The Suprachiasmatic Nucleus Changes the Daily Activity of the Arcuate Nucleus \(\alpha\)-MSH Neurons in Male Rats

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Timing of metabolic processes is crucial for balanced physiology; many studies have shown the deleterious effects of untimely food intake. The basis for this might be an interaction between the arcuate nucleus (ARC) as the main integration site for metabolic information and the suprachiasmatic nucleus (SCN) as the master clock. Here we show in male rats that the SCN influences ARC daily neuronal activity by imposing a daily rhythm on the \(\alpha\)-MSH neurons with a peak in neuronal activity at the end of the dark phase. Bilateral SCN lesions showed a complete disappearance of ARC neuronal rhythms and unilateral SCN lesions showed a decreased activation in the ARC at the lesioned side. Moreover light exposure during the dark phase inhibited ARC and \(\alpha\)-MSH neuronal activity. The daily inhibition of ARC neuronal activity occurred in light-dark conditions as well as in dark-dark conditions, demonstrating the inhibitory effect to be mediated by increased SCN (subjective) day neuronal activity. Injections into the SCN with the neuronal tracer cholera toxin B showed that \(\alpha\)-MSH neurons receive direct projections from the SCN. The present study demonstrates that the SCN activates and possibly also inhibits depending on the moment of the circadian cycle ARC \(\alpha\)-MSH neurons via direct neuronal input. The persistence of these activity patterns in fasted animals demonstrates that this SCN-ARC interaction is not necessarily satiety associated but may support physiological functions associated with changes in the sleep-wake cycle. (Endocrinology 155: 525–535, 2014)

Physiological and behavioral processes exhibit circadian fluctuations with peaks at different time points, with a clearly synchronized temporal order imposed by the suprachiasmatic nucleus (SCN) (1, 2). Destruction of the SCN induces a complete loss of all these rhythmic functions even under light-dark (LD) conditions (3–6). Maintenance of these cycling patterns in physiology is due to the SCN ability to communicate information through neuronal projections to hypothalamic target nuclei (2, 7), resulting in synchronization of hormonal and autonomic output with the rhythmic changes in behavior. In this respect connections between the SCN and the ARC have also been described (8–10), with little knowledge on the relevance of these connections.
perimental data, has shown a strong relationship between metabolic health and the time of food consumption (25, 26). Earlier studies in addition have shown that the arcuate nucleus not only is involved in the control of food intake but also has an important contribution in cardiovascular regulation and energy expenditure, both of which have an important circadian component. The fact that our society has had such an increase in the occurrence of the metabolic syndrome, as illustrated by the increase of cardiovascular and diabetes type 2 diseases, has stimulated interest in the interaction between circadian- and metabolic-regulated processes.

Considering the essential role of the ARC in maintaining energy balance and the importance of the day-night rhythmicity in this respect, the aim of the present study was to investigate the functional relation between the SCN and the ARC by analyzing its connectivity and its diurnal neuronal activity pattern and to study the functional nature of this interaction. Our results show that α-MSH neurons have a daily pattern in neuronal activity, which is organized by the SCN, whereby the SCN excites and probably inhibits α-MSH neuronal activity in a different temporal pattern.

Materials and Methods

Animals

Adult male Wistar rats (Rattus norvegicus, 250–280 g), obtained from the general bioterium of the Faculty of Medicine of Universidad Nacional Autónoma de México, were housed in individual cages under LD 12-hours light, 12-hours dark conditions (light on at 7 h considered Zeitgeber time [ZT]0). Water and regular laboratory rodent diet (Purina, Chow 5001; Woodstock) were provided ad libitum throughout the study, unless otherwise stated. Rats were adapted 1 week to housing conditions before starting the experimental protocol. The Committee for Ethical Evaluation at Institute for Biomedical Research, Universidad Nacional Autónoma de México, approved experiments that were carried out in strict accordance with the Mexican norms for animal handling (Norma Oficial Mexicana NOM-062-ZOO-1999).

