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Pup circadian rhythm entrainment—effect of maternal ganglionectomy or pinealectomy

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Abstract

In rodents, during late embryonic and early neonatal development, circadian rhythms develop in synchrony with those of their mothers, which in turn are synchronized with the environmental photoperiod.

This paper examines the effect of maternal ganglionectomy (pineal gland sympathetic denervation) or extirpation of the pineal gland on pups' drinking rhythms, a behavior that is continuously monitored in individual animals starting after weaning and studied up to 3 weeks later. Maternal ganglionectomy or pinealectomy performed on the 7th day of gestation significantly disrupts rat pups' drinking behavior, within and among litters. In both treatments, circadian rhythm characteristics of the free-running period (tau), phase, amplitude and alpha were significantly altered compared to those of the control pups born from sham-operated mothers. With the exception of the alpha component, both maternal treatments have similar effects. When melatonin was given to the mothers instead of the endogenous pineal secretory activity for 5 days during the late period of gestation, this treatment reversed the effects of maternal ganglionectomy and pinealectomy. These observations, together with previous studies of our group, indicate that the maternal superior cervical ganglia and pineal gland are necessary components of the mechanism for maternal synchronization, and that maternal melatonin may, directly or indirectly, affect the performance of the pups' central oscillator during early pup rat development.

Keywords: Pup circadian rhythm; Maternal entrainment; Pineal gland; Superior cervical ganglia; Melatonin

1. Introduction

In mammals, the photoperiodic synchronization of the circadian system is accomplished through a complex neuroendocrine mechanism. A circadian pacemaker localized in the hypothalamic suprachiasmatic nuclei (SCN) integrates environmental light signals and generates circadian rhythms synchronized to daily variations in light intensity [1,2]. The SCN contains multiple autonomous single-cell circadian oscillators that are coupled together, generating a single rhythm [3].

In rodents, during late embryonic and early neonatal development, circadian rhythms develop in synchrony with those of their mothers, which in turn are synchronized with the environmental photoperiod. Evidence of this maternal entrainment phenomenon has been obtained by analyzing the circadian rhythms of rat pups reared by foster mothers and exposed to constant lighting from birth. Measuring the circadian phase of pineal serotonin *N*-acetyltransferase (NAT) activity in a population of 10-day-old rats, the pup's NAT activity is close to that of the biological mother when the photoperiod during gestation was different from that of the foster mother [4,5]. The same phenomenon is found in evidence from plasma corticosterone [6], body temperature [7], parotid α-amylase (AMY) and testicular malate dehydrogenase (MDH) [8,9].

Nevertheless, the major evidence of maternal entrainment is the mother–pup phase synchrony observed in rat pups' drinking behavior, and in hamster and mouse locomotor rhythms [10–13]. With the circadian phase of each pup synchronized to that of its mother, there is circadian phase synchrony within a litter. Circadian phase synchrony is also observed among different

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litters if their mothers were exposed to the same photoperiod during gestation [14].

Some studies have focused on identifying the nature of the maternal signal(s). Maternal SCN lesions during early gestation disrupt hamster and rat mother–pup synchronization [13]. The pineal NAT activity rhythms in 10-day-old rats are lost, while locomotor rhythms in young hamsters and drinking rhythms in rat pups show a different circadian phase distribution when compared with pups born from sham-operated mothers [15,13,16]. Since the SCN lesions disrupt several circadian rhythms, a maternal circadian signal must be involved in the transmission of photoperiodic information to fetal or neonatal pups. Remarkably, melatonin was the first maternal hormonal signal suggested for the transfer of maternal photoperiodic information during mammal development [17].

In rats, Reppert and Schwartz [18] examined the role of several maternal hormones in mediating prenatal entrainment by testing whether removal of any maternal endocrine glands (pineal, pituitary, thyroid, adrenal, ovaries) early in the gestation would have similar effects to maternal SCN lesions. Even though a pinealectomy-like effect was reported on daily values of fetal SCN-2-deoxy-D-glucose uptake [18], authors did not find similar results in postnatal pineal NAT activity rhythms. It was suggested that the maternal signal might be redundant, with several maternal rhythms acting in concert to entrain the fetal and/or neonatal circadian system. Thus, eliminating any one of these hormonal signals would not be enough to disrupt maternal entrainment [19].

