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Seasonal variations of Substance P in the striatum of the female rat are affected by maternal and offspring pinealectomy

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ABSTRACT

The effect of pinealectomy (PIN-X) and PIN-X + melatonin treatment during pregnancy (PIN-X + MEL) 100 μg/100 g body weight on Substance P (SP) in the striatum was investigated in offspring female rats. Female offspring were divided into control and PIN-X at the neonatal period, and studied at days 31 and 60. PIN-X mother/control offspring showed a positive influence on striatal SP values in winter, at both ages and in spring at day 31. However, this effect of maternal PIN-X was not observed in summer or fall. The effect of PIN-X on the offspring showed a positive effect in spring at day 31 and summer at the two ages studied. This effect was not observed in fall or winter. Two generations, PIN-X mother/PIN-X offspring, altered the effect of mother or offspring PIN-X and decreased the SP values in winter, spring and summer. Only striatal SP at day 60 in fall was increased. In two generations PIN-X, the striatal SP values were similar to those observed in control mother/control offspring. The effect of PIN-X + MEL treatment on mothers during pregnancy was inhibitory for the intact offspring and stimulatory for PIN-X offspring. In conclusion, the results indicate that maternal and offspring PIN-X seem to exert a rotative and positive seasonal influence from winter to spring to summer. Two generations PIN-X disrupted this rotative circuit and in fall a compensatory discharge of SP was observed.

The laboratory rat, although a nonphotoperiodic rodent, shows circannual variations in the absence of photoperiod or temperature cues, which can occur even under constant environmental conditions. In the laboratory rat, seasonal changes in hormones [34] or morphometric changes of hypothalamic nuclei [26] were found. This phenomenon of seasonal variations was reflected in pineal melatonin production in rodents under constant environmental conditions. Female Fischer and male Wistar rats kept under constant environmental conditions show seasonal rhythms of pineal activity [2–4]. One possible explanation for this phenomenon could be the influence of geomagnetic fields. The horizontal component H, of the geomagnetic field, may act as a seasonal zeitgeber because H shows a similar seasonal rhythm to melatonin production of female rats during one year, and changes in the direction and intensity of H can affect pineal activity [4].

Substance P (SP) and its receptor have a widespread distribution in the CNS of mammals, which is the major source of this peptide. Immunohistochemical studies have demonstrated the expression of SP [7], and also the presence of SP receptors in the striatum of the rat [28]. In rats, both the density and the distribution of SP-containing neurons change during the period just before birth, the days after birth, and into the adult period. Sp-staminal cells and fibers reached maximum levels between postnatal days 5 and 15, then the density generally decreased [15]. In female and male rat offspring, the maternal pineal gland and prenatal melatonin treatment produced major alterations in the postnatal development of neurokinin A (NKA) and SP in frontal cortex, and striatum [31]. In male offspring, the developmental pattern of the tachykinins NKA and SP in frontal cortex was season-dependent [32].

Regarding the question of the role played by the maternal pineal gland on fetal biological setting, the possibility that the source of melatonin to the fetuses comes from the mother, has been demonstrated in rats [18]. While the biological time clock of rat pups seems to be determined by their mother [13] the mechanism by which the maternal pineal gland plays a role in the entrainment of seasonal variations in the offspring, still remains unclear. In the present study, we attempted to investigate the influence of both maternal and offspring pineal glands on the entrainment of the SP seasonal variations of the striatum.

Female Wistar rats from our colony of the Faculty of Medicine, University of Oviedo, Spain, were housed under controlled environmental conditions for 12 h:12 h photoperiod (lights on at 08.00 a.m.), at a room temperature of approximately 23 °C in a humidity-controlled environment, and they were fed standard rat chow

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and water *ad libitum*. Mother rats were divided into the following groups: control ($n = 37$), pinealectomized (PIN-X) ($n = 29$) and pinealectomized + melatonin (PIN-X + MEL) ($n = 39$). Pinealectomy was carried out two weeks before mating. The study was performed during the four seasons of the year. Mother rats were mating pairs with one male, the possible pregnancy being monitored by the presence of vaginal spermatozoa. During pregnancy mother rats were kept individually in polypropylene cages at the beginning of each season, on day 20 of December, March and June and day 22 of September in 2006–2007. Since pregnancy in the rat lasts 21 days, with this schedule, offspring up to 60 days of age could be studied in the corresponding 3 months of each season, and offspring were studied in the last 2 months of each season. At delivery, the pup litter was adjusted to 12 pups per dam by cross-fostering some pups from larger litters within treatment groups. Pups remained with the mother until weaning on day 21 (day of birth = day 0). Female rats were studied at 31 days of age, juvenile period; and on day 60 when the female rat is sexually mature.

