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Day/night expression of MT_1 and MT_2 receptors in hypothalamic nuclei of the primate *Sapajus apella*

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ABSTRACT

Melatonin is involved in the temporal organization of several physiological and behavioral events, controlled by hypothalamic nuclei, like sleep, feeding, reproduction and metabolic modulation and acts through two types of high-affinity G protein-coupled membrane receptors: MT_1 and MT_2. This study aimed to investigate the expression of MT_1 and MT_2 receptors proteins in four hypothalamic nuclei, i.e., SCN, supraoptic (SON), paraventricular (PVN) and anteroventral periventricular nuclei (AVPV), of the diurnal primate *Sapajus apella* using immunohistochemistry. Since these areas are involved in the expression of biological rhythms, they are candidates to have variations in their neurochemistry, so the MT_1 and MT_2 expression has been analyzed at a point in light and another in the dark phase. Both receptors were found to have day/night differences in the four hypothalamic nuclei with an apparent inverse expression in the SCN compared with the other areas. These differences could be related to the idea that the individual should be prepared to respond by different ways to melatonin signal within the several processes and can contribute to the efficacy of melatonin ligands or melatonin in therapies.
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1. Introduction

Via a pathway that involves the suprachiasmatic nucleus (SCN), the dark/light cycle determines the peak production of melatonin by the pineal gland in the plasma and cerebrospinal fluid (CSF) during the night, ensuring a neuroendocrine signal of darkness to the body (Reiter et al., 2014). Melatonin, in turn, acts by feeding back on the SCN (Gillette and McArthur, 1996), and possibly on other brain areas, contributting to the temporal organization of various physiological and behavioral events such as sleep (Arendt and Skene, 2005), locomotor activity, mood, anxiety, thermogenesis, immune responses, cardiac functions, blood pressure (Buijs and Kalsbeek, 2001; Xia et al., 2008), reproduction (Chai et al., 2013), feeding behavior and energy metabolism (Cipolla-Neto et al., 2014; Reiter et al., 2012) some of these controlled by the hypothalamic areas.

Among a wide variety of functions (Claustrat et al., 2005; Ekmekcioglu, 2006), melatonin can act on various brain areas directly (Dubocovich et al., 2003; Lall et al., 2012; Stehle et al., 2003) per high-affinity G-protein coupled receptors in the membrane, melatonin receptor 1 (MT_1) and melatonin receptor 2 (MT_2) (Ekmekcioglu, 2006) wherein its physiological or therapeutic efficacy depends directly on the expression of these receptors (Wu et al., 2007).

Regarding their presence in the central nervous system (CNS), MT_1 or MT_2 receptors have already been identified in hypothalamic (Lacoste et al., 2015; Wu et al., 2013), and others brain areas (Drew et al., 2001; Lacoste et al., 2015; Reppert et al., 1995) with certain variability in different species (Stankov et al., 1991). Considering that, novel therapeutic strategy in several treatments involves the action of MT_1 and MT_2 agonists or antagonists these descriptions are crucial to understand the melatonin effects. Despite, most of the studies available on melatonin receptors expression are in rodents, and so far, one study in humans investigated day/night differences in the MT_1 and MT_2 expression in the SCN (Wu et al., 2013).

To analyzing this issue in a diurnal model (new world primate) with social habits, intense maternal care, complex fine motor skills, and well developed frontal lobes (Fragaszy and Adams-Curtis, 1991; Lavalle, 1999), would add relevant information to knowledge

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in this area. The *Sapajus apella* has been used to the circadian timing system study and shows several behavioral and anatomical brain characteristics that are similar to those of humans (Terborgh, 1983).

Our hypothesis is that the expression of these receptors could be responsive to extrinsic environmental changes as day/night cycle or intrinsic changes as melatonin content in order to adapt the functioning of the hypothalamic areas. Following this reasoning, the aim of the present study was to describe day/night MT_1 and MT_2 expression proteins in the SCN, anteroventral periventricular nuclei (AVPV), paraventricular (PVN) and supraoptic (SON) nuclei in the primate *Sapajus apella*.

2. Methods

2.1. Animals

Brains of 6 adult male *Sapajus apella*, obtained from the Center of Tufted Capuchin Monkey Procreation of the São Paulo State University (UNESP), Araçatuba, SP, Brazil, were used. The animals were housed under natural light and were fed with a controlled diet that consisted of eggs, fruit, a granulated ration with protein, and dried corn; water was provided *ad libitum*.

The sunrise time during the experiments was at approximately 0600 h and was considered the Zeitgeber time 0 (ZT 0) as a reference; the sunset time started at approximately 1800 h. The "procedures involving animals use" were compliant with the "Guidelines for the care and use of mammals in neuroscience and behavioral research (2003)" and all experiments were approved by the local ethic committee (IACUC) for animal use of the São Paulo State University (CEUA-FOA, protocol number 2013-00259).

2.2. Tissue collection

The animals were anesthetized with sodium thiopental (30 mg/kg, i.p.) and euthanized at ZT 2 (day time point) and ZT 14 (night time point, anesthetized in dim light), N = 3 per ZT. Following the protocol described in Campos et al. (2014), after transcardiac perfusion the brains were cryoprotected and cryosectioned, and the sections of 30 μm were stored as 10 different stepwise series in anti-freeze solution.