To test the hypothesis that maternal melatonin acts as a signal during early development, Davis and Manion [20] injected SCN-lesioned pregnant hamsters with melatonin during gestation. The prenatal melatonin injection restored the phase synchrony of pups' locomotor activity within and among litters. This result shows that exogenous melatonin given to the mother during gestation can entrain pups' circadian rhythms, but it did not demonstrate that maternal melatonin is a physiological signal mediating entrainment of the fetus [21].

Neurons of the superior cervical ganglia (SCG) provide sympathetic innervations to the pineal gland [22], and pineal melatonin biosynthesis is under sympathetic system control [23–25]. In rats, bilateral superior cervical ganglionectomy (SCGx) destroys the pineal sympathetic terminals and suppresses the rhythm of melatonin biosynthesis [23,26,27]. We have observed that SCGx in rats, before mating or early in the gestation, disrupts maternal synchronization in the circadian rhythms of young pups' parotid AMY and testicular MDH activities. The daily enzymatic profiles did not show a significant circadian acrophase when they were analyzed in a time-group population of 25-day-old pups born from ganglionectomized mothers [28,9,29]. The disruptive effects only occurred when the maternal ganglionectomy was carried out on or before the 11th day of gestation [30]. Daily doses of melatonin to ganglionectomized mothers during late gestation generated a circadian phase in the pup's daily enzyme activity profile, but only when it was injected after the 11th day of gestation [30]. It was suggested that maternal synchronization of these pup circadian rhythms begins very early in rat fetal development, and that rhythms of melatonin secretion from the maternal pineal gland play an important role. However, several questions remain to be elucidated: (1) We do not know if maternal pinealectomy could have a similar effect on pup circadian rhythms to that of maternal ganglionectomy, since superior cervical ganglia provide sympathetic innervation to a number of intra- and extracranial structures including the pineal gland, cephalic blood vessels, choroid plexus, eye, carotid bodies and the salivary and thyroid glands [31,32]. (2) The disruptive effects of maternal ganglionectomy have been observed on AMY and MDH rhythms in rat pups. It is not clear whether there was a loss of the pup rhythm or a lack of phase synchronization between individual pups' circadian rhythms.

In the present work, we examined the effect of maternal ganglionectomy and pinealectomy on pups' drinking rhythms, a behavior that can be continuously monitored in individual animals for several days after weaning. The circadian rhythm characteristics of the free-running period (tau), phase, amplitude and alpha in both treatments were significantly affected compared to those of the control pups born from sham-operated mothers. In addition, maternal melatonin treatments during the late period of gestation reverse the effects of maternal ganglionectomy and pinealectomy. With the exception of the alpha parameter, both maternal treatments have similar effects.

2. Materials and methods

2.1. Animals and general procedure

Three-month-old female Wistar rats, kept under constant temperature (23±1 °C), synchronized to a 14:10 light/dark cycle (light from 06:00 to 20:00 h), with food (laboratory chow) and water "ad libitum", were used. Estrous cycles were determined by a daily examination of vaginal smears and the animals were mated on the night of ovulation.

On gestational day 18, pregnant rats were transferred to individual cages and exposed to constant dark beginning at the time of lights off. Mothers were injected with melatonin late in gestation, at the time of the endogenous pineal secretion, for 5 days, a period considered adequate to cause entrainment [9,20,27]. After delivery, mothers and pups remained in constant dark conditions. On postnatal day 3 (P3), each litter was reduced to eight, mainly male, pups. After the day of weaning (P21), mothers and pups were housed in individual cages and monitored for drinking activity for 24 days. All manipulations were done under dim red light.

2.2. Pup groups

Four to six litters were included in each of the following experimental pup groups:

1. Pups from ganglionectomized mothers (SCGx)
   Rat pups born from superior cervical ganglionectomized mothers on the 7th day of gestation.
2. Pups from ganglionectomized mothers treated with melatonin (SCGx+mel)
   Rat pups born from ganglionectomized mothers injected with a daily dose of 1 mg/kg body weight (s.c., 19:00 h) from the 17th to the 2lst days of gestation.

