First evidence of melatonin receptors distribution in the suprachiasmatic nucleus of tree shrew brain

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Abstract

OBJECTIVE: In this study we investigated the distribution of melatonergic receptors in the tree shrew brain. The psychosocial stress in the tree shrew is a validated model of depression with disturbance of circadian rhythms.

METHODS: Given the role of melatonin in the modulation of circadian rhythms, we determined by autoradiography the distribution of binding sites of 2-[^{125}I]-MLT, a ligand for the two melatonergic receptors MT_1 and MT_2, in the tree shrew brain focusing mostly on the suprachiasmatic nucleus (SCN), the biological clock involved in resynchronization of circadian rhythms. We also analyzed the distribution of 2-[^{125}I]-MLT in other brain areas involved in regulation of mood.

RESULTS: Specific binding of 2-[^{125}I]-MLT was found in the SCN. In addition, several structures in the tree shrew brain were labeled, among them the pars tuberalis, the cerebellum, structures of the hippocampal formation and dopaminergic areas such as the caudate putamen and nucleus accumbens, providing other potential targets of psychosocial stress in the tree shrew, in addition to the SCN.

CONCLUSIONS: The demonstration of melatonergic receptors in these brain areas supports their probable involvement in the behavioural, neuroendocrine and circadian rhythms disturbances observed in the psychosocial stress model of depression in the tree shrew.

ORIGINAL ARTICLE

INTRODUCTION

Agomelatine is a potent melatonergic (MT_1/MT_2) receptor agonist [1] and 5-HT_{2C} antagonist [2] that has been proved to be effective antidepressant in animal models [3–6] and in patients with major depressive disorder [7–9]. One of the core symptoms of depression is a disturbance of biological rhythms [10]. Indeed, the SCN, the site of the biological clock, has been shown to be critical for the agomelatine effects in the social defeat model in the rat [11]. Because entrainment of circadian rhythms is mediated by an action on melatonergic receptors in the SCN and because a melatonin antagonist is able to antagonize the effects of agomelatine in the chronic mild stress [3] and in the learned helplessness model of depression [6], it is hypothesized that at least a part of the antidepressant effects of agomelatine is obtained by an action through high-affinity melatonergic binding sites in the SCN. Indeed, subordinate male tree shrews develop during periods of daily social stress symptoms that are known from many depressed patients such as persistent hyperactivities of both the hypothalamic-pituitary-adrenal axis and sympathetic nervous system, disturbances in sleeping patterns and circadian rhythms, and reduced motor activity. Some of these parameters can be normalized by antidepressants thus supporting the view of the tree shrew social stress paradigm as a validated model for major depression [12,13].

Recently it has been shown that in chronic psychosocially stressed tree shrews, agomelatine regulates body temperature, circadian rhythms, cortisol levels, normalizes locomotor activity and resynchronizes disturbed sleep pattern [14]. To test the hypothesis of the involvement of SCN melatonergic binding sites in the antidepressant effects of agomelatine, we determined whether high-affinity (MT_1/MT_2) specific binding of 2-[^{125}I]-MLT is observed in the SCN of the tree shrew brain. Moreover, the distribution of 2-[^{125}I]-MLT binding was also investigated in other brain areas to identify other sites where agomelatine could act upon structures potentially associated with depression.

MATERIAL AND METHODS

Animal husbandry

The experiment was conducted with 4 adult female tree shrews (*Tupaia balangeri*) from the breeding colony at the German Primate Center (Göttingen, Germany). The animals were maintained in a 12:12 light:dark cycle (from 8:00 a.m. to 8:00 p.m.), for details of housing see [15]. All animal experimentations were approved by the Government of Lower Saxony, Germany.

Autoradiography.

Animals were sacrificed by decapitation (within 2 h after lights-on), their brains were quickly removed and frozen over liquid nitrogen. Frozen frontal brain sections (15µm) were generated throughout the brain using a microtome-cryostat (Leitz Kryostat 1720) at –20 °C. These sections were collected on TESPA (3-amino-propyl-ethoxy-silane; SIGMA-Aldrich, France) gel coated slides, and returned to –80 °C until the time of incubation. Analyses were performed on 2 brain sections distant of 45 µm, every 600 µm along the brain.

For 2-iodo-melatonin binding, sections were first washed with PBS buffer (0.01 M; Na_2HPO_4 0.1 M, NaH_2PO_4 0.1 M, NaCl 9 g/l, pH 7.4) at 4 °C, incubated for 1 h at room temperature with 200 µl of PBS contained 2-[^{125}I]-MLT 280 pM (specific activity of 2 000 Ci/mmole), rinsed twice at 4 °C with PBS (2 and 3 minutes), fixed with 4% paraformaldehyde at 4 °C for 10 minutes and dipped in water 10 minutes. Adjacent sections were incubated with melatonin 10 µM (Flucka, SIGMA-Aldrich, France) to assess non specific binding [16]. Autoradiograms were generated with air-dried sections placed in X-ray cassettes with hyperfilm Biomax MR (Amersham, France). [^{125}I] microscale standards were generated with 7 concentrations of 2-[^{125}I]-MLT in solution in pure ethanol dispersed on thin layer chromatography silicate gel (Macherey-Nagel, France). Exposure time was 28 days at room temperature. To identify histological structure, autoradiographical sections were stained by the Klüver and Barrera methods [17] and compared with tree shrew brain atlas [18] and rat brain atlas [19]. The intensity of binding was assessed by an image analysis system (Biocom Histo 500, France). After removal of the non specific levels measured on the control section incubated with cold ligands, the mean grey density was transformed in cpm using the microscale standards and data were converted to fmol/$mg_{protein}$ as described previously [20].

RESULTS

Specific binding of the 2-[^{125}I]-MLT was widely distributed in tree shrew brain with density ranging from 1 to 17 fmol/$mg_{protein}$. Specific binding was observed in the suprachiasmatic nucleus (SCN, 4.22±0.78 fmol/$mg_{protein}$ n=4; Figure 1A–B; Figure 2B), and in structures with density closed to that of the SCN, between 7 and 4 fmol/$mg_{protein}$: cochlear nucleus and the cerebellar lobule 2 (Figure 2F), the medial preoptic area (Figure 2B), the prepositus nucleus (not shown), the lateral/medial hypothalamic area (Figure 2C), the nucleus accumbens (not shown), the area of the premammillary nuclei/arcuate nucleus and the posterior thalamus nucleus (Figure 2C.), the caudate nucleus (Figure 2A–B), the pontine reticular nucleus (Figure 2F), the olfactory tubercle (Figure 2A.), the cerebral arteries