Pinealectomy to mothers was carried out according to the procedure previously described [10,31,32]. All mothers used in the study were examined after weaning to verify the completeness of the PIN-X. Offspring of the mothers with failed pinealectomies were removed from the group, not taken into consideration for the experiment, and new mothers were prepared for the next year at the same season.

Pinealectomy to pups was performed with a method previously described, with some modifications [8]. By day 5 post partum life, female pups were separated from their dam and subjected to hypothermia. This procedure resulted in cessation of respiration and heartbeat. The female pups were put under a heating lamp (60 W) for recovery. When the pups were active, they were returned to the maternal cage. The efficiency of the method was examined at the moment of the sacrifice. Female rats with failed pinealectomy were not taken into consideration for the experiment and new offspring were prepared for the next year in the same season.

Considering previous findings [18] in which 20 μCi of ^{3}H-acetyl-melatonin was administered to pregnant rats, and that each fetus contained about 0.1% (20nCi) of the injection dose, 100 μg Mel/100 g body weight was used as a standard dose in the present study. Melatonin treatment was given to mother rats during pregnancy by subcutaneous injections at the end of the light phase.

The striatum samples were dissected from the brain and processed as previously described [31,32]. Substance P (SP) concentration in striatal extracts was determined by radioimmunoassay, as previously described [12,31]. For statistical analysis, the data were analyzed by means of two way analysis of variance (ANOVA), and showed a positive interaction between the maternal and the offspring related treatments ($F = 3.481$; $p < 0.004$), and variable season-type ($F = 4.401$; $p < 0.000$). But there were no significant differences for the variable season ($F = 0.667$; $p < 0.573$) and the variable age ($F = 0.024$; $p < 0.876$). The post hoc test for comparing groups was obtained by Tukey's method, in each season and for each age. The level of significance was set as $p < 0.05$.

Values of each group were expressed as means ± S.E.M. (Table 1).

Spring, 31-day-old offspring. The striatal SP value of control mother/control offspring group was significantly lower ($p < 0.001$) than the control mother/PIN-X offspring. This significant difference was not observed between PIN-X mother/control offspring vs. PIN-X mother/PIN-X offspring, or between PIN-X + MEL mother/control offspring vs. PIN-X + MEL mother/PIN-X offspring. The control mother/control offspring group showed significantly lower ($p < 0.001$, $p < 0.05$, respectively) SP values compared to the PIN-X mother/control offspring and to the PIN-X + MEL mother/control offspring. However, no significant differences were observed when the control mother/PIN-X offspring was compared to the PIN-X mother/PIN-X offspring or to PIN-X + MEL mother/PIN-X offspring.

Spring, 60-day-old offspring. The striatal SP value was significantly higher ($p < 0.001$) in the PIN-X mother/control offspring compared to the PIN-X mother/PIN-X offspring, and significantly lower as compared to control mother/control offspring ($p < 0.05$). The PIN-X mother/Pin-X offspring showed significantly lower values ($p < 0.01$) compared to control mother/PIN-X offspring and PIN-X + MEL mother/PIN-X offspring. Also, the PIN-X + MEL mother/PIN-X offspring SP value was significantly higher ($p < 0.05$) compared to PIN-X + MEL mother/control offspring.

Summer, 31-day-old offspring. As it was found in spring, the SP value of the control mother/control offspring group was significantly lower ($p < 0.05$) compared to the control mother/PIN-X offspring. A significantly lower ($p < 0.001$) SP value was found in PIN-X + MEL mother/control offspring compared to PIN-X + MEL offspring/PIN-X offspring. However, no significant differences between PIN-X mother/control offspring vs. PIN-X mother/PIN-X offspring were found. The PIN-X mother/PIN-X offspring showed significantly lower SP values ($p < 0.05$) compared to the control mother/PIN-X offspring and to PIN-X + MEL mother/PIN-X offspring.