3. Pups from pinealectomized mothers (Px)
   Rat pups born from mothers pinealectomized on the 7th day of gestation.
4. Pups from pinealectomized mothers treated with melatonin (Px+mel)
   Rat pups born from pinealectomized mothers injected with a daily dose of 1 mg/kg body weight of melatonin (s.c., 19:00 h) from the 17th to the 21st days of gestation.
5. Pups of sham-operated mothers (Control)
   Rat pups born from superior cervical ganglionectomized or pinealectomized sham-operated mothers were used as control groups.

2.3. Drinking activity recording

Circadian rhythms of drinking behavior were individually recorded using an infrared detector. Drinking activity was detected when the head of the rat interrupted an infrared light beam, located in front of the water dispenser. Through an interface and software, electrical signals from 15-min time bins were transformed into numerical values and accumulated in a database. In all the experiments, the drinking behavior was continuously monitored for 24 days. A double-plotted actogram of each animal's drinking rhythms was obtained [33] and is shown in Fig. 1. Activity onset was defined as the first bout of activity preceded by at least 2 h with no sustained activity. Activity offset was considered as the last bout of activity that occurred before a 2-h period with no sustained activity. This rule was chosen for calculating phase (instantaneous stage of an oscillation within a cycle) at the day of weaning, period (tau; portion of time taken to complete one cycle), alpha (duration of behavioral activity) and amplitude (difference between maximum and mean value in a sinusoidal oscillation) values by the DISPAC software (DISPAC, Vega Gonzalez and Aguilar Roblero, UNAM). Data from the first 3–4 days were excluded from analysis in order to control for possible effects of drinking acclimation on activity patterns. The phase of activity offset on the day of weaning was taken as the interception on that day of an eyefit or regression line through the activity offsets of the subsequent 3 weeks of recording. Tau was determined by estimating the time (in hours) between successive activity offsets during at least 10 days of clear activity patterns. Alpha was determined by calculating the time (in hours) between activity onset and offset. Data from consecutive days were averaged to determine values for phase, tau, alpha and amplitude.

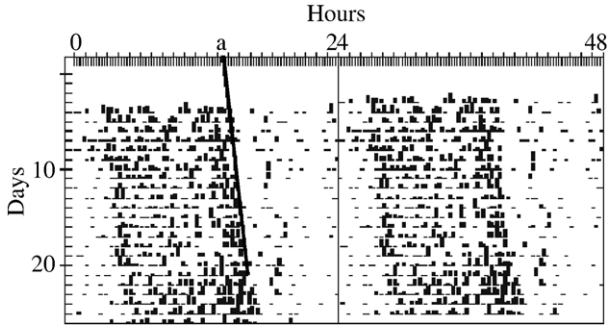

Fig. 1. Double-plotted actogram (temporal profile) of drinking behavior of pups reared by a pinealectomized dam kept in constant darkness from the day of delivery. The rhythmic components were measured with DISPAC software (DISPAC, Vega Gonzalez and Aguilar Roblero, UNAM). The phase of activity offset on the day of weaning is the interception of a line through subsequent activity offsets (a). Tau was determined by estimating the time (in hours) between successive activity offsets during at least 10 days of clear activity patterns. Alpha was determined by calculating the time (in hours) between activity onset and offset. Data from consecutive days were averaged to determine values for phase, tau, alpha and amplitude. Data from the first 3–4 days was excluded from analysis in order to control for possible effects of drinking acclimation on activity patterns.

2.4. Maternal superior cervical ganglionectomy

Pregnant mothers were ganglionectomized on the 7th day of gestation, since previous studies demonstrated that maternal bilateral SCG on the 7th, 10th or 11th days of gestation disrupted, the circadian rhythm of testicular MDH activity in pups reared under constant conditions. In contrast, no disruptive effect was observed when mothers were denervated on the 12th or 14th day of gestation [30].

The animals were anaesthetized with chloral hydrate (5 mg/kg b.w.; Sigma), and the operation was conducted using a microsurgery microscope. After medial incision in the submandibular region, salivary gland and muscles were displaced to expose the sympathetic cervical ganglion located under the bifurcation of the carotid artery. Bilateral removal of the ganglia was performed and the subsequent Horner's syndrome was observed. As a control, sham-operated mothers were treated similarly, but without removing the superior cervical ganglia.