Stereotactic surgeries

Bilateral (n = 10) or unilateral (n = 12) SCN lesions were performed under a mixture of ketamine (40–80 mg/kg) with xylazine (5–8 mg/kg) injected im. For SCN lesions animals were performed as mentioned without current (sham bilateral, n = 4, and sham unilateral, n = 4).

The tracer Cholera Toxin B (CtB) 0.5% conjugated with Alexa Fluor-555 fluorescent dye (Molecular Probes) was unilaterally injected (0.1 μL) in the SCN of 12 rats with a Hamilton microsyringe using the same coordinates mentioned above. After the injection, the syringe was left in place for 10 minutes to minimize leakage. Missed SCN injections were used as controls to verify tracing and target specificity.

Immunocytochemical staining

All rats were deeply anesthetized with a lethal dose of sodium pentobarbital and perfused intracardially with 0.9% saline followed by a solution of 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Brains were removed and kept in fixative at 4°C for overnight postfixation and equilibrated 48 hours with 30% sucrose in 0.1 M PBS. Brains were cut in 40-μm coronal sections with a cryostat and placed in 30% sucrose. Sections used for immune labeling were collected and rinsed in 0.1 M PBS.

Six ARC sections per animal, two anterior, two medial, and two posterior, were incubated with rabbit anti-c-Fos primary antibody (1:40 000; Calbiochem) overnight under constant shaking at 4°C; subsequently, sections were rinsed and incubated at room temperature with biotinylated donkey-antirabbit serum (1:200; Jackson) for 1 hour, rinsed, and incubated in avidin-biotin complex (1:500 Vector Laboratories) for 1 hour. Product visualization was obtained with 0.01% diaminobenzidine (DAB), 0.05% nickel ammonium sulfate, and 0.01% hydrogen peroxide for 6 minutes. A second staining was performed on the same sections with sheep anti-α-MSH primary antibody (1:10 000, Millipore) overnight at constant shaking at 4°C, followed by incubation with biotinylated donkey antisheep (1:200; Jackson) for 1 hour and then rinsed and incubated in avidin-biotin complex (1:500); the same procedure was followed for the second staining without nickel.

Six (anterior-posterior) SCN sections of bilateral, unilateral lesioned, and control rats were stained for vasoactive intestinal peptide (VIP; 1:2000) (27). The SCN of CtB-injected animals was also incubated with rabbit CtB primary antibody following the same immunohistochemical procedure described before to examine the position of the SCN lesions and CtB injections, respectively.

Immunofluorescence staining

ARC sections of intact animals were incubated with rabbit anti-VIP or anti-gastric releasing peptide (GRP) 1:2000 (27, 28) together with sheep anti-α-MSH primary antibodies overnight at 4°C. Sections were rinsed with PBS and incubated with secondary antibodies Alexa 555 conjugated AffiniPure Donkey anti-Rabbit and Alexa 484 or 647 conjugated AffiniPure Donkey anti-Sheep (1:200, Jackson ImmunoResearch) for 1 hour, then rinsed and placed on gelatinized glass slides, coverslipped in glycerol (50%)-PBS (50%), and analyzed with the Laser Scanning Microscope 5 Pascal confocal microscope (Zeiss).

Statistical analysis

Data are presented as mean ± SEM for each group; the results were considered statistically significant when P < .05. To determine the presence of daily differences in ARC c-Fos neuronal activity and α-MSH activation under both ad libitum and fasting conditions, a series of nonrepeated measures one-way ANOVA was performed. If significant, ANOVA was followed by Tukey's
post-hoc to identify differences between individual group means. To compare the curves obtained for ARC c-Fos neuronal activity and α-MSH activation under ad libitum and fasting conditions, a two-way ANOVA was performed and was followed by Bonferroni’s post-hoc if required.

To determine the effect of SCN bilateral lesions at ZT22, unpaired Student’s t tests were performed.