Nissl stain was performed in one series of each animal to cytoarchitectural control. For each animal, all of the coronal sections of other series representing the entire extent of the hypothalamus were placed in rostrocaudal order. After this, sections representing the same rostrocaudal levels of the hypothalamic nuclei from each animal, containing the areas of interest for this study were processed for each antibody.

2.3. Immunohistochemistry

The brain sections were processed using MT_1 goat polyclonal (1: 200, Santa Cruz Biotechnology sc13179, TX, USA) and MT_2 goat polyclonal (1: 200, Santa Cruz Biotechnology sc28453, TX, USA) receptors and vasoactive intestinal polypeptide (VIP) rabbit polyclonal (1: 250, Abcam ab8556, Cambridge, UK) antibodies. Following the protocol described in Campos et al. (2014), the slices were pre-incubated for 2 h in TBS-TX containing 2% normal serum. Then, a combination of the MT_1 and VIP or MT_2 and VIP primary antibodies were added for 48 h. Next, the sections were washed in buffer and incubated for 2 h in Alexa 488 − donkey-anti-rabbit (1: 200, Jackson immuno research, 711-545-152, West Grove, PA, USA) and Cy3- donkey-anti-goat (1: 200, Jackson immuno research, 705-165-147, West Grove, PA, USA) fluorescent secondary antibodies specific for each species of primary antibody. The nuclei of the cells were stained with 6-diamidino-2-phenylindole (DAPI) (Sigma Chemical, D9542, St. Louis MO, USA), and the slices were analyzed by epifluorescence microscopy.

The preadsorption of the anti-MT_1 and anti-MT_2 antibodies with the immunogenic peptides MEL-1A-R (N-20) P (Santa Cruz Biotechnology, sc13179 peptide, TX, USA), and MEL-1B-R (G-20) P (Santa Cruz Biotechnology, sc28453 peptide, TX, USA) eliminated any positive staining in the hypothalamic sections. These control reactions and the results showing different patterns of expression in different areas supported the specificity of the antibodies in this study.

2.4. Data analysis

The brain sections were analyzed under a light field (Olympus BX50 microscope), and the images were acquired using the cellSens software (USA). The numbers of neurons were counted, using the tool cellcounting of the digital image processing and analysis software ImageJ (McMaster Biophotonics Facility, Canada).

For each animal, five coronal sections of one series in which the SCN, PVN, AVPV and SON were present were analyzed. The number of sections in which the areas was present varied among them depending of it rostrocaudal extension (eg. SCN was present in three sections, SON in five). In order to allow the assembly of the image containing the entire structures several pictures were taken with a magnification of 20×. To avoid overlapping fields the assembly performed by the cellSens software was manually checked and corrected if necessary.

The delimitation of the boundaries of each nucleus was based in the clusters of cell nuclei stained with DAPI and cells stained by Nissl staining (0.5% thionine in distilled water). In the SCN, the ventrolateral (VL) and the dorsomedial (DM) divisions were based in the location of VIP imunorreactive neurons, that classically marks the VL portion in rodents and primates (Abrahamson and Moore, 2001; Cassone et al., 1988; Ramanathan et al., 2006; Reghunandanan and Reghunandanan, 2006; Rocha et al., 2014; Campos et al., 2014) and by the description of previous studies which characterized these divisions in this same species (Rocha et al., 2014; Campos et al., 2014).

All visible IR cells (MT_1-IR, MT_2-IR, VIP-IR, MT_1/VIP-IR, and MT_2/VIP-IR) were counted in each image. The data were expressed as the means ± standard error of the mean of the IR cell number counted in all sections of each nucleus and the One-way analysis of variance followed by Newman-Keuls Multiple Comparison Test was applied to compare the data.

3. Results

The anatomical localization and organization of the hypothalamic nuclei, SCN, AVPV, PVN and SON, were identified as clusters of neurons arranged into longitudinal zones (in mediolateral direction), which were termed the periventricular, medial and lateral zones of the preoptic area of the hypothalamus (Fig. 1).

In the SCN, the cell number quantification indicated that both melatonin receptors were mainly expressed at night (MT_1 99.9 ± 2.9; MT_2 164.9 ± 7.9) and less expressed in the day time point (MT_1 39.7 ± 3.1; MT_2 80.1 ± 3.4) (Fig. 2). The immunofluorescence analysis revealed a complex organization of the both melatonin receptors inside this nucleus in different levels (rostral level, Fig. 3; caudal level, Fig. 4). In the day time point, the MT_1 receptor was mainly expressed in the DM subdivision of the SCN (DM 31.8 ± 2.9; VL 8.0 ± 1.2, $p < 0.0001$) (Fig. 3B and H), showing few MT_1-IR cells colocalizing with vasoactive intestinal polypeptide (VIP-IR) cells that in this level are expressed in the VL subdivision (Figs. 3 C and 5 I). At the night time point, there was a higher number ($p < 0.0001$) of MT_1-IR cells in the DM (52.0 ± 2.9)