2.5. Maternal pinealectomy

Pinealectomy was performed on the 7th day of gestation. The rats were anaesthetized with chloral hydrate (5 mg/kg b.w.; Sigma) and mounted on stereotaxic equipment. The skin was incised, a hole was made in the skull at the lambda point and the pineal gland was removed from the surrounding sinus with fine forceps. The control sham-pinealectomized rats were treated similarly, with the exception that the bone plug was not removed.

2.6. Statistical analysis

The most appropriate method for calculating an average phase and determining if it is significant (i.e., if the distribution is non-random) was performed by circular statistics. The Rayleigh test [34] was used to determine whether a given phase distribution in an experimental pup group was significantly different from a uniform distribution. For perfect synchrony $r=1$ and for complete scatter $r=0$. The Mardia–Watson–Wheeler test [34] was applied to assess significant differences between the phase groups' distribution. One-way analysis of variance (ANOVA) was used to determine the maternal treatment effects on pup free-running period, alpha and amplitude values. Post-hoc comparisons were made using Newman–Keuls test. Non-parametric statistical (Kruskal–Wallis test) analyses were used when a circadian parameter did not have a normal distribution. For all tests, significance was set at the $p<0.05$ level.

3. Results

3.1. Pups circadian phase distribution

The phase distributions for drinking behavior of pups born from superior cervical ganglionectomized (SCGx), sham-ganglionectomized (Control) and melatonin-treated ganglionectomized (SCGx+mel) mothers were plotted relative to clock time on the day of weaning as shown in Fig. 2. In all groups examined, the circadian phase distribution was significantly different from uniform ($p<0.05$, Rayleigh test) and clustered near those of the mother's circadian phase. The SCGx pup group showed a significant difference of mean circadian phase (μ) and angular deviation (s) distribution ($\mu \pm s = 10{:}27 \pm 2{:}36$ h, $n=34$) compared to those of the control ($8{:}15 \pm 0{:}48$ h, $n=21$) and SCGx+mel ($7{:}55 \pm 0{:}56$ h, $n=21$) groups ($p<0.01$, Mardia–Watson–Wheeler test). The degrees of synchronization of the

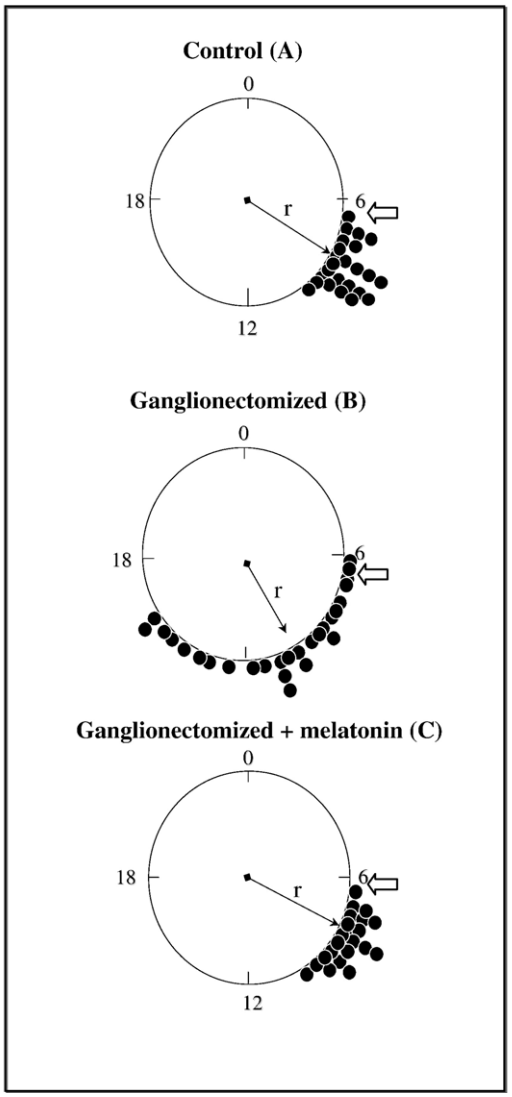

Fig. 2. Phase distribution for drinking behavior of pups born from (A) sham-ganglionectomized, (B) superior cervical ganglionectomized (SCGx) and (C) melatonin-treated ganglionectomized (SCGx+mel) mothers, plotted relative to clock time. The large circles represent the 24 h of the weaning day and each of the small circles the phase of individual pups. The mean phase of the pups group is indicated by the arrow within the large circle. The length of the arrow (r) indicates the degree of synchrony of the pup group. The mean phase of the mothers is indicated by the arrow outside the large circle. The phases are significantly different from uniform distribution in sham-SCGx, SCGx, SCGx+mel ($p<0.05$, Rayleigh test). The mean phase and angular deviation distribution in the SCGx pup group are significantly different from those of sham-SCGx and SCGx+mel groups ($p<0.01$, Mardia–Watson–Wheeler test).