Nonrepeated measures one-way ANOVAs were done to determine whether ARC activity of SCN sham and unilateral SCN lesions had significant differences. Because both ARC sides of either sham or SCN unilateral lesions (SCNx) animals correspond to the same animal and same section level, to compare the differences between sides we additionally performed a paired t test to compare both ARC sides of the same brain in sham and SCNx rats.

To determine if light affects ARC neuronal activity, nonpaired t tests were performed; finally, differences between constant darkness at Circadian time (CT) 2 compared with ZT2 and 22 were examined through one-way ANOVA.

Experimental Procedure

Determination of ARC daily activity pattern

Rats were killed at six time points along the LD cycle (ZT2, 6, 10, 14, 18, and 22, n = 4 for each time point) in ad libitum or 48 hours fasting before killing. Quantification of the total c-Fos immunoreactivity (IR) neurons within the ARC (three sections per animal, anterior, medial, and posterior) was performed with ImageJ software by determining ARC area by free-hand selection of the quantification area for each side of the ARC. Background was subtracted; threshold was determined, and particle analysis was set for particles of 1.0–2.0 circularity and 500–800 pixels.

The number of α-MSH neurons colocalizing with c-Fos was counted by two observers blind to the treatment, and only those α-MSH neurons that presented a clear double mark of c-Fos (blue nuclei) and α-MSH staining (brown soma) were included as colocalizing neurons (Figure 1A).

ARC activation after SCN bilateral lesions

To ensure the completeness of the bilateral SCN lesions (SCNx), the locomotor activity of the animals was recorded during the next 2 weeks after surgery to discard the reappearance of the activity rhythm and only the animals with an arrhythmic activity profile were included in the experiments. The lack of rhythm in lesioned animals was determined in LD because small remnants of the SCN are known to inhibit the locomotor activity, resulting in LD rhythmic masking locomotor activity profiles (29). In addition, VIP immunohistochemistry was performed to detect SCN remnants; animals that showed VIP-IR were discarded from further analysis (Figure 2B).

The brains of nonrhythmic animals (n = 4) were collected at ZT22 for comparison purposes because intact animals showed the highest activation of the ARC at this time point; no other time point was taken into account for this experiment since SCNx rats present a complete lack of rhythms and show equal physiology and behavior at all time points also in LD (30). The ARC neuronal activation was quantified as described above.

ARC activation after SCNx

SCNx were performed using one electrode and analyzed for changes in c-Fos expression in the ARC, depending on the lesion side. To ensure that SCNx compose only one side of the SCN, we selected only animals presenting a locomotor activity rhythm under LD conditions and accepted only animals with an intact SCN on one side and a complete lesioned SCN on the other side as determined by VIP staining (n = 3).

ARC response to 1-hour light pulse

One-hour light pulse of 100 lux was given in animals after 48 hours of fasting during the dark phase from ZT21 to ZT22 (n = 4). Animals were killed at ZT22; analysis of the ARC activation was performed as described previously and compared with ZT22 48-hour fasted intact animals.

Endogenous inhibition of the ARC

ARC activation was determined in animals subjected to constant dark con-
ditions or dark-dark conditions (DD) for 2 days before killing at CT2 (n = 3) and CT22 (n = 3). ARC and α-MSH neuronal activity was compared animals obtained in LD at ZT2 and ZT22.

SCN projections to α-MSH neurons in the ARC
To determine SCN projections to α-MSH neurons, the neuronal retrograde and anterograde tracer CtB was injected into the SCN; rats were killed after 7 days. The ARC was analyzed for CtB-positive fibers together with α-MSH staining. Moreover, to investigate the possible transmitters involved, GRP and VIP innervation pattern on α-MSH neurons in the ARC was determined.

Results
α-MSH neurons show a PEAK of activity at the end of the dark period
The number of c-Fos-positive cells in the ARC of ad libitum animals showed a daily peak (Figure 1C; time effect, $F_{[5,18]} = 25.6, P < .0001$), with a c-Fos IR maximum during the last part of the active phase at ZT22 and the lowest at ZT10 ($P < .001$).