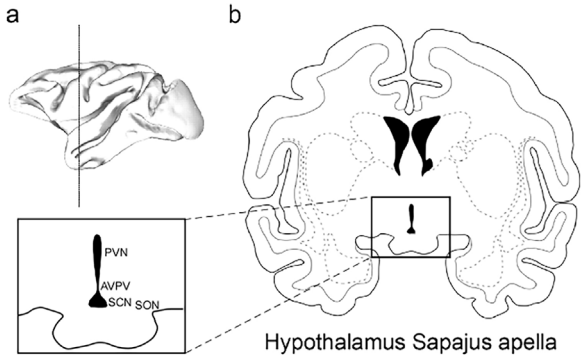

Fig. 1. Drawings showing the hypothalamic region where the suprachiasmatic nucleus (SCN), supraoptic (SON), paraventricular (PVN) and anteroventral periventricular nuclei (AVPV) can be visualized in the brain of the primate *Sapajus apella*. In A, lateral view of the monkey brain indicating one of the antero-posterior levels analyzed. In B, a coronal slice showing one of the anteroposterior levels in which the nuclei were analyzed.

than in the day time point (31.8 ± 2.9), however, at night, the MT_1 expression was found also increased in the VL region of the SCN (47.9 ± 3.2) (Fig. 3D–H) which was characterized by the presence of colocalizations between MT_1-IR and VIP-IR cells (Figs. 3 F and 5 I). The same pattern of expression was shown for the MT_2 receptors, since at day time point MT_2 receptors were predominantly in the dorsomedial region of the SCN (DM 69.4 ± 3.7; VL 10.6 ± 1.5, $p < 0.0001$) (Fig. 4B, C and H). At night, there was also an increase of MT_2-IR cells in the ventrolateral subdivision (Fig. 4E, F and H).

Summarizing, there was a prevalence of both MT_1-IR and MT_2-IR cells in the DM region comparing with the VL region in the day time point (Figs. 3 H and 4 H). At night, contrary to MT_2, there was no difference in the MT_1 expression between the subregions (Figs. 3 H and 4 H). The double staining between VIP and the melatonin receptors was predominant at night for both receptors with a more impressive increase for MT_2/VIP colocalization

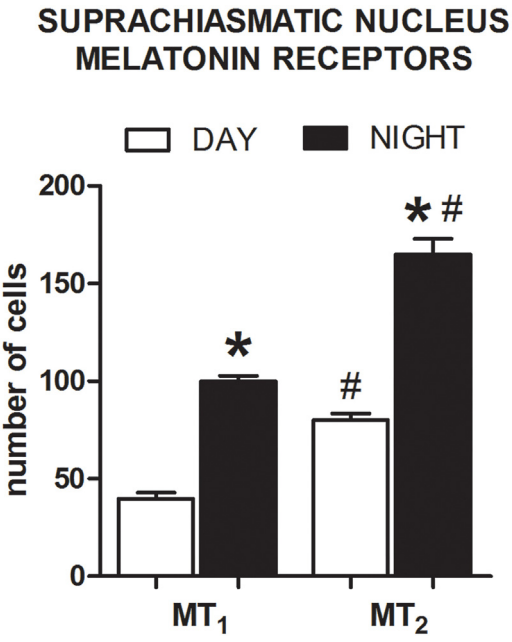

Fig. 2. Number of MT_1- and MT_2-IR cells in the suprachiasmatic nucleus (SCN) of the primate *Sapajus apella*. The graph indicates that both melatonin receptors are primarily expressed at night than in the day time. The MT_2 receptor showed a more expressive presence in this nucleus at both periods compared to the MT_1 receptor. N = 3 per time point, *means day ≠ night, $p < 0.0001$; # means $MT_1 \neq MT_2$, $p < 0.0001$.

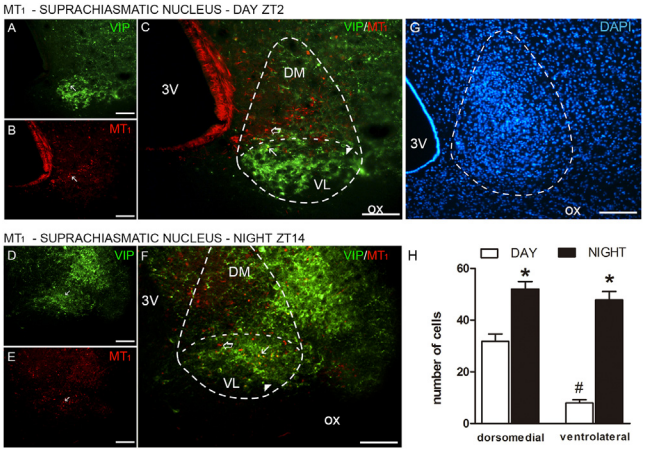

Fig. 3. Temporal distribution of the MT_1 receptor in the suprachiasmatic nucleus (SCN) of the primate *Sapajus apella*. Photomicrographs of frontal brain sections at the most rostral level of the SCN showing VIP-IR cells (A and D) (green) and MT_1-IR cells (B and E) (red) at two periods, one in the day time point (A, B and C) and at night (D, E and F). Panels C and F show the colocalization (yellow) between the MT_1-IR and VIP-IR cells (VIP/MT_1). In G, the SCN boundaries are represented by DAPI staining (blue). In H, the graph shows the means ± standard error of the mean of the MT_1-IR cells in the dorsomedial and ventrolateral SCN regions of monkeys perfused in the day and at night time points. N = 3 per time point, *means day ≠ night, $p < 0.0001$; # means dorsomedial ≠ ventrolateral, $p < 0.0001$. The unfilled arrows indicate the MT_1-IR cells; the filled arrows indicate colocalization (MT_1/VIP-IR cells); and the arrowheads indicate the VIP-IR cells. Bar = 100 μm. Optic chiasm (OX); third ventricle (3 V); dorsomedial (DM); ventrolateral (VL). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 5I). Furthermore, MT_2 showed a more expressive presence ($p < 0.0001$) in this nucleus compared with MT_1 at both periods (Fig. 2).