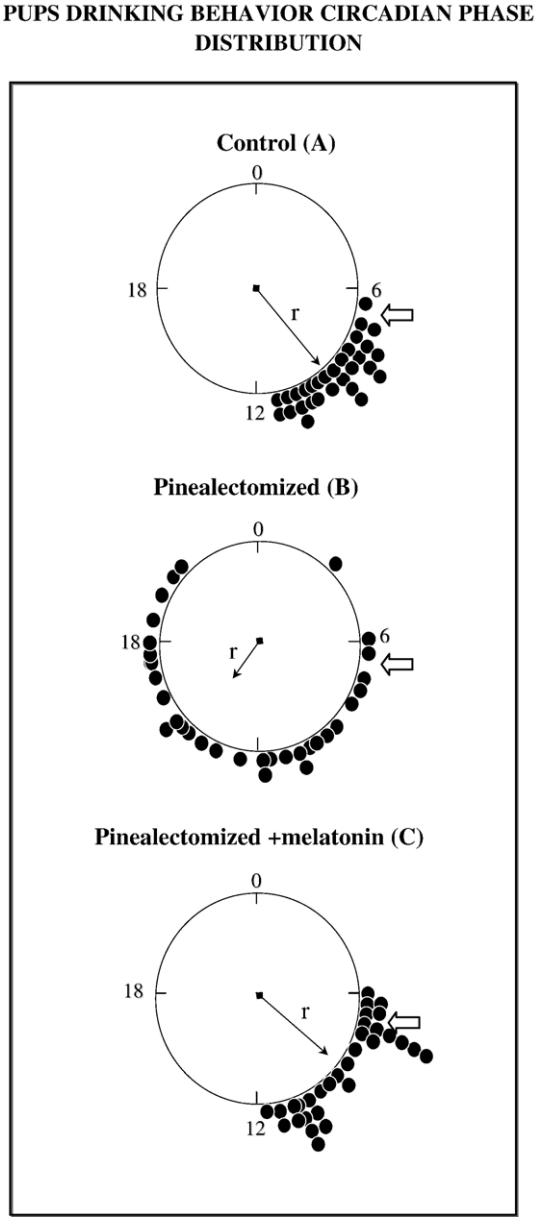

Fig. 3. Phase distribution for drinking behavior of pups born from (A) sham-pinealectomized (control), (B) pinealectomized (Px) and (C) melatonin-treated pinealectomized mothers (Px+mel), plotted relative to real clock time. Conventions as for Fig. 2. Control and Px+mel groups phase distribution are significantly different from uniform ($p<0.05$, Rayleigh test). The mean phase and angular deviation distribution in the Px pup group are significantly different from those of the sham-Px ($p<0.001$) and Px+mel groups ($p<0.01$, Mardia–Watson–Wheeler test).

SCGx+mel and control groups were 99% and 98%, respectively, while that of the SCGx group was 76%.

Fig. 3 shows the phase distribution for drinking behavior of pups born from mothers subjected to Px and Px+mel, and sham-pinealectomized mothers, plotted relative to clock time on the day of weaning. The phase distribution of the control and Px+mel groups was significantly different from uniform ($p<0.05$, Rayleigh test) and clustered around the mother's circadian phase. On the other hand, the phase distribution of the Px group was uniform ($p>0.05$, Rayleigh test; $\mu \pm s = 13{:}41 \pm 3{:}48$ h, $n=34$) and significantly different from those of the control (9:20±1:14 h, $n=32$, $p<0.001$) and Px+mel (8:34±1:34 h, $n=33$) ($p<0.01$, Mardia–Watson–Wheeler test) groups. The mean circadian phase of the Px+mel group was not significantly different from that of the control group ($p>0.05$). The degrees of synchronization of the Px+mel and control groups were 85% and 90%, respectively, while that of the Px group was only 46%.