Summer, 60-day-old offspring. The striatal SP value was significantly lower ($p < 0.01$) in control mother/control offspring group compared to the control mother/PIN-X offspring group. Similarly, in the PIN-X + MEL mother/control offspring, SP was significantly lower vs. PIN-X + MEL mother/PIN-X offspring and control mother/control offspring ($p < 0.01$, $p < 0.05$, respectively). No significant differences between PIN-X mother/control offspring and PIN-X mother/PIN-X offspring were found. SP in the PIN-X mother/PIN-X offspring was significantly lower ($p < 0.01$, $p < 0.001$, respectively) compared to control mother/PIN-X offspring and to PIN-X + MEL mother/PIN-X offspring ($p < 0.001$). In the control mother/PIN-X offspring SP values were significantly higher ($p < 0.05$) than in PIN-X + Mel mother/PIN-X offspring.

Fall, 31-day-old offspring. In fall, the significantly lower SP value of control mother/control offspring vs. control mother/PIN-X offspring, as previously observed, was not evident. However, significantly lower SP values in the PIN-X + Mel mother/control offspring compared to PIN-X + MEL mother/PIN-X offspring and to PIN-X mother/control offspring ($p < 0.01$, $p < 0.05$, respectively) were observed. The control mother/control offspring SP value was significantly higher ($p < 0.001$) compared to PIN-X + MEL mother/control offspring.

Fall, 60-day-old offspring. In the control mother/control offspring, striatal SP was significantly higher ($p < 0.01$) compared to PIN-X mother/control offspring and to PIN-X + MEL mother/control offspring. In the PIN-X mother/PIN-X offspring, SP was significantly higher ($p < 0.001$) compared to PIN-X mother/control offspring and to control mother/PIN-X offspring. The PIN-X + MEL mother/PIN-X offspring showed the highest SP value of all the year with significant differences vs. PIN-X + MEL mother/control offspring, PIN-X mother/PIN-X offspring and control mother/PIN-X offspring ($p < 0.01$, $p < 0.5$, respectively).

Winter, 31-day-old offspring. The control mother/control offspring SP value was significantly lower ($p < 0.01$) compared to the PIN-X mother/control offspring and PIN-X + MEL mother/control offspring groups. In PIN-X mother/control offspring, SP was significantly higher ($p < 0.001$) than in PIN-X mother/PIN-X offspring. The highest value of SP was observed in PIN-X + MEL mother/PIN-X offspring, showing significant differences ($p < 0.01$) vs. control mother/PIN-X offspring and PIN-X mother/PIN-X offspring.

Winter, 60-day-old offspring. Striatal SP in the control mother/control offspring was significantly lower ($p < 0.01$; $p < 0.05$) compared to PIN-X mother/control offspring and to PIN-X + Mel mother/control offspring. In the PIN-X mother/control offspring, striatal SP was significantly higher ($p < 0.05$) compared to PIN-X mother/PIN-X offspring. In the PIN-X + MEL mother/PIN-X

Table 1
Seasonal variations of SP concentrations in the striatum of female control and pinealectomized (Px) offspring of control, Px or Px + MEL (100 μg/100 g body weight) treated mother rats during pregnancy. Values are mean + SEM, *N* = number of cases.