The present results showed at ZT22 the localization of c-Fos IR mainly in the dorsolateral ARC coinciding with the presence of α-MSH neurons. Their activation was determined with double immunohistochemistry for c-Fos and α-MSH IR (Figure 1, A and B, left panel). Double-labeled c-Fos/α-MSH neurons showed a 24-hour activity pattern (Figure 1D; $F_{[5,18]} = 11.26, P < .0001$), with the highest activation at ZT22 and the lowest at ZT10 ($P < .05$). Because food intake in rats mainly occurs during the dark phase of the LD cycle and α-MSH neurons are activated by food intake–associated signals (31), the observed activation of α-MSH neurons could be triggered by feeding and not by direct inputs from the SCN. Therefore we examined c-Fos expression in the ARC after 48-hour fasting; despite the lack of food intake, the ARC c-Fos-IR pattern persisted (time effect, $F_{[5,18]} = 17.61, P < .0001$) with differences between ZT10 and ZT22 ($P = .001$). Even when differences between ad libitum and fasting were found (effect of conditions, $F_{[1,5]} = 3.24, P < .05$) at ZT14 ($P < .01$) and ZT22 ($P < .01$) with fasted animals presenting lower c-Fos levels (Figure 1C). The daily acti-
vation pattern was conserved even in the absence of food intake. In addition, no differences between ad libitum and fasted rats in α-MSH cells expressing c-Fos were observed (Figure 1D; $F_{1,5} = 0.02, P = .89$).

The persistence in the peak of c-Fos expression in α-MSH neurons at ZT22 during fasting conditions suggests that food intake is not the only factor involved in the rhythm in α-MSH neuronal activity. However, fasting does not abolish the rhythm of other important signals for ARC activity, such as ghrelin, leptin, glucose, and corticosterone (3, 32), which could represent an important humoral pathway for the SCN to promote a rhythm of neuronal activity in the ARC.

**SCNxx eliminate ARC activity peak at ZT22**

To further determine whether the peak in ARC activation at ZT22, observed under ad libitum and fasting conditions, was SCN dependent, we performed SCNxx and examined the neuronal activation of the ARC. Only complete lesioned animals as determined by actograms and postmortem analysis of VIP staining were included (Figure 2A).

Because SCNxx (n = 4) rats do not have a clear feeding pattern and eat during the entire 24 hours, our animals were fasted 48 hours before killing to prevent ARC activity changes due to random food intake. After the SCNxx, all animals killed at ZT22 showed a nearly complete disappearance of c-Fos staining in the ARC (Figure 2C, $P = .0004$, and Figure 2D, $P = .0003$), demonstrating that the presence of the SCN is essential to drive ARC activity.

ZT22 was the only sampled time point of SCNxx animals because several studies have demonstrated that SCN lesioned animals lack all rhythms including feeding (24, 33), indicating that the ARC also lacks rhythmicity, and that the maximal activation at ZT22 is dependent on the SCN integrity.

The ARC is also responsive to humoral signals that oscillate, and these rhythms have been demonstrated to be coordinated in a circadian pattern by the SCN (5, 34, 35), whether the present diminishment in c-Fos in the ARC and in α-MSH neurons is due only to the lack of rhythmicity in humoral signals or to the loss of direct innervation from the SCN to the ARC remains unclear.
Unilateral SCN lesion prevents ipsilateral activation of the ARC

To determine whether the communication between the SCN and the ARC is at least partly neuronal, SCNx (n = 3) were performed. The efficiency of the lesion was determined in two ways: first the animals needed to present locomotor activity rhythm under LD conditions (Figure 3A) and the SCN needed to be only unilateral ablated as determined by VIP staining (Figure 3B).

After SCNx both sides of the ARC showed a significant decrease in neuronal activation as compared with sham animals at ZT22 (Figure 3, C and D; F(3,8) = 9.42, P = .005 for total c-Fos and F(3,8) = 12.47, P = .002 for c-Fos/α-MSH). However, the side of the ARC corresponding to the SCN lesion side presented even less c-MSH. Experiment conducted after a 48-hour fast for all groups. NLP, no light pulse; LP, light pulse.