Two out of five litters born from pinealectomized mothers showed a uniform phase distribution ($p>0.05$, Rayleigh test). Maternal ganglionectomy or pinealectomy did not have any effect on mothers' circadian phase distribution (data not shown).

3.2. Pups free-running circadian period

The box plot of the free-running period (tau) of control, SCGx and SCGx+mel pup groups is shown in Fig. 4. Drinking behavior of pups born from superior cervical ganglionectomized mothers showed a different distribution of tau compared to those of sham-operated mothers. Nonparametric analysis indicated a significant difference between mean values of the SCGx pup group (23.97±0.045 h, $n=34$) and those of the control (24.04±0.005 h, $n=45$, $p<0.003$) and SCGx+mel (24.07±0.006 h, $n=21$, $p<0.05$) groups (Kruskal–Wallis test). There was a significant difference in the percentage of variance between these groups ($p<0.0001$). No significant difference was observed between control and SCGx+mel groups ($p>0.05$).

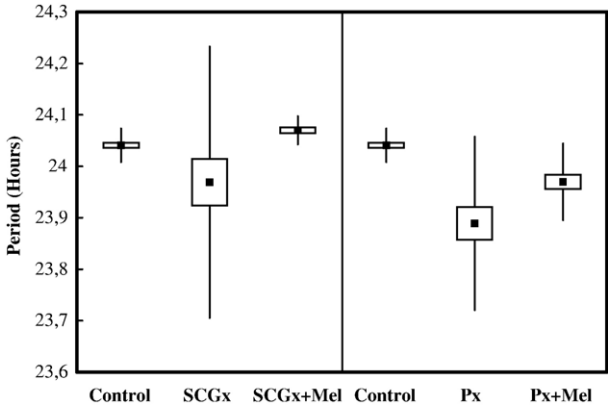

Fig. 4. Box plot of circadian period of drinking behavior in pups born from sham-operated (control), ganglionectomized or pinealectomized mothers and the effect of melatonin treatment. Those of sham-operated mothers are all the pups born from shamganglionectomized and sham-pinealectomized mothers (no statistical differences were observed between these groups). The mean value for each group is indicated by the square within the box. The box indicates the limits of the S.E.M. and the vertical line represents the S.D. The pups' group from SCGx mothers is significantly different from those of the control ($p<0.003$) and SCGx+mel ($p<0.05$) groups and the pups' group from Px mothers is significantly different from those of the sham-Px ($p<0.0001$) and Px+mel ($p<0.006$) (ANOVA Newman–Keuls test) groups.

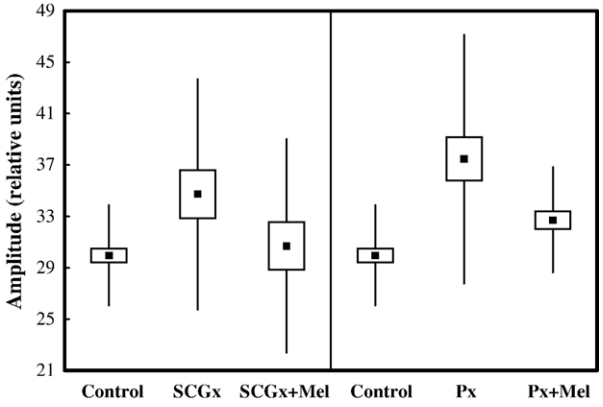

Fig. 5. Box plot of the circadian amplitude of drinking behavior in pups born from sham-operated (control), ganglionectomized or pinealectomized mothers and the effect of melatonin treatment. Conventions as for Fig. 4. Control and Px+mel groups are significantly different from Px group ($p<0.001$).

The free-running tau values of pups born from mothers subjected to Px, Px+mel and those sham-operated are also shown in Fig. 4. These pup groups showed a normal statistical distribution of free-running period with significant differences of $F(2,100)=17.95$, $p<1.10^{-7}$. The pinealectomized pup group (23.89±0.032 h, $n=34$) was significantly different from the control (24.041±0.005 h, $n=45$) and Px+mel (23.97±0.014 h, $n=33$) groups ($p<0.0001$ and $p<0.006$, respectively) (Newman–Keuls test) and there was a significant difference in the percentage of variance among the same groups ($p<0.0001$). Maternal ganglionectomy or pinealectomy did not have any effect on mother's free-running circadian period (data not shown).