	SP pg/ml	
	Age 31 days old	Age 60 days old
Spring		
Control mother/control offspring (C–C)	5.10 ± 0.81 (*N* = 12)	29.74 ± 2.52 (*N* = 12)
Control mother/PIN-X offspring (C–Px)	18.00 ± 4.70 (*N* = 12)	13.84 ± 3.04 (*N* = 11)
PIN-X mother/control offspring (Px–C)	19.66 ± 4.10 (*N* = 12)	19.12 ± 5.00 (*N* = 12)
PIN-X mother/PIN-X offspring (Px–Px)	8.94 ± 2.73 (*N* = 12)	7.35 ± 1.33 (*N* = 10)
PIN-X + MEL mother/control offspring (Px + Mel–C)	18.68 ± 5.41 (*N* = 11)	8.66 ± 2.20 (*N* = 11)
PIN-X + MEL mother/PIN-X offspring (Px + MEL + Px)	12.94 ± 2.45 (*N* = 11)	20.61 ± 7.46 (*N* = 12)
31 days old: C–C vs. Px–C: $p < 0.001$, C–C vs. C–Px: $p < 0.01$, C–C vs. Px + MEL–C: $p < 0.05$.		
60 days old: Px–C vs. Px–Px: $p < 0.001$, Px–Px vs. C–Px and vs. Px + MEL–Px: $p < 0.01$, Px–C vs. C–C and Px + MEL–Px vs. Px + MEL–C: $p < 0.05$.		
Summer		
Control mother/control offspring (C–C)	16.16 ± 4.23 (*N* = 12)	8.30 ± 1.72 (*N* = 11)
Control mother/PIN-X offspring (C–Px)	42.96 ± 10.38 (*N* = 11)	47.29 ± 1.28 (*N* = 12)
PIN-X mother/control offspring (Px–C)	21.63 ± 9.07 (*N* = 12)	3.70 ± 1.26 (*N* = 11)
PIN-X mother/PIN-X offspring (Px–Px)	9.10 ± 2.91 (*N* = 12)	2.52 ± 0.48 (*N* = 11)
PIN-X + MEL mother/control offspring (Px + Mel–C)	6.44 ± 2.56 (*N* = 12)	1.85 ± 0.95 (*N* = 11)
PIN-X + MEL mother/PIN-X offspring (Px + MEL + Px)	21.29 ± 5.41 (*N* = 12)	15.05 ± 4.63 (*N* = 12)
31 days old: Px–Px vs. C–Px: $p < 0.001$, Px–Px vs. Px + MEL–Px and Px + MEL–C vs. Px + MEL–Px: $p < 0.01$, C–C vs. C–Px: $p < 0.05$.		
60 days old: Px–Px vs. Px + Mel–Px: $p < 0.001$, C–C vs. C–Px, Px–Px vs. C–Px and Px + Mel–C vs. Px + Mel–Px: $p < 0.01$, C–C vs. Px + MEL–C; C–Px vs. Px + MEL–Px: $p < 0.05$.		
Fall		
Control mother/control offspring (C–C)	10.65 ± 4.20 (*N* = 10)	23.48 ± 9.63 (*N* = 12)
Control mother/PIN-X offspring (C–Px)	12.42 ± 4.82 (*N* = 12)	10.85 ± 4.90 (*N* = 12)
PIN-X mother/control offspring (Px–C)	27.37 ± 4.41 (*N* = 12)	4.11 ± 1.74 (*N* = 12)
PIN-X mother/PIN-X offspring (Px–Px)	12.50 ± 5.19 (*N* = 11)	42.37 ± 10.51 (*N* = 11)
PIN-X + MEL mother/control offspring (Px + Mel–C)	1.71 ± 0.31 (*N* = 11)	12.57 ± 2.42 (*N* = 11)
PIN-X + MEL mother/PIN-X offspring (Px + MEL + Px)	23.82 ± 6.92 (*N* = 12)	92.58 ± 29.59 (*N* = 10)
Age, 31 days old: C–C vs. Px + Mel–C: $p < 0.001$, Px–C vs. Px + Mel–C: $p < 0.01$, Px + Mel–C vs. Px + Mel–Px: $p < 0.001$.		
Age, 60 days old: C–C vs. Px–C, C–C vs. Px + Mel–C, C–Px vs. Px + Mel–Px and C–Px vs. Px–Px: $p < 0.01$, Px–Px vs. Px + Mel–PX: $p < 0.05$, Px–C vs. Px–Px: $p < 0.001$, Px + Mel–C vs. Px + Mel–Px: $p < 0.01$.		
Winter		
Control mother/control offspring (C–C)	8.92 ± 3.20 (*N* = 11)	5.43 ± 1.75 (*N* = 12)
Control mother/PIN-X offspring (C–Px)	7.80 ± 4.24 (*N* = 12)	11.93 ± 4.61 (*N* = 12)
PIN-X mother/control offspring (Px–C)	31.28 ± 11.79 (*N* = 8)	52.04 ± 20.86 (*N* = 10)
PIN-X mother/PIN-X offspring (Px–Px)	5.13 ± 0.77 (*N* = 12)	4.58 ± 1.76 (*N* = 10)
PIN-X + MEL mother/control offspring (Px + Mel–C)	18.16 ± 6.36 (*N* = 12)	33.03 ± 13.51 (*N* = 11)
PIN-X + MEL mother/PIN-X offspring (Px + MEL + Px)	38.12 ± 11.79 (*N* = 12)	26.42 ± 9.29 (*N* = 10)
Age, 31 days old: C–C vs. Px–C and C–C vs. Px + Mel–C: $p < 0.01$; Px–C vs. Px–Px: $p < 0.001$, Px + Mel–Px vs. C–Px and Px + Mel–Px vs. Px–Px: $p < 0.01$.		
Age, 60 days old: C–C vs. Px–C: $p < 0.01$, C–C vs. Px + Mel–C and Px–C vs. Px–Px: $p < 0.05$, Px + Mel–Px vs. C–Px: $p < 0.05$, Px + Mel–Px vs. Px–Px: $p < 0.01$.		

offspring striatal SP was significantly higher ($p < 0.05$; $p < 0.01$, respectively) compared to control mother/PIN-X offspring and to PIN-X mother/PIN-X offspring.