In contrast to the SCN, there was a predominance of MT_1 and MT_2 expression for the PVN, AVPV and SON in the day time point.

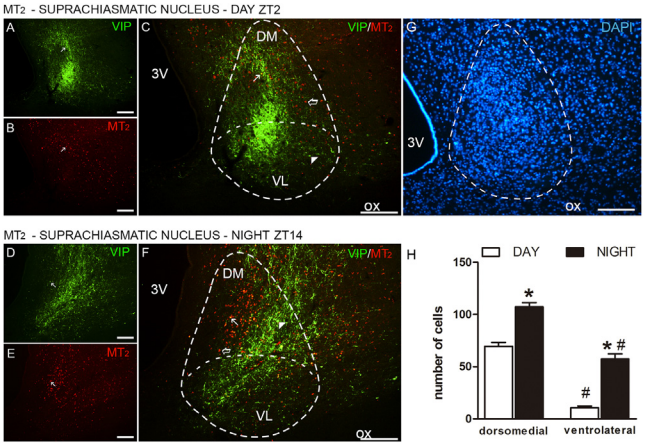

Fig. 4. Distribution of the MT_2 receptors in the suprachiasmatic nucleus (SCN) of the primate *Sapajus apella*. Photomicrographs of frontal brain sections showing VIP-IR cells (A and D) (green) and MT_2-IR cells (B and E) (red) at the most caudal level at two periods, one during the day (ZT2) (A, B and C) and another at night (ZT14) (D, E and F). Panels C and F show the colocalization (yellow) between the MT_2-IR and VIP-IR cells (VIP/MT_2). In G, the SCN boundaries are represented by DAPI staining (blue). In H, the graph shows the means ± standard error of the mean of the number of MT_2-IR cells in the dorsomedial and ventrolateral SCN regions of monkeys perfused in the day and at night time points. N = 3 per time point, *means day ≠ night, $p < 0.0001$; # means dorsomedial ≠ ventrolateral, $p < 0.0001$. The unfilled arrows indicate the MT_2-IR cells, the filled arrows indicate colocalization (MT_2/VIP-IR cells), and the arrowheads indicate the VIP-IR cells. Bar = 100 μm. Optic chiasm (OX); third ventricle (3 V); dorsomedial (DM); ventrolateral (VL). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