Interestingly the onset of SCN activity seems to have an inhibitory effect on the ARC as observed by the loss of c-Fos activation in the ARC at ZT2 (Figure 1). This observation suggests that not only is the SCN necessary for the induction of c-Fos expression in the ARC during the night but that activity of the SCN might be responsible for its inhibition at the beginning of the resting phase. Therefore in the next series of experiments we investigated whether exposure of the animals to light might change ARC neuronal activity.

Loss of ARC activity by light at CT 22

Photic signals especially at night are known to activate SCN neurons (36, 37), to phase shift SCN activity, and to change its neuronal output, resulting, for example, in the inhibition of melatonin secretion by γ-aminobutyric acid (GABA)-ergic signaling (5, 38). Because we observed that the SCN induced maximal neuronal activity in the ARC at the end of the dark period and that the ARC showed an immediate decrease in neuronal activity after light onset (Figure 1), we examined whether light exposure during the night would be able to change ARC neuronal activity.

One-hour light pulse from ZT21–22 immediately decreased ARC c-Fos expression (P = .001) and the number of the α-MSH cells colocalizing with c-Fos (P = .0007) as compared with ZT22 without light (Figure 4). The observed diminishment of c-Fos in the ARC by light suggests that the decreased ARC c-Fos expression at ZT2 is provoked by light-induced SCN neuronal activity, resulting in a similar inhibition as observed at ZT2. It seems unlikely that hypothetical direct retinal projections to the ARC are responsible for this inhibition because it is known that the retino-hypothalamic projections are excitatory (39).

To demonstrate that ARC inhibition at ZT2 is associated with SCN endogenous activity, we quantified ARC activation in animals killed after 2 days in constant darkness (DD) and fasted at CT2 and 22 and compared with their respective fasted controls killed in LD.

Irrespective whether animals were killed under DD or LD conditions, a marked difference was observed between (subjective) daytime and nighttime killed animals (F(3,10) = 13.84, P = .0007), with no differences between ZT or CT killed animals. The persistence of the rhythm in DD and fasted at CT2 and 22 was not different between ZT22 and ZT2 (P < .05) remained, although the difference was somewhat smaller than observed between ZT22 and ZT2 (P < .01), indicating greater amplitude in LD condition. Nevertheless, the persistence of the rhythm in DD conditions demonstrates it to be a true circadian phenomenon.

Figure 4. One-hour light pulse form ZT21–22 decreases ARC activation (63) (A). Representative ARC c-Fos and α-MSH sections at ZT22 without light and after a 1-hour light pulse (right) it is clear that the c-Fos black dots are strongly diminished after the light pulse, which was shown after quantification in (B). Total ARC activation (c-Fos counts) after the light pulse (ZT22) and (C) ARC α-MSH activation (c-Fos and α-MSH colocalization counts) after light pulse (ZT22). Experiment conducted after a 48-hour fast for all groups. NLP, no light pulse; LP, light pulse. ***, P < 0.01; ***, P < 0.001.
SCN terminals contact ARC α-MSH neurons

The SCN features a wide range of neuronal populations; the two most important for photic reception are VIP and GRP neurons (40), making them good candidates for neuronal populations involved in the transmission of light to the α-MSH neurons.

VIP (Figure 6A) terminals are in close apposition with most α-MSH neurons in the ARC nucleus. VIP innervation in the ARC was less dense than that observed for GRP; for GRP (Figure 6B) we observed that all α-MSH neurons received contacts with GRP fibers. However, lesioning the SCN resulted in the loss of all VIP innervation from the ARC, suggesting that all VIP in the ARC is derivated from the SCN; this was not the case for GRP fibers, however, as the GRP innervation in the ARC was diminished after SCN lesion but did not disappear, suggesting that also GRP from other sources may influence the ARC. To provide further evidence for the direct neuronal interaction of the SCN with ARC neurons, we injected the neuronal tracer CtB in the SCN (Figure 6, C and D) and determined its projections to the ARC with emphasis on the analysis of α-MSH neurons. Injections were considered successful when they were inside the SCN (n = 3).

