R.Y. Moore, and S.M. Reppert. (Eds.), The Suprachiasmatic Nucleus: The Mind's Clock. (pp.341 -348). New York:Oxford.

Ralph, M.R., Foster, R.G., Davis, F.C., & Menaker, M. (1990). Transplanted suprachiasmatic nucleus determines circadian period. Science, 747,975978.

Ralph, M.R., Hurd, M.W., Takeuchi, J., Melo, L., Mathur, A., & Golombek, D.A. (1996). Regulation and integration in the mammalian circadian system. In: R.M. Buijs, A. Kalsbeek, H.J. Romijn, C.M.A. Pennartz, and M. Mirmiran (Eds.), Progress in Brain Research, Volume 11, Hypothalamic Integration of Circadian Rhythms. (pp. 191-204). Amsterdam:Elsevier.

Ralph, M.R., & Mrosovsky, N. (1992). Behavioral inhibition of circadian responses to light. Journal of Biological Rhythms, 7, 353-359.

Rea, M.A. (1989). Light increases Fos-related protein immunoreactivity in the rat suprachiasmatfcnuclei. Brain Research Bulletin, 23, 577-581.

Rea, M.A., Barrera, J., Glass, J.D. & Gannon, R.L. (1995). Serotonergic potentiation of photic phase shifts of the circadian activity rhythm. NeuroReport, 6:1289-1292.

Rea, M.A., Buckley, B., & Lutton, L.M. (1993). Local administration of EAA antagonists blocks light-induced phase shifts and c-fos expression in the hamster SCN. American Journal of Physiology, 265, R1191-R1198.

Rea, M.A., Glass, J.D., & Colwell, C.S. (1994). Serotonin modulates photic responses in the hamster suprachiasmatic nuclei. Journal of Neuroscience, 14, 3635-3642.

Reebs, S.G., Lavery, R.J., & Mrosovsky, N. (1989). Running activity mediates the phase-advancing effects of dark pulses on hamster circadian rhythms. Journal of Comparative Physiology, A, 165, 81 l-81 8.

Reebs, S.G., & Mrosovsky, N. (1989). Effects of induced wheel running on the circadian activity rhythm of Syrian hamsters:Entrainment and phase-response curve. Journal of Biological Rhythms, 4, 39-48.

Reppert, S.M. (1992). Prenatal development of a hypothalamic biological clock. Progress in Brain Research, 93, 119-131.

Richardson, J.S. (1991). Animal models of depression reflect changing views on the essence and etiology of depressive disorders in humans. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 15, 199-204.

Rosenwasser, A.M. (1989). Effects of chronic clonidine administration and withdrawal on free-running circadian activity rhythms. Pharmacology,Biochemistry & Behavior, 33: 291-297.

Rosenwasser, A.M. (1990). Free-running circadian activity rhythms during long-term clonidine administration in rats. Pharmacology, Biochemistry & Behavior, 35, 35-39.

Rosenwasser, A.M. (1996). Clonidine shortens free-running circadian period in both constant light and constant darkness. Physiology & Behavior, 60, 373-379.

Rosenwasser, A.M., & Adler, N.T. (1986). Structure and function in circadian timing systems:Evidence for multiple coupled circadian oscillators. Neuroscience and Biobehavioral Reviews, 10, 431-448.

Rosenwasser, A.M., & Hayes, M.J. (1994). Neonatal desipramine treatment alters free-running circadian drinking rhythms in rats. Psychopharmacology, 115: 237-244.

Rosenwasser, A.M., Vogt, L.J., Pellowski, M.W. (1995). Circadian phase shifting induced by clonidine injections in Syrian hamsters. Biological Rhythm Research, 26, 1-19.

Rosenwasser, A.M., & Wirz-Justice, A. (1997). Circadian rhythms and depression:Clinical and experimental models. In P.H. Redfem and B. Lemmer (Eds.), Physiology and Pharmacology of Biological Rhythms, (pp.457-486). Berlin:Springer-verlag.

Rusak, B. (1977). The role of the suprachiasmatic nuclei in the generation of circadian rhythms in the golden hamster, *Mesocricetus auratus*. Journal of Comparative Physiology, 118, 145-164.

Rusak, B., Meijer, J.H., & Harrington, M.E. (1989). Hamster circadian rhythms are phase-shifted by electrical stimulation of the geniculohypothalamic tract. Brain Research, 493, 283291.