The biological significance of the different levels of SP in the striatum at the four seasons may be related to the overall presence of SP with its receptors in the CNS of mammals. This peptide plays a very important role as a neurotransmitter/neuromodulatory agent [24]. Tachykinins are involved in the central control of several autonomic functions, affective and emotional life and high cerebral functions related to learning and memory [27]. Previously reported evidence has suggested that brain SP contributes to pain perception and elaboration [10,19,21].

Comparisons of the same type of treatment at the different seasons and age influence. Only in winter, the quiescent season, a similar SP development from 31- to 60-day-old rats was observed between the control mother/control offspring group and control mother/PIN-X offspring group. The PIN-X + MEL mother/control offspring SP development from 31- to 60-day-old rats was similar to the control mother/control offspring group in summer and fall.

The suprachiasmatic nucleus (SCN) of the hypothalamus contains a circadian pacemaker that controls a variety of physiological and behavioral rhythms [1,9]. The SCN is connected to the pineal gland by a multisynaptic pathway that synchronizes the melatonin circadian rhythm [23,29]. Melatonin receptors have been described in the SCN of the hypothalamus [33] and have a direct effect on the circadian pacemaker [1]. PIN-X abolishes the melatonin circadian rhythm [25].

The results of the striatal SP concentrations in the four seasons of the year were affected by maternal PIN-X or offspring PIN-X depending on the season of the year. In winter at both ages studied and spring at day 31, it was observed that PIN-X mother/control offspring had significantly increased striatal SP concentrations compared to control mother/control offspring. All these results indicate the influence of maternal melatonin during the uterine life on the postnatal development of SP in the striatum. An influence of the maternal PIN-X on the development of SP in the striatum and frontal cortex was previously found in male offspring [31,32]. In rodents during late embryonic and early neonatal development, circadian rhythms develop in synchrony with those of their mothers. Maternal pinealectomy performed on day 7 of gestation significantly disrupted the rat pupsï drinking behavior. In these animals, when melatonin was given to mothers for 5 days during the late period of gestation, this treatment reversed the effects of maternal PIN-X [6]. There is evidence that the maternal pineal gland influenced prenatal and postnatal development of offspring, and suppressed the incidence of spontaneous malformations [17]. An influence of the maternal pineal gland on postnatal ontogeny of the hormones involved in the neuroendocrine–reproductive axis was found in both female and male developing rats [11].

In summer, the maternal PIN-X did not show influences on the striatal SP in offspring as compared to control mothers. Instead,

the SP value was affected by the PIN-X offspring. The effect of PIN-X offspring on SP values started in spring at day 31 and this can be interpreted as a burst after the quiescent season. The climax of such an effect was observed in summer and established at both prepubertal and adult ages. After the summer increase, the effect of PIN-X offspring on SP values started to decrease during fall up to winter. The positive influence of the PIN-X offspring was observed in the opposite half of the year in comparison with the influence of maternal PIN-X, observed in winter. This clearly indicates a seasonal distribution of the influence of the pineal gland. Rat pineal glands were tested for tumor-inhibiting activity in an in vitro microassay using erythroleukemia cells. The highest activity was detected in summer and the smallest inhibition and even a stimulation were observed in winter [5]. A seasonal variation in the development and growth of mammary carcinogenesis induced by 7,12-dimethyl/benz[a]anthracene was found in Sprague–Dawley rats [22]. The effect of PIN-X offspring indicates that entrainment of seasonal rhythms in rats is not only dependent on the maternal biological clock, but also on their own melatonin rhythm. It is known that the rat pineal gland generates its own melatonin circadian rhythm [30]. Indeed, individual murine pups, born to arrhythmic mothers in constant darkness without external zeitgeber, developed normal circadian rhythms, but their clocks were less synchronized to each other compared to wild-type animals [16].