Injections into the SCN resulted in relatively dense projections to the lateral ARC exhibiting frequent neuronal contacts with α-MSH neurons (Figure 6E), whereas injections that were just above or lateral to the SCN did not show such projections. The SCN input was most dense at the same side of the injection but also contralateral projections were present, indicating that the neuronal connections between the SCN and the ARC are in the majority but not limited to one side. We also observed colocalization with CtB traced fibers in the ARC for GRP, indicating the GRP origin from the SCN (Figure 6F).

Discussion

The present data demonstrate that the SCN has the capacity to stimulate neuronal activity of the ARC (ZT22) and probably to inhibit it during the day (ZT2); in addition, it shows that the α-MSH neuronal activity follows the same pattern. Furthermore, because unilateral lesions of the SCN resulted in the decrease of ARC α-MSH activity mainly at the side ipsilateral to the lesion, we conclude that the rhythm in ARC neuronal activity is largely derived from neuronal inputs from the SCN. Also, the observation that neuronal tracing from the SCN results not only in ipsilateral but also in some contralateral staining as well may explain the dissimilar diminishment of c-Fos after unilateral lesion of the SCN. Because c-Fos also decreases at the contralateral site of the lesion, it cannot be excluded that also circulating signals such as the hormones corticosterone or melatonin may contribute to this rhythm in ARC activity. Further evidence of SCN-ARC projections was revealed by CtB injections showing a marked association of VIP and GRP with α-MSH cell bodies and CtB tracer colocalization with GRP fibers, indicating that SCN-ARC neuronal pathways are at least partly responsible for the observed activation.

Studies have evaluated GRP effects in the ARC and shown that bombesin-like peptides increase the firing rate of both neuropeptide Y and proopiomelanocortin neurons (41, 42), suggesting that GRP originating from the SCN may activate ARC α-MSH neurons. A major problem for this hypothesis is that GRP-VIP neurons in the SCN are activated by light (37), the condition whereby the ARC α-MSH neurons are inhibited. Moreover in the SCN, VIP and GRP mRNA levels present a peak in the middle of the light phase (43, 44), suggesting that VIP and GRP might be responsible for the inhibitory effect observed during the onset of the subjective day. This would agree with the observation that VIP inhibits dopamine neurons in the ARC (45).

In view of reports that demonstrate oscillatory activity in the ARC in vitro (46), it is important to consider that in SCNxx animals the ARC may show a free running rhythm that prevents seeing its activation. Considering that SCNxx resulted in the loss of all rhythmicity including food intake (33) and considering that food intake is importantly driven by the ARC (47), we assume that the ARC in SC-
Nxx animals loses its rhythmicity. Moreover all our SC-
Nxx animals showed the same low activity of the ARC; if
the ARC were free running, one would expect at least one
animal with a higher activity of c-Fos. Still, the combina-
tion of these studies demonstrating the rhythmic proper-
ties of the ARC and the present study showing the SCN-
ARC interaction give a fascinating picture of the
importance of rhythms in the ARC. Nonetheless it has also
been demonstrated that ARC lesions also induce arrhyth-
mic food intake when animals are exposed to constant
darkness (48, 49), suggesting that communication be-
tween the SCN and the ARC is important in both direc-
tions and that the ARC is also crucial for the SCN activity (8, 9, 48, 49) to
sustain food intake when synchronizing photic signals are absent.