Schwartz, W.J., Gross, R.A., & Morton, M.T. (1987). The suprachiasmatic nuclei contain a tetrodotoxin-resistant circadian pacemaker. Proceedings of the National Academy gf Sciences. USA. 84, 1694-I 698.

Selim, M., Glass, J.D., Hauser, U.E., & Rea, M.A. (1993). Serotonergic inhibition of light-induced fos protein expression and extracellular glutamate in the suprachiasmatic nuclei. Brain Research, 621, 181-I88.

Shibata, S., Tsuneyoshi, A., Hamada, T., Tominaga, K., & Watanabe, S. (1992a). Effect of substance P on circadian rhythms of firing activity and 2-deoxyglucose uptake in the rat suprachiasmatic nucleus in vitro. Brain Research, 597, 257-263.

Shibata, S., Tsuneyoshi, A., Hamada, T., Tominaga, K., & Watanabe, S. (1992b). Phase-resetting effect of 8-OH-DPAT, a serotoninl A receptor agonist, on the circadian rhythm of firing rate in the rat suprachiasmatic nuclei in vitro. Brain Research, 582, 353-356.

Shimomura, K. & Menaker, M. (1994). Light-induced phase shifts in tau mutant hamsters. Journal of Biological Rhythms, 9, 97-110.

Shiori, T., Takahashi, K., Yamada, N. & Takahashi, S. (1991). Motor activity correlates negatively with free-running period, while positively with serotonin contents in SCN in free-running rats. Physiology & Behavior, 49: 779-786.

Shirakawa, T., & Moore, R.Y. (1994). Glutamate shifts the phase of the circadian neuronal firing rhythm in the rat suprachiasmatic nucleus in vitro. Neuroscience Letters, 178, 47-50.

Shiromani, P.J., Klemfuss, H., Lucero, S. & Overstreet, D.H. (1991). Diurnal rhythm of core body temperature is phase advanced in a rodent model of depression. Biological Psychiatry, 29, 923-930.

Shiromani, P.J., & Overstreet, D.H. (1994). Free-running period of circadian rhythms is shorter in rats with a genetically upregulated central cholinergic system. Biological Psychiatry, 36, 622-626.

Shiromani, P.J., Overstreet, D.H., Levy, D., Goodrich, C.A., Campbell, S.S., & Gillin, J.C. (1988). Increased REM sleep in rats selectively bred for cholinergic hyperactivity. Neuropl,/chopharmacology. 3 3 .

Smale, L., Michels, K.M., Moore, R.Y., & Morin, L.P. (1990). Destruction of the hamster serotonergic system by 5,7-DHT: Effects on circadian rhythm phase, entrainment and response to triazolam. Brain Research. 515, 9-19.

Smith, RD., Turek, F.W., & Takahashi, J.S. (1992). Two families of phase-response curves characterize the resetting of the hamster circadian clock. American Journal of Physiology. 262. R1149-R1153.

Souetre, E., Salvati, E., Belugou, J.L., Pringuey, D., Candito, M., Krebs, B., Ardisson, J.L. & Dar-court, G. (1989). Circadian rhythms in depression and recovery: Evidence for blunted amplitude as the main chronobiological abnormality. Psychiatric Research. 28: 263-278.

Spear, L.P., & File, S.E. (1996). Methodological considerations in neurobehavioral teratology. Pharmacology Biochemistry & Behavior. 55. 455-457.

Srkalovic, G., Selim, M., Rea, M.A., & Glass, J.D. (1994). Serotonergic inhibition of extracellular glutamate in the suprachiasmatic nuclear region assessed using in vivo brain microdialysis. Brain Research 656. 302-308.

Stephan F. K. (1984). Phase shifts of circadian rhythms in activity entrained to food access. Physiology & Behavior. 32, 663-71.

Stephan, F.K., & Zucker, I. (1972). Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesion. Proceedings of the National Academy of Sciences. USA. 69, 1583-l 586.

Stewart, K.T., Rosenwasser, A.M., Hauser, H., Volpicelli, J.R., & Adler, N.T. (1990). circadian rhythmicity and behavioral depression I: Effects of stress. Physiology & Behavior. 48, 149-l 55.

Summer, T., Ferraro, J., & McCormack, C. (1984). Phase-response and Aschoff illuminance curves for locomotor activity rhythm of the rat. American Journal of Physiology. 246. R299-R304.

Sumova, A., Maywood, E.S., Selvage, D., Ebling, F.J.P., & Hastings, M.H. (1996). Serotonergic antagonists impair arousal-induced phase shifts of the circadian system of the Syrian hamster. Brain Research.709. 88-96.