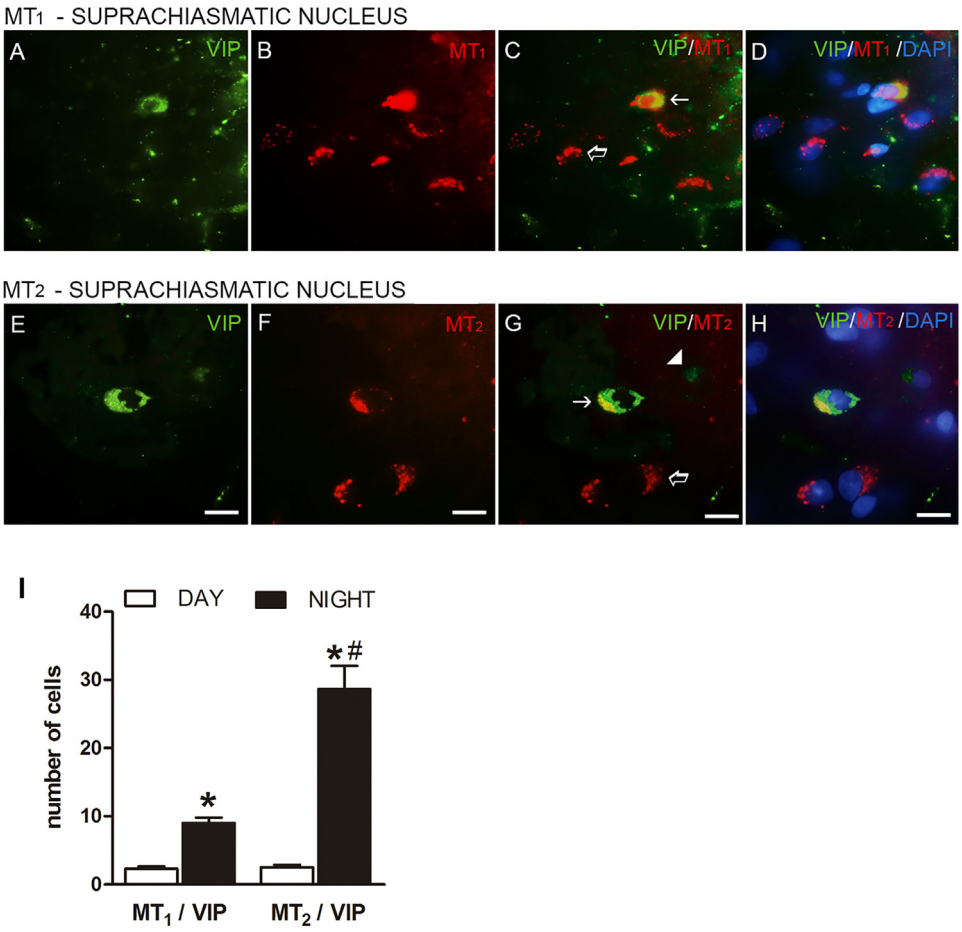

Fig. 5. Double labeling of VIP and melatonin receptors in the suprachiasmatic nucleus of the primate *Sapajus apella*. A-H represent high magnification photomicrographs of frontal brain sections showing VIP-IR cells (green), MT_1-IR cells or MT_2-IR cells (red) and VIP/MT_1-IR colocalization (yellow) (C and G) at ZT 14. D and H represent the overlap of the double labeled cells (VIP/MT_1 or VIP/MT_2) with 6-diamidino-2-phenylindole (DAPI) (blue). The cell nuclei were stained with DAPI (H). The graph (I) shows the means ± standard error of the mean of the number of MT_1/VIP and MT_2/VIP cells of mokeys perfused in the day and at night time points. N = 3 per time point, *means MT_1/VIP day ≠ MT_1/VIP night, $p < 0.05$; MT_2/VIP day ≠ MT_2/VIP night, $p < 0.0001$; # means MT_1/VIP ≠ MT_2/VIP, $p < 0.0001$. The unfilled arrows indicate the melatonin receptor-immunoreactive cells; the filled arrows indicate colocalization (MT_1/VIP-IR cells or MT_2/VIP-IR cells); and the arrowheads indicate the VIP-IR cells. Bar = 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In the PVN, both melatonin receptors were mainly expressed ($p < 0.0001$) in the day time point (MT_1 105.3 ± 2.7; MT_2 112.3 ± 2.6) and less expressed at night (MT_1 30.1 ± 1.7; MT_2 46.8 ± 2.6) (Fig. 6F). The MT_1-IR cells were expressed throughout the nucleus in the daytime (Fig. 6B). On the contrary, at the night point there were few MT_1-IR cells dispersed inside the nucleus, (Fig. 6C). MT_2-IR cells were observed in this nucleus primarily concentrated in the central region at both time points (Fig. 6D and E). Furthermore, MT_2 showed a more expressive presence ($p < 0.0001$) in this nucleus compared with MT_1 at night (Fig. 6F).

In the AVPV, both melatonin receptors were mainly expressed ($p < 0.0001$) in the day time point (MT_1 84.6 ± 2.1; MT_2 143.4 ± 3.9) and less expressed at night (MT_1 40.1 ± 3.4; MT_2 65.8 ± 4.3) (Fig. 7F). Furthermore, MT_2 showed a more expressive presence ($p < 0.0001$) in this nucleus compared with MT_1 at both periods (Fig. 7B–F).

The SON showed a high expression of both melatonin receptors throughout the nucleus in the day time point (MT_1 155.8 ± 6.4; MT_2 159.2 ± 9.4) when the IR cells showed a strong staining for both receptors inside the nucleus (Fig. 8B–D and H–J) and less expressed at night (MT_1 31.1 ± 1.1; MT_2 49.5 ± 2.6) (Fig. 8N) when MT_1 and MT_2 staining were weak and dispersed inside the nucleus (Fig. 8E–G and K–M). Furthermore, MT_2 showed a more expressive presence ($p < 0.05$) in this nucleus compared with MT_1 at night (Fig. 8N).

In addition to the antibody controls, the specificity of MT_1 and MT_2 labeling in these nuclei was demonstrated by the absence of MT_1 and MT_2 receptor expression in others areas at the same coronal level as the SCN, such as the bed nucleus of the stria terminalis (BNST) and the septohypothalamic nuclei (SHy). The absence of expression of both receptors was shown in both day and night time points in these areas.

4. Discussion

The high expression of MT_1 and MT_2 receptors proteins showed in the SCN, PVN, AVPV and SON, and the lack of expression in adjacent hypothalamic areas located at the same antero-posterior levels indicated that melatonin receptors expression in hypothalamus is local specific in the diurnal primate *Sapajus apella* as well as in rodents (Gupta et al., 2013; Lacoste et al., 2015) and humans (Wu et al., 2006, 2013).

Although we found a distinct difference in the distribution pattern between the MT_1 and MT_2 receptor proteins inside the different hypothalamic nuclei, the expression of both melatonin receptor proteins in the SCN seems to be more expressive at night (Fig. 2).

