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It is well established that the mammalian suprachiasmatic nucleus (SCN) is a biological pacemaker which entrains the activity of organisms to their environment and controls circadian rhythmicity. However, neither the nature of these coupling signal(s) from the SCN, nor their target(s) in the brain are well understood. Fiber efferents from the SCN reach nearby hypothalamic regions, suggesting a coupling role for neural efferent pathways. The SCN produces diffusible signals that reach nearby hypothalamic sites and the cerebrospinal fluid, suggesting a role for a diffusible efferent pathway. We consider the possibility of redundant coupling signals of the SCN, and review evidence suggesting that diffusible elements may be sufficient to sustain locomotor rhythmicity in adult animals and to restore locomotor rhythmicity in lesioned hamsters bearing SCN grafts. We also provide data for the occurrence of signals that synchronize oscillators in irrespective of initial phase. The distinct role of neural and diffusible SCN coupling signals, and the role of SCN-driven systems (pineal melatonin rhythms, body temperature) remains to be explored.

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Efferent Signals of the Suprachiasmatic Nucleus

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Key Words: SCN coupling signals, diffusible signals, ^{14}C-deoxyglucose, hamsters, hypothalamic "island", neural efferents, neural grafts, pacemaker cells, SCN, SCN transplants

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Abstract: It is well established that the mammalian suprachiasmatic nucleus (SCN) is a biological pacemaker which entrains the activity of organisms to their environment and controls circadian rhythmicity. However, neither the nature of these coupling signal(s) from the SCN, nor their target(s) in the brain are well understood. Fiber efferents from the SCN reach nearby hypothalamic regions, suggesting a coupling role for neural efferent pathways. The SCN produces diffusible signals that reach nearby hypothalamic sites and the cerebrospinal fluid, suggesting a role for a diffusible efferent pathway. We consider the possibility of redundant coupling signals of the SCN, and review evidence suggesting that diffusible elements may be sufficient to sustain locomotor rhythmicity in adult animals and to restore locomotor rhythmicity in lesioned hamsters bearing SCN grafts. We also provide data for the occurrence of signals that synchronize oscillators irrespective of initial phase. The distinct role of neural and diffusible SCN coupling signals, and the role of SCN-driven rhythmic systems (pineal melatonin rhythms, body temperature) remains to be explored.

Introduction: The suprachiasmatic nucleus (SCN) of the mammalian brain is a biological clock that controls circadian rhythmicity in a very broad array of physiological and behavioral responses. In a widely used conceptualization, pacemaker cells lie within the SCN, and signals from pacemakers reach target regions by neural efferents. In fact, the nature of the efferent signals from the SCN is obscure. A fairly conservative approach suggests that if an output pathway is present, it probably serves a function. This argument suggests that we might have to reconceptualize our model of the efferent signals of the SCN to include two fundamentally distinct types of mechanisms, neural and humoral, whereby output signals of the SCN can reach targets (Fig 1).

Neural efferents: The first type of efferent pathway involves neural projections of the SCN. Very briefly, Watts (1991) describes a projection pattern with six anatomical components that projecting to intra- and extra-hypothalamic targets in the rat. The major projection of fibers, and the major terminal field for SCN efferents is the subparaventricular zone This is part of a multisynaptic pathway thought to regulate pineal melatonin rhythms. The function of the remaining five efferent components is unknown.

Diffusible efferents: The second type of efferent pathway involves a diffusible signal(s) from the SCN. For example, the SCN has a circadian rhythm of arginine vasopressin (VP) expression, and SCN neurons are the source of the circadian rhythm of VP in the CSF (for review, see Majzoub, Robinson, and Emanuel, 1991). VP exhibits a prominent daily rhythm in the CSF in several mammals. A circadian rhythm of VP release is seen in SCN explants *in vitro* (Earnest and Sladek, 1986), and transplantation of fetal SCN into VP-deficient Brattleboro rats, normally lacking in this peptide,

results in normal diurnal variation in CSF VP levels (Earnest et al., 1989). The rhythm is abolished following SCN lesions, but survives transection of most SCN efferents, with a damped amplitude (Reppert, Schwartz and Uhl, 1987). It should be noted that the precise function of VP secretion by the SCN is unknown. It is not thought however, to mediate the expression of circadian rhythmicity (for review see Majzoub et al., 1991; Reppert et al., 1987).

The foregoing studies indicate that cells of the SCN can release peptides into the extracellular fluid and the CSF, providing evidence that neurons of this nucleus can release diffusible signals. Circadian rhythms have been described in a host of other neuropeptides and neuromodulators, providing a plethora of candidate output signals of pacemaker cells (Takeuchi et al., 1992; Shinohara et al., 1991). The function of these and other as yet unidentified potential diffusible signals remains to be explored.