Takatsuji, K., Miguel-Hidalgo, J.J., & Tohyama, M. (1991). Substance P-immunoreactive innervation from the retina to the suprachiasmatic nucleus in the rat Brain Research.568.223-229.

Tominaga, K., Shibata, S., Ueki, S. & Watanabe, S. (1992). Effects of 5-HT_{1A} receptor agonists on the circadian rhythm of wheel-running activity in hamsters. European JournalofPharmacology.314: 79-84.

Trachsel, L., Heller, H.C., & Miller, J.D. (1995). Nicotine phase-advances the circadian neuronal activity rhythm in rat suprachiasmatic nuclei explants. Neuroscience. 65. 797-803.

Treep, J.A., Abe, H., Rusak, B., & Goguen, D.M. (1995). Two distinct retinal projections to the hamster suprachiasmatic nucleus. JournalofBiological Rhythms.10. 299307.

Turek, F.W., Pinto, L.H., Vitaterna, M.H., Penev, P.D., Zee, P.C., & Takahashi, J.S. (1995). Pharmacological and genetic approaches for the study of circadian rhythms in mammals. FrontiersinNeuroendocrinology. 16.191-223.

Vagell, M.E., McGinnis, M.Y., Possidente, BP, Narasimhan, V.N., & Lumia, A.R. (1991). Olfactory bulbectomy increases basal suprachiasmatic cyclic AMP levels in male rats. BrainResearch Bulletin. 27.839-842.

Van Reeth, O., & Turek, F.W. (1989). Stimulated activity mediates phase shifts in the hamster circadian clock induced by dark pulses or benzodiazepines. Nature. 339.49-51.

Velazquez-Moctezuma, J. & Ruiz, O.D. (1992). Neonatal treatment with clomipramine increased immobilii in the forced swim test:An attribute of animal models of depression. Pharmacology. Biochemistry & Behavior. 42: 737-739.

Vindlacheruvu, RR., Ebling, F.J.P., Maywood, ES., & Hastings, M.H. (1992). Blockade of glutamatergic neurotransmission in the suprachiasmatlc nucleus prevents cellular and behavioral responses of the circadian system to light. European Journal of Neuroscience. 4.673-679.

Vogel, G., Hagler, M., Hennessey, A., & Richard, C. (1996). Dose-dependent decrements in adult male rat sexual behavior after neonatal chlorimipramine treatment. Pharmacology Biochemistry & Behavior. 54:605-609.

Vogel, G., Hartley, P., Neill, D., Hagler, M., & Kors, D. (1966). Animal depression model by neonatal clomipramine:Reduction of shock induced aggression. Pharmacology, Biochemistry & Behavior. 31.103-106.

Vogel, G.W., Minter, K., & Woolwine, B. (1986). Effects of chronically administered antidepressant drugs on animal behavior. Physiology & Behavior. 36.659666.

Vogel, G., Neill, D., Hagler, M., & Kors, D. (1990). A new animal model of endogenous depression:A summary of present findings. Neuroscience & Biobehavioral Reviews. 14: 85-91.

Vogel, G., Neill, D., Hagler, M., Kors, D., & Hartley, P. (1990). Decreased intracranial self stimulation in a new animal model of endogenous depression. Neuroscience & Biobehavioral Reviews. 14.65-68.

Vogel, G., Neill, D., Kors, D., & Hagler, M. (1990). REM sleep abnormalities in a new animal model of endogenous depression. Neuroscience & Biobehavioral Reviews. 14, 77-83.

Vogel, G.W., Vogel, F. (1982). A new animal model of human endogenous depression. Sleep Research.11. 222a.

Wakshlak, A., & Weinstock, M. (1990). Neonatal handling reverses behavioral abnormalities induced in rats by prenatal stress. Physiology & Behavior. 48. 289-292.

Wang, H., & Morris, J.F. (1996). Presence of neuronal nitric oxide synthase in the suprachiasmatic nuclei of mouse and rat. Neuroscience. 74. 1059-l 068.

Watts, A.G. (1991). The efferent projections of the suprachiasmatic nucleus:Anatomical insights into the control of circadian rhythms. In D.C. Klein, R.Y Moore, and S.M. Reppert. (Eds.), Jhe Suprachiasmatic Nucleus-The Mind's Clock. (pp. 77-106). New York:Oxford.