The SCN, main circadian oscillator, showed in the *Sapajus apella*, a MT_1 labeling higher and more widespread at night than in the day

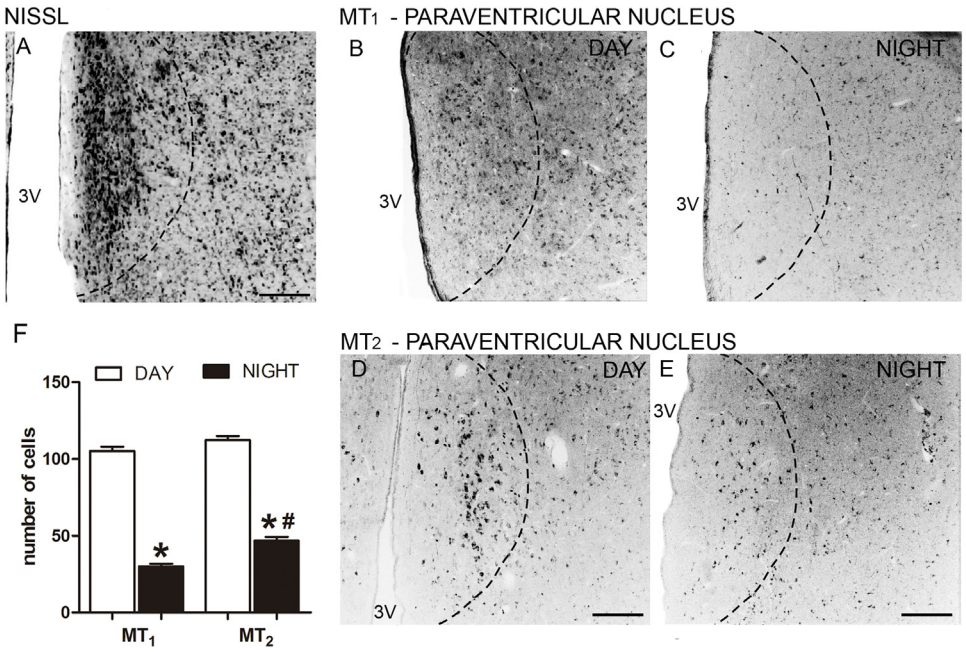

Fig. 6. Distribution of the MT_1 and MT_2 receptors in the paraventricular nucleus of the primate *Sapajus apella*. In A, photomicrographs of the Nissl-stained coronal brain sections. MT_1-IR cells (B and C) and MT_2-IR cells (D and E) at two periods (day and night). The colors were changed to gray scale. In F, the graph shows the means ± standard error of the mean of the number of MT_1- and MT_2-IR cells of monkeys perfused in the day and night time points. N = 3 per time point, *means day ≠ night, $p < 0.0001$; # means $MT_1 \neq MT_2$, $p < 0.0001$. Bar = 100 μm. Third ventricle (3 V).

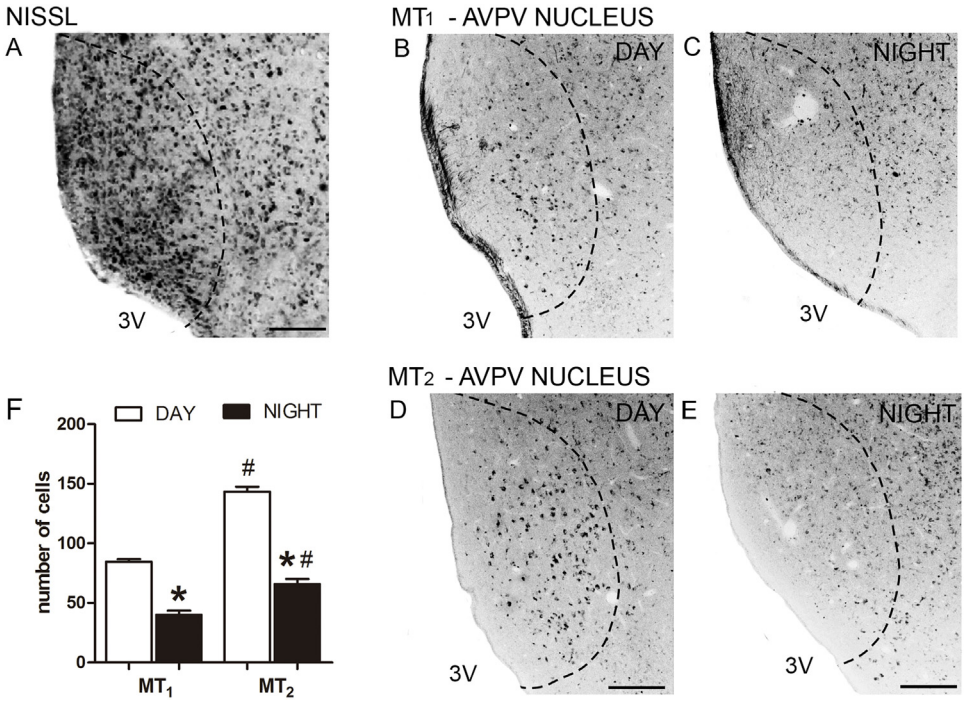

Fig. 7. Distribution of the MT_1 and MT_2 receptors in the anteroventral periventricular nucleus (AVPV) of the primate *Sapajus apella*. Photomicrographs of the Nissl-stained frontal brain sections are shown in A. MT_1-IR cells (B and C) and MT_2-IR cells (D and E) at two time points (day and night). The colors were changed to gray scale. In F, the graph shows the means ± standard error of the mean of the number of MT_1- and MT_2-IR cells of monkeys perfused in the day and night time points. N = 3 per time point, *means day ≠ night, $p < 0.0001$; # means $MT_1 \neq MT_2$, $p < 0.0001$. Bar = 100 μm. Third ventricle (3 V).