Efferents modulating hamster locomotor rhythms: Several lines of evidence suggest indirectly that a diffusible signal(s) from the SCN can sustain locomotor rhythmicity in hamsters. Previous work in our laboratory and others demonstrates that SCN-lesioned hamsters recover locomotor rhythmicity following grafting of anterior hypothalamic tissue containing the SCN into the third ventricle (reviewed in Lehman et al., 1991). In such grafts, few if any neural connections between graft and host can be demonstrated (Lehman et al., 1987). SCN grafts are functional irrespective of their precise attachment sites at various loci in the third ventricle or in the lateral ventricle near the foramen of Monro. This is important as the grafted SCN makes local connections with normal targets that lie near the attachment site of the graft, but no single graft reestablishes all normal efferent connections (Canbeyli et al., 1991;

Wiegand and Gash, 1988). Hamsters bearing an "SCN island" created with a Halasz knife, recover free running locomotor rhythms, often at the phase seen prior to transection of SCN efferents. Together these lines of evidence suggest that either any neural connection from the SCN to extra-SCN targets is sufficient to sustain rhythmicity, or that none are necessary (see Hakim et al., 1991 for further discussion).

In order to study communication among oscillators, we implanted intact hamsters with fetal SCN grafts (Philpot et al., 1989). Most often, no alteration at all was seen in circadian locomotor rhythmicity. In these intact grafted animals, it seems likely that normal targets of the native SCN remained occupied by host neurons, thereby limiting targets available for the grafted SCN cells. When the *in situ* SCN of these grafted hamsters were lesioned several weeks after implantation, the animals continued to free-run, often with no change of phase, suggesting that the host and donor SCN had become synchronized with each other.

To pursue this possibility more directly, we used ^{14}C-deoxyglucose (2-DG) to measure metabolic activity of the SCN in intact hamsters implanted with donor fetal SCN tissue, and asked whether a grafted SCN can phase shift or be phase shifted by the host SCN (Servière et al., 1992). A positive result in either direction would provide evidence of communication between grafted and host oscillators. Donor and host were maintained in opposite LD cycles, such that mid-day of the donor was mid-night of the host. Two phase reference points were sampled to determine metabolic activity (CT 05 and CT 14): The first day after implantation in the third ventricle, the metabolic activity of the donor and host SCN were depressed (likely due to the 2 consecutive treatments with anesthesia required for grafting surgery on one day and for 2DG injections the next day). Fourteen days (or longer) after grafting, the phase of

metabolic activity of the grafted SCN had assumed that of the host in every animal, irrespective of the attachment site of the graft. The results indicate first, that a signal from the host animal resets the phase of grafted SCN and not vice versa. Second, the distance of the graft from the host SCN did not affect the ability of the host to phase shift the graft, a result consistent with a diffusible signal, though this conclusion is constrained by the fact that all grafts were located within the third ventricle.

The fact that a signal from the host SCN entrained the grafted SCN indicates that an output (coupling) signal of the oscillator can act as an input (entraining) signal for another oscillator. Perhaps this result is expected, in that a wealth of data suggests that circadian oscillators become mutually coupled if their periods are similar. It is noteworthy however, that in our study, the native SCN always controls phase, and that the relative positions of the oscillators is not important in determining whether they are able to synchronize. It is also noteworthy that the results do not address the issue of whether control of phase by the host SCN is achieved by a neural or a diffusible signal, or whether the mediating pathway is direct or indirect. Thus, it appears that the native SCN continues to entrain all the physiological and behavioral rhythms of the animal. The grafted SCN need only receive entraining input from one of these systems to become synchronized to the native SCN. For example, circulating melatonin, corticosteroids, signals from meals, sleep-wake cycles, body temperature cues, etc., could all/each provide entraining cues. The actual significance of these potential synchronizing signals remains to be explored.

Conclusion: While we are quite sure that pacemaker cells of the SCN regulate circadian rhythms, the output signal(s) of these cells are not at

all understood. Evidence to date suggests the possibility that the stability of circadian rhythmicity rests on a foundation of redundant regulatory mechanisms, both neural and diffusible.

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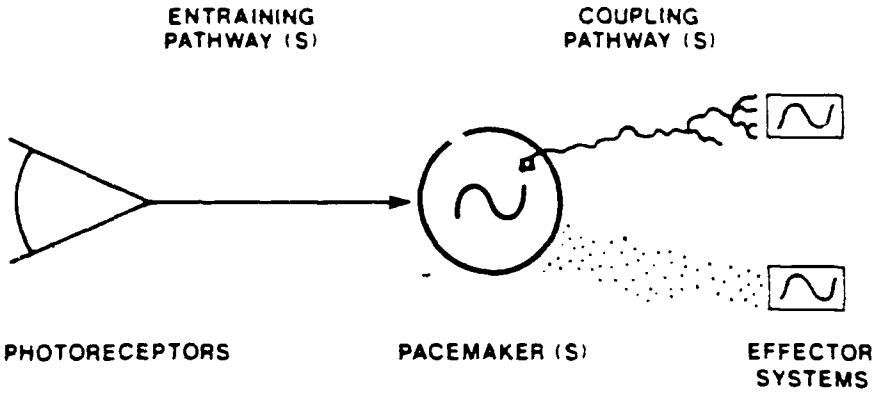
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