3.3. Pup circadian amplitude

The box plot of circadian amplitude of SCGx (34.71±1.87, $n=23$), control (29.96±0.54, $n=45$) and SCGx+mel (30.70±1.86, $n=20$) pup groups are shown in Fig. 5. The SCGx mean value was slightly greater than those of control and SCGx+mel but there was no significant difference among them. The melatonin treatment did not reduce the dispersion observed in the SCGx group. The box plot of amplitude values of Px (37.46±1.68, $n=33$), control (29.96±0.54, $n=45$) and Px+mel (32.72±0.70, $n=34$) pup groups are also shown in Fig. 5. All these groups present a normal distribution of amplitude values. The pinealectomized mean value was significantly greater than those of the sham ($p<0.0001$) and Px+mel ($p<0.002$) pup groups (Newman–Keuls test). No significant difference was observed between the control and Px+mel groups.

3.4. Pup circadian alpha

The box plot of the circadian alpha of SCGx (13.43±0.16 h, $n=30$), sham-ganglionectomized (12.21±0.038 h, $n=44$) and

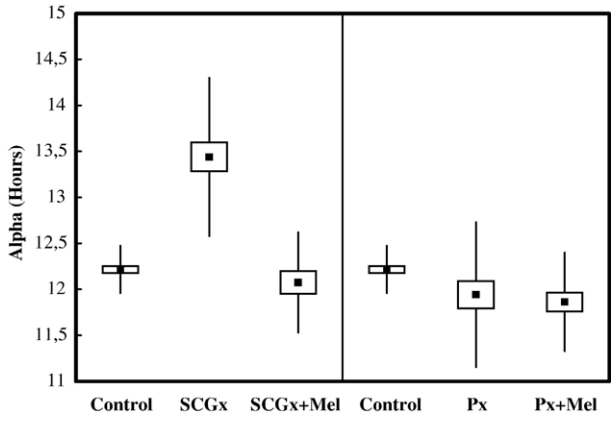

Fig. 6. Box plot of circadian alpha of drinking behavior in pups born from sham-operated (control), ganglionectomized or pinealectomized mothers and the effect of melatonin treatment. Conventions as for Fig. 4. Control and SCGx +mel groups are significantly different from SCGx group ($p<0.0001$). The Px group is significantly different from the control group ($p<0.05$) (ANOVA Newman–Keuls test).

SCGx+mel (12.37±0.075 h, $n=23$) pup groups are shown in Fig. 6. All pup groups showed a normal distribution of alpha values. That of the SCGx group was significantly higher than those of the control and SCGx+mel groups ($p<0.0001$, Newman–Keuls test) and there was a significant difference in the percentage of variance among these groups ($p<0.01$). No significant difference was observed between the SCGx+mel and control pup groups.

Fig. 6 also shows the box plot of alpha values of pups born from pinealectomized (11.89±0.168 h, $n=33$), sham-pinealectomized (12.2±0.038 h, $n=44$) and melatonin-treated pinealectomized mothers (11.84±0.126 h, $n=34$). All of these groups showed a normal distribution of alpha values. The mean alpha value of those pinealectomized was significantly lower than that of the control pup group ($p<0.05$, Newman–Keuls test). There was a significant difference in the percentage of variance between the same groups ($p<0.003$). No significant difference was observed between the Px+mel and Px pup groups.

4. Discussion

The present results further demonstrate that the maternal superior cervical ganglia and pineal gland are necessary components of the mechanism for maternal synchronization. These findings match our previous results [9,28–30]. Here, we have shown for the first time that maternal ganglionectomy and pinealectomy early in gestation disrupt rat pups' drinking behavior, within and among litters, compared to pups born from control mothers (sham-operated).

Several works on maternal entrainment have been particularly concerned to study phase expression, one of the rhythmic parameters of daily variation. The effect of maternal pinealectomy during gestation on the phase of hamster wheel-running and on rat pups' pineal NAT activity rhythm had not been previously demonstrated (cited by 35). Here we show that maternal pinealectomy or sympathetic pineal denervation, performed on the 7th day of gestation, has a clear effect on tau, phase, amplitude and alpha of pups' drinking behavior rhythms.