time when the MT_1 expression was just concentrated in the dorsomedial portion of the nucleus. The results in rodents also showed few MT_1-positive neurons in the SCN ventral division at day time (Lacoste et al., 2015) and MT_1 mRNA highest expression at the beginning of the night (ZT 13) (Poirel et al., 2002). Considering that melatonin concentration peaks at night in both species (diurnal primates or nocturnal rodents) this could be an indicative that the melatonin plasma concentration itself could be the regulating factor in the temporal variation of MT_1 expression (Fig. 9) (Gauer et al., 1994; Masana et al., 2000; Poirel et al., 2002). However, results in diurnal rodents showing no significant effect of time in the expression of the MT_1 receptor protein in the SCN

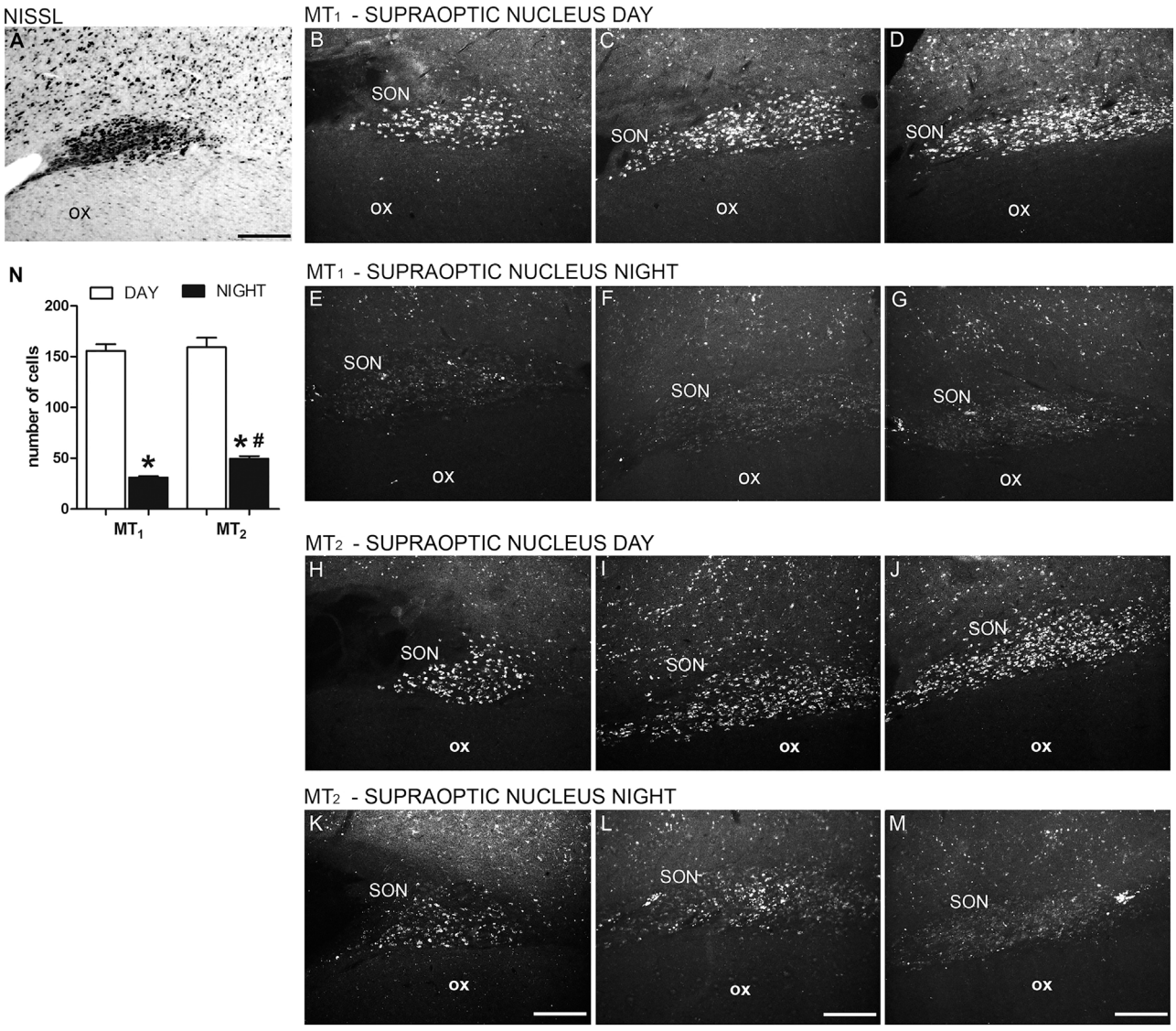

Fig. 8. Distribution of the MT_1 and MT_2 receptors in the supraoptic nucleus (SON) of the primate *Sapajus apella*. Panel A shows a photomicrograph of a Nissl-stained frontal section of the brain. MT_1-IR cells (B, C and D) in the day time and (E, F and G) at night. MT_2-IR cells (H, I and J) in the day and (K, L and M) night in three different anteroposterior levels of the SON. The colors were changed to gray scale. In N, the graph shows the means ± standard error of the mean of the number of MT_1- and MT_2-IR cells of monkeys perfused in the day and night time points. N = 3 per time point, *means day ≠ night, $p < 0.0001$; # means $MT_1 \neq MT_2$, $p < 0.005$. Optic chiasm (OX), Bar = 100 μm.