Weber, E.T., Gannon, R.L., Michel, A.M., Gillette, M.U., & Rea, M.A. (1995). Nitric oxide inhibitor blocks light-induced phase shifts of the circadian activity rhythm, but not c-fos expression in the suprachiasmatic nucleus of the Syrian hamster. Brain Research, 692. 137-142.

Weber, E.T., & Rea, M.A. (1997). Neuropeptide Y blocks light-induced phase advances but not delays of the circadian activity rhythm in hamsters. Neuroscience Letters, 231. 159162.

Wee, B.E., & Turek, F.W. (1989). Carbachol phase shifts the circadian rhythm of locomotor activity in the Djungarian hamster. Brain Research, 505. 209-214.

Wehr, T.A., & Wirz-Justice, A. (1982). Circadian rhythm mechanisms in affective illness and in antidepressant drug action. Pharmacopsychiatry, 15. 31-39.

Weinstock, M., Fride, E., & Hertzberg, R. (1988). Prenatal stress effects on functional development of the offspring. Progress in Brain Research, 73. 319-331.

Welsh, D.K., Logothetis, D.E., Meister, M., & Reppert, S.M. (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing patterns. Neuron, 14. 697-706.

Wickland, C., & Turek, F.W. (1991). Phase shifting effects of acute increases in activity on circadian locomotor rhythms in hamsters. American Journal of Physiology, 261. R1109-R1117.

Wickland, C., & Turek, F.W. (1994). Lesions of the thalamic intergeniculate leaflet block activity-induced phase shifts in the circadian activity rhythm of the golden hamster. Brain Research, 660.293-300.

Willner, P. (1984). The validity of animal models of depression. Psychopharmacology, 83. I-16.

Willner, P. (1997). The chronic mild stress procedure as an animal model of depression: Valid, reasonably reliable, and useful. Psychopharmacology, 134, 371-377.

Wirz-Justice, A. & Campbell, I.C. (1982). Antidepressant drugs can slow or dissociate circadian rhythms. Experientia, 38:1301-1309.

Wollnik, F. (1992). Effects of chronic administration and withdrawal of antidepressant agents on circadian activity rhythms in rats. Pharmacology Biochemistry & Behavior, 43, 549-561.

Wollnik, F., Brysch, W., Uhlmann, E., Gillardon, F., Bravo, R., Zimmerman, M., Schlingensiepen, K.H., & Hergegen, T. (1995). Block of c-Fos and JunB expression by antisense oligonucleotides inhibits light-induced phase shifts of the mammalian circadian clock. European Journal of Neuroscience, 7, 388-393.

Wu, J.C., & Bunney, W.E. (1990). The biological basis of an antidepressant response to sleep deprivation and relapse: Review and hypothesis. American Journal of Psychiatry, 147, 14-21.

Yamada, N., Shimoda, K., Takahashi, K., & Takahashi, S. (1990). Relationship between free-running period and motor activity in blinded rats. Brain Research Bulletin, 25, 115-119.

Yannielli, P.C., Cadeiras, M., Cardinali, D.P. & Golombek, D.A. (1998). Effects of acute clomipramine administration on Syrian hamster circadian rhythms. Society for Research on Biological Rhythms Abstract.

Yannielli, P.C., Cutrera, R.A., Cardinali, D.P., & Golombek, D.A. (1998). Neonatal clomipramine treatment of Syrian hamsters: Effect on the circadian system. European Journal of Pharmacology, 349, 143-150.

Yavari, P., Vogel, G.W. & Neill, D.B. (1993). Decreased raphe unit activity in a rat model of endogenous depression. Brain Development, 611:31-36.

Zatz, M., & Herkenham, M.A. (1981). Intraventricular carbachol mimics the phase-shifting effect of light on the circadian rhythm of wheel-running activity. Brain Research, 212, 234-238.

Zorrilla, E.P. (1997). Multiparous species present problems (and possibilities) to developmentalists. Developmental Psychobiology, 30, 141-150.

BIOGRAPHY OF THE AUTHOR

Suzanne M. Dwyer was born in Sydney, Nova Scotia on May 25, 1971. She graduated from Sydney Academy High School in 1989, and then attended Saint Mary's University in Halifax, Nova Scotia. She graduated with a Bachelor of Science, majors in Biology and Psychology, in 1998. The following year, she returned to Saint Mary's and completed an honors year in Psychology . In 1994, she entered the doctoral program in Experimental Psychology at the University of Maine. She is a candidate for the Doctor of Philosophy degree in Psychology from the University of Maine, in December, 2000.