The circadian mean phase and the angular deviation of the SCGx and Px pup groups were significantly greater than those of the control group and widely scattered over a range of 12 and 15 h, respectively (Figs. 2B and 3B), while the control values were clustered in a range of 3 to 5 h (Figs. 2A and 3A), with a greater phase dispersion in the Px group. Moreover, the tau values of pups' free-running in the absence of rhythmic prenatal melatonin signals (SCGx and Px groups) were significantly scattered and lower than those of control pups ($p<0.001$, Fig. 4). Thus, two of the most important parameters of pups' behavior rhythms show a significant increase in the spread of their values.

In addition, for both maternal treatments, the pups' amplitude values were considerably scattered and higher than those of controls (Fig. 5). However, these treatments had opposite effects on the pups' alpha values. They were higher in the SCGx group but lower in the Px group, compared to those of the controls (Fig. 6). Since SCG innervates a number of intra- and extracranial structures [29,30], it is possible that its removal may affect several system, thus explaining the different effects on pup's alpha drinking behavior between SCGx and Px.

The results suggest that there is a decrease in the degree of synchronicity within and among litters and clearly show that the absence of a rhythmic maternal melatonin signal produces asynchronous but robust individual circadian drinking activity, indicating that this is not the result of stopping the pups' clock mechanism. However, normal temporal synchronization within and among litters was lost without a rhythmic maternal melatonin signal.

When melatonin was given to SCGx or Px mothers late in gestation, it clearly reversed the effects of both maternal ganglionectomy and pinealectomy on the pups' free-running circadian period and phase of drinking activity, and reduced significantly their spreads (Figs. 2C, 3C and 4). In other words, the results obtained show that melatonin injections given to the mothers during gestation are effective in clustering the circadian periods and the phases of offspring studied up to 3 weeks after weaning. These results match those of our previous biochemical studies. The disrupting effects of ganglionectomy on parotid α-AMY and testicular MDH activities rhythms were not observed when SCGx mothers were injected with melatonin, suggesting that the effects of maternal SCGx on daily enzymatic rhythms were a consequence of the absence of circadian melatonin entrainment within and among litters [28,9,30]. Comparable results were found when SCN-lesioned hamsters were injected with melatonin during gestation: the offspring's wheel-running rhythms showed circadian phase synchrony within and among litters [20,35,36].

The mean values of tau and those of phase and amplitude show opposing tendencies, i.e., when tau diminished as a consequence of the decrease in endogenous circulating maternal melatonin, phase and amplitude increased, and vice versa (Figs. 2B, 3B, 4 and 5, respectively), suggesting a possible correlation among these parameters. Nevertheless, alpha does not appear to have any association with the rest of the rhythm parameters. As it was suggested [37,38], probably, each rhythms parameters is independently controlled.

Melatonin has been proposed as an efferent hormonal output of the circadian clock [39], since pineal melatonin can modulate the clock function through direct action on hormone receptors in the SCN [40]. Thus, when melatonin was given to Px or SCGx adult rats under forced desynchronization conditions (22 h LD cycle) [41], the hormone modified the period of the locomotor activity rhythm [42]. In the rat, locomotor activity follows the same pattern as drinking behavior (cited by 42). Since the melatonin given to the mother affects the pups' free-running period of drinking behaviour, and this period is an intrinsic property of the central oscillator, our results would indicate that the maternal melatonin may act directly on the fetal SCN, producing changes in the length of the period, narrowing the distribution of the pups' free-running period within and among litters and bringing it closer to the period of the control pups. These findings are compatible with the idea that maternal melatonin increases the internal coupling of the fetal multioscillatory system.

Probably our results on pup drinking behavior after maternal SCGx and Px may be the expression of desynchronization of the SCN during the embryonic stage. However, maternal entrainment under circadian control may arise from a more complex mechanism than a single hormonal output. This implies that central and peripheral oscillators should all interact in defining the behavior of the organism. Further studies will be necessary to elucidate this question.

Our results suggest that the maternal pineal gland and its hormone, directly or indirectly, affect the performance of the pups' central oscillator and confirm that melatonin plays a functionally relevant entrainment role during early pup rat development, being necessary for the prenatal establishment of young pup circadian rhythms, in synchrony with the social and physical environment.

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