(Gupta et al., 2013) are contrary to this hypothesis and indicate that it is not yet clear what is the factor determining for MT_1 temporal variations found in some species.

For the MT_2, in the present study, was shown that in the SCN there is a day/night variation in the expression of this receptor with a peak at nighttime. This result is in agreement with results in humans (Wu et al., 2013). MT_2 receptors in the SCN are considered to be responsible for the phase-shifting and entrainment effects of melatonin on circadian rhythms (Liu et al., 1997; Pfeffer et al., 2012). Thus the rhythmic expression of the MT_2 receptors in the SCN supports its role in the regulation of biological circadian rhythms of primates.

Summaring, these findings suggest that, in primates, both receptors could interact in chronobiotic events and reinforce the importance of these targets for drug discovery in circadian-dependent pathologies in the SCN (Pévet, 2016).

In rodents, despite some contradictory results about the ausence (Lacoste et al., 2015; Waly and Hallworth, 2015) or the presence of MT_2 subtype in the SCN (Dubocovich et al., 1998; Hunt et al., 2001; Lacoste et al., 2015; Ochoa-Sanchez et al., 2011; Odo et al., 2014), the activation of the MT_2 by melatonin mediates phase advances in circadian rhythms of wheel running activity in the C3H/HeN mouse (Dubocovich et al., 1998); mainly at the light/dark or dark/light transitions (Hunt et al., 2001).

Among the hypothalamic areas outside the SCN, the data from the present study showed MT_1 and MT_2 receptor proteins in the PVN, SON and AVPV mainly at the beginning of the day (Fig. 9). The strong expression at daytime in these nuclei in the primate diurnal *Sapajus apella* agrees with results of MT_1 and MT_2 receptor mRNAs in hypothalamus of nocturnal rodents (Odo et al., 2014) indicating that there is no temporal diference in melatonin receptors expression between diurnal and nocturnal animals in hypothalamic areas outside of SCN.

The MT_1 and MT_2 labeling in the PVN and SON, which are involved directly and indirectly in the secretion of oxytocin and vasopressin indicates a possible role of melatonin in these hormones regulation (Wu et al., 2013).

Another area that presented melatonin receptors, the AVPV, receives direct projections from the SCN and also is involved in the integration of the neurons that synthesize hormones and

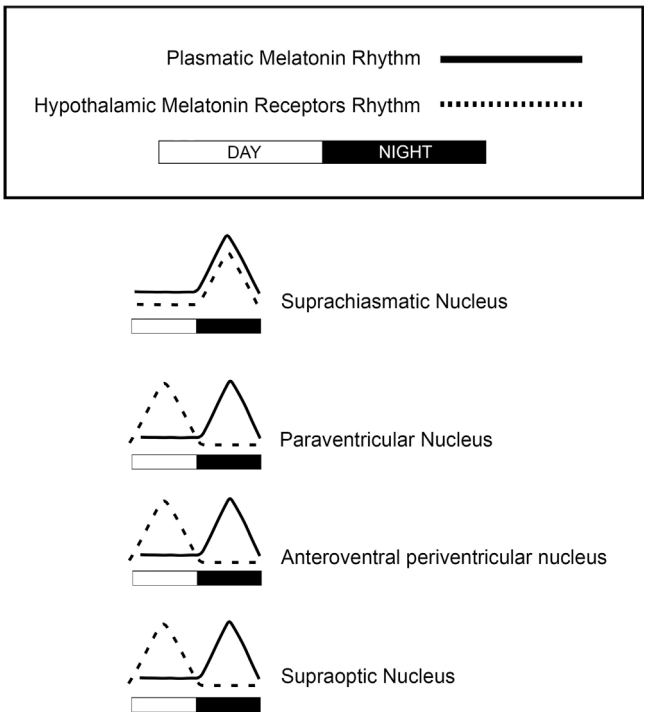

Fig. 9. Schematic representation of the temporal (day/night) distributions of plasma melatonin, as previously described in humans (Scholtens et al., 2016), versus the melatonin receptor-IR cells in the *Sapajus apella* hypothalamic nuclei: suprachiasmatic (SCN), supraoptic (SON), paraventricular (PVN) and anteroventral periventricular nuclei (AVPV). The black lines indicate the plasma melatonin rhythm and the dotted lines indicate the melatonin receptors day/night variations.

environmental signals (Vida et al., 2010). Therefore, the presence of both receptors in this area in the present study reinforces that melatonin may be involved in this type of hormones regulation in diurnal animals.

4 Summarizing, the day/night expression of the melatonin receptors in the PVN, SON and AVPV seems to be opposite that in the SCN, this difference indicate that the individual should be prepared to respond to the melatonin signal by different ways within the several processes. These day/night melatonin receptors expression differences must be taken into account for improving melatonin and its analogs therapeutic in several pathologies that affect brain areas.

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