Notably the only other identified SCN targets that up until now show
a similar activation and inhibition pattern are the preautonomic PVN
neurons that control melatonin secretion in a multisynaptic circuit (5,
38). These PVN neurons are activated at night by SCN glutamatergic
terminals that stimulate melatonin secretion and are inhibited by a
GABA-ergic SCN projection that is activated by light, suggesting that the
activation and inhibition as presently seen in a similar timeframe in
the ARC may be induced by comparable signals from the SCN. Al-
though we cannot exclude that the loss of ARC activation at ZT2 or at
ZT22 induced by light is induced by a lack of stimulatory input from the
SCN, we would like to propose that the SCN projections to the ARC pro-
vide a similar activation inhibition pattern to the ARC as they offer to
the PVN. On the other hand, in prin-
ciple it is possible that other retinal
targets within or outside the hypo-
thalamus (50, 51) also may provide
an indirect inhibitory input to the
ARC. The fast disappearance of c-
Fos after the light exposure may also
favor an SCN or retinal target-medi-
ated inhibition rather than simple
lack of excitation; future studies will
need to clarify this interaction.

In addition to these ARC neuro-

Figure 6. SCN fibers contact α-MSH neurons. (A) VIP and (B) GRP IR fibers (red) in the ARC
contact α-MSH (green) neurons in the ARC. (C) DAB-Ni section of the SCN with CtB injection, (D)
CtB traced fibers (red) originating from the SCN in close relation with α-MSH IR cells (blue), (E
and F) CtB (red) and GRP (green) colocalizing (yellow) in the ARC (arrows), n = 4. III, third
ventricle; OC, optic chiasm.

nal activity changes imposed directly by the SCN, circu-
lating metabolites or hormones can activate or inhibit
ARC neurons in a circadian manner (52). Many of these
compounds also have a clear 24-hour rhythm directly or
indirectly driven by the SCN (53) with receptors in the
ARC (49, 54, 55), indicating that the temporal ARC ac-
tivation can also be promoted by humoral pathways or
through multisynaptic neuronal relays such as via the dor-
somedial hypothalamic nucleus (56).

Independent of the feeding condition, 60% of the ARC
α-MSH neurons are activated at ZT22, illustrating the
involvement of these α-MSH neurons in functions espe-
cially associated with the end of the activity period, probably supporting the circadian control of energy balance. Because α-MSH neurons are also activated after food intake, this activation by the SCN in the absence of food shows that the SCN will activate the ARC in agreement with the daily physiology to support functions that as a rule are associated with satiety. It is known that when food intake occurs outside the time indicated by the SCN, this may lead in the long run to obesity and diabetes (57, 58); one of the explanations that now surge from our study is that in such a situation the ARC is not suitably prepared for the ingestion of food, which may result in inadequate autonomic and hormonal responses associated with the food intake. Also, the coexpression of a wide variety of transmitters, like glutamate, GABA, dynorphin, or acetylcholine in the α-MSH neurons (59), suggests their possible involvement in a wide variety of physiological functions other than the direct inhibition of food intake.

The present study concurs with earlier studies that also have shown rhythmicity in certain populations of ARC neurons (46, 60–62); together these data show that the SCN not only provides an output to synchronize behavioral, hormonal, and autonomic functions but also may gate and prepare the ARC for the access of sensory circulating information. Thus, the activity of the ARC is on the one hand driven by circulating metabolic information and on the other hand directly driven by the SCN. Furthermore, it is known that the ARC is also able to influence the functionality of the SCN, and its neuronal projections are shown to be able to influence not only the neuronal activity of the SCN but also its circadian phase (9, 49, 63). It is tempting to speculate that this ARC-SCN reciprocal interaction is essential to maintain a well-balanced metabolic circadian profile, which might be another explanation that desynchronization of circadian and metabolic signals, for example, by food intake outside the time indicated by the SCN, may result in the metabolic syndrome.

Acknowledgments

This research was supported by Grants DGAPA IN-209711 and CONACyT 79797 and comprises a PhD thesis for the Doctoral in Investigación Biomédica of the Universidad Nacional Autónoma de México, supported by the CONACyT PhD student grant. The authors thank Dr Carolina Escobar, Fac. de Medicina Universidad Nacional Autónoma de México and Dr Roberto Salgado-Delgado, Fac. De Ciencias Universidad de San Luis for their suggestions and Miguel Tapia for his technical assistance.

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Disclosure Summary: The authors have nothing to disclose.

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