In fall, the significant influence of PIN-X mother or PIN-X offspring on the female offspring SP development, previously observed, was not evident. However, in fall, the effect of two generations, PIN-X, PIN-X mother/PIN-X offspring at day 60, resulted in significantly increased striatal SP concentrations compared with PIN-X mother/control offspring and control mother/PIN-X offspring. This positive effect of two generations PIN-X on striatal SP was only observed in this season, followed by a decrease again in winter. It is worth mentioning that SP values of two generations PIN-X, PIN-X mother/PIN-X offspring were very similar to those of control mother/control offspring with the exception observed in spring and fall at day 60. For the first time we examined the effect of two generations, mother and offspring PIN-X. In this group the pups did not receive the melatonin signal cue on the SCN during the fetal development or after birth, and the melatonin cue on the entrainment of circadian and seasonal rhythmicity was not present. Different studies have considered if the biological clock that controls rhythmicity is set during the fetal development or after the newborn is exposed to light–dark stimulus. It was found that the biological clock for pineal N-acetyltransferase enzyme rhythm, necessary for melatonin biosynthesis, is generated independently of the environmental schedules [13]. The observation suggests that in the absence of the light–dark cycle, the clock time of pups is determined by their mothers so as to synchronize their own clock time [13]. In Syrian hamsters exogenous melatonin can entrain the free running clock [14]. The c-fos clock gene responsible for entrainment of circadian rhythms showed statistically significant expression in the rat SCN by photic stimulation at circadian time 22 on postnatal day 1. But hybridization above background levels can be detected in the SCN of some rat pups earlier in the subjective night [20]. The rhythms in clock gene expression in the rat SCN develop mostly postnatally [29].

If we compare these previous references with our results, it can be inferred that the entrainment of the rhythmicity has two components, one experienced in utero and the second in postnatal life.

From our data, a mechanism related to the control of seasonal variations by the pineal gland could be in place. When the maternal and the offspring pineal glands are functionally active, the normal sequence of the seasons results in small changes and only at the end of spring and fall, at day 60, were high concentrations of SP found. This indicates that, in control rats, the entrainment of seasonal variations is not established up to the adult age. This pattern was altered by the effect of the maternal or offspring PIN-X in a season-dependent manner. When a biological clock, maternal or offspring cue is lacking, the resulting entrainment of seasonal variations is altered. Therefore, both maternal and offspring clock signals are necessary for the development of the seasonal variations. However, when both clock signals are absent, SP in the striatum remains at low levels during the four seasons and at the end of the fall this circuit is altered, showing a compensatory discharge of SP. It seems that under two generations PIN-X, the organism could not recognize the external zeitgeber signal and remains depressed for most of the year. The information obtained from the maternal and offspring pineal glands on the mechanisms of seasonal variations, suggests a suppressive effect of the pineal gland on their development. The influence of maternal and postnatal PIN-X was investigated on affective responses in Siberian hamsters. Maternal PIN-X increased rearing behavior and postnatal PIN-X increased locomotor behavior in the open field test [35].

The maternal melatonin treatment was administered to PIN-X mothers in order to provide the replacement for the lack of the endogenous melatonin secretion. Melatonin was only administered during pregnancy and not during the lactation period. The effect of maternal melatonin administration was different in the intact- or PIN-X-offspring. For the PIN-X + MEL mother/control offspring, maternal melatonin treatment affected striatal SP concentrations in winter, summer and fall in 31-day-old rats, decreasing the effect of PIN-X mother/control offspring. For the PIN-X + MEL mother/PIN-X offspring, as the offspring did not produce endogenous melatonin, the response was different. Maternal melatonin treatment during pregnancy resulted in a stimulatory influence at day 60 in winter, spring, summer and fall, with a burst of SP secretion, and at day 31 in winter and summer, a reversal of the inhibitory effect of PIN-X mother/PIN-X offspring was observed. These results indicate that the offspring pineal gland participates in the normal development of the seasonal changes.

In conclusion, the present results indicate that the PIN-X of the mother increases striatal SP concentrations in winter at both ages and in spring at day 31, but not in summer or fall. PIN-X of the offspring affects striatal SP concentrations in spring at day 31 and summer at both ages studied, but not in fall or winter. The effect of two generations, PIN-X, PIN-X mother/PIN-X offspring, resulted in low SP concentrations throughout the year with the exception observed at day 60 in fall. The maternal PIN-X + MEL treatment on the seasonal variations of the offspring was dependent on the own melatonin rhythm of the offspring. In the control offspring with endogenous melatonin rhythm the effect was only observed at the prepubertal age. However, in the PIN-X offspring without endogenous melatonin rhythm, the effect was observed up to the adult age with a burst in SP secretion.

References

[1] S.M. Amstrong, Melatonin and circadian control in mammals, Experientia 45 (1989) 932–938.
[2] M.G. Balemans, F.A. Bary, W.C. Legerstee, J. Van Benthem, Seasonal variations in HIOMT activity during the night in the pineal gland of 21 day old male Wistar rats, J. Neural Transm. 49 (1980) 107–116.
[3] H. Bartsch, C. Barstch, F. Deerberg, D. Mecke, Seasonal rhythms of 6-sulphatoxymelatonin (aMT6s) excretion in female rats are abolished by growth of malignant tumors, J. Pineal Res. 31 (2001) 57–61.
[4] H. Bartsch, C. Bartsch, D. Mecke, T.H. Lippert, Seasonality of pineal melatonin production in the rat: possible synchronization by the geomagnetic field, Chronobiol. Int. 11 (1994) 21–26.
[5] H. Bartsch, C. Bartsch, D. Gupta, Tumor-inhibiting activity in the rat pineal gland displays a circannual rhythm, J. Pineal Res. 11 (1990) 171–178.
[6] S.L. Bellavía, A.R. Carpentieri, A.M. Vaqué, A.F. Macchione, N.T. Vermouth, Pup circadian rhythm entrainment-effect of maternal ganglionectomy or pinealectomy, Physiol. Behav. 89 (2006) 342–349.

[7] C.L. Brandon, H. Steiner, Repeated methylphenidate treatment in adolescent rats alters gene regulation in the striatum, Eur. J. Neurosci. 18 (2003) 1584–1592.
[8] K.D. Cairncross, A. Parks Ann, A.V. Everitt, P. Destefanis, C.A. Maxwell, The use of hypothermia in surgical pinealectomy of the neonate rat, Physiol. Behav. 45 (1989) 663–665.
[9] F.R. Cardoso, F.A. de, O. Cruz, D. Silva, C.M. Cortez, A simple model for circadian timing by mammals Braz, J. Med. Biol. Res. 42 (2009) 122–127.
[10] A. Carlsson, M. Ohsawa, M. Hallberg, F. Nyberg, J. Kamei, Substance P(1–7) induces antihyperalgesia in diabetic mice through a mechanism involving the naloxone-sensitive sigma receptors, Eur. J. Pharmacol. 626 (2010) 250–255.
[11] B. Díaz López, M.D. Colmenero Urquijo, E. Díaz Rodríguez, A. Arce Fraguas, A. Esquifino Parras, B. Marín Fernández, Effect of pinealectomy and melatonin treatment during pregnancy on the sexual development of the female and male rat offspring, Eur. J. Endocrinol. 132 (1995) 765–767.
[12] L. Debeljuk, J.N. Rao, A. Bartke, Developmental changes of tachykinins in the hypothalamus and anterior pituitary of female Siberian Hamsters from prepuberty to adulthood, Peptides 16 (1995) 827–831.
[13] T. Deguchi, Ontogenesis of a biological clock for serotonin: acetyl coenzyme A N-acetyltransferase in pineal gland of rat, Proc. Nat. Acad. Sci. U.S.A. 72 (1975) 2814–2818.
[14] M.H. Hastings, G.E. Duffield, F.K. Ebling, A. Kidd, E.S. Maywood, I. Schurov, Non-photic signaling in the suprachiasmatic nucleus, Biol. Cell 89 (1997) 495–503.
[15] S. Inagaki, M. Sakanaka, S. Shiosaka, E. Senba, K. Takatsuki, H. Takagi, Y. Kawai, H. Minagawa, M. Tohyama, Ontogeny of substance P-containing neuron system of the rat: immunohistochemical analysis. I. Forebrain and upper brainstem, Neuroscience 7 (1982) 251–277.
[16] C. Jud, U. Albrecht, Circadian rhythms in murine pups develop in absence of a functional maternal circadian clock, J. Biol. Rhythm 21 (2006) 149–154.
[17] T. Kachi, D. Tanaka, S. Watanabe, R. Suzuki, Y. Tonosaki, H. Fujieda, Physiological pineal effects on female reproductive function of laboratory rats: prenatal development of pups, litter size and estrous cycle in middle age, Chronobiol. Int. 23 (2006) 289–300.
[18] D.C. Klein, Evidence for the placental transfer of ^{3}H-acetyl-melatonin, Nat. New Biol. 237 (1972) 11–18.
[19] H. Kohara, S. Tajima, M. Yamamoto, Y. Tabata, Angiogenesis induced by controlled release of neuropeptide substance P, Biomaterials 31 (2010) 8617–8645.
[20] L.E. Leard, E.S. Macdonald, H.C. Heller, T.S. Kilduff, Ontogeny of photic c-fos mRNA expression in rat suprachiasmatic nuclei, Neuroreport 20 (1994) 2683–2687.
[21] H. Liu, H. Gong, Z. Liu, G. Liu, Z. Li, Effects of acute exposure of alpha 1- and alpha 2-adrenoreceptor agonist or antagonist on capsaicin-evoked substance P release from dorsal root ganglion neurons in vitro, Pharmazie 65 (2010) 47–50.
[22] W. Löscher, M. Mevissen, B. Häussler, Seasonal influence on.7 12-dimethylbenz[a]anthracene-induced mammary carcinogenesis in Sprague–Dawley rats under controlled laboratory conditions, Pharmacol. Toxicol. 81 (1997) 265–270.
[23] R.Y. Moore, D.C. Klein, Visual pathways and central control of a circadian rhythm in pineal serotonin_N-acetyltransferase activity, Brain Res. 71 (1974) 17–34.
[24] M. Otsuka, K. Yoshioka, Neurotransmitter functions of mammalian tachykinins, Physiol. Rev. 73 (1993) 229–308.
[25] R.W. Pelham, A serum melatonin rhythm in chickens and its abolition by pinealectomy, Endocrinology 96 (1975) 543–546.
[26] D. Peschke, J. Teichmann, E. Peschke, Pineal influence on annual nuclear volume changes in ventromedial hypothalamic nucleus (VMH) neurons of the male Wistar rat, Chronobiol. Int. 17 (2000) 15–28.
[27] C. Severini, G. Improta, G. Falconieri-Erspamer, S. Salvadori, V. Erspamer, The tachykinin peptide family. The American Society for Pharmacology and Experimental Therapeutics, Pharmacol. Rev. 54 (2002) 285–322.
[28] R. Shigemoto, S. Nakaya, R. Nomura, R. Ogawa-Meguro, H. Ohishi, T. Kaneko, S. Nakanighi, N. Mizuno, Immunocytochemical localization of substance P in the rat striatum, Neurosci. Lett. 153 (1993) 157–160.
[29] A. Sumová, Z. Bendová, M. Sládek, Z. Kovácíková, R. El-Hennamy, K. Laurinová, H. Illnerová, The rat circadian clockwork and its photoperiod entrainment during development, Chronobiol. Int. 23 (2006) 237–243.
[30] P.L. Tang, S.F. Pang, The ontogeny of pineal and serum melatonin in male rats at mid-light and mid-dark, J. Neural Transm. 72 (1988) 43–53.
[31] N. Vázquez Moreno, L. Debeljuk, E. Díaz Rodríguez, C. Fernández Álvarez, B. Díaz López, Seasonal changes of SP and NKA in frontal cortex, striatum and testes in the rat. Role of maternal pineal gland, Peptides 25 (2004) 997–1004.
[32] N. Vázquez Moreno, L. Debeljuk, E. Díaz Rodríguez, C. Fernández Álvarez, B. Díaz López, Influence of maternal pineal gland on the developmental pattern of neurokinin A (NKA) and substance P (SP) in male-rat-offspring: relationship to the season of the year, Neurosci. Lett. 368 (2004) 243–248.
[33] D.R. Weaver, S.A. Rivkees, S.M. Reppert, Localization and characterization of melatonin receptors in rodent brain by in vitro autoradiography, J. Neurosci. 9 (1989) 2581–2590.
[34] C.C. Wong, K.D. Döler, M.J. Atkinson, H. Geerlings, R.D. Hesch, A. von zur Mühlen, Circannual variations in serum concentrations of pituitary, thyroid, parathyroid, gonadal and adrenal hormones in male laboratory rats, J. Endocrinol. 97 (1983) 179–185.
[35] J.L. Workman, Z.M. Weil, C.R. Tuthill, R.J. Nelson, Maternal melatonin increases depressive-like responses in Siberian hamster offspring, Behav. Brain Res. 189 (2008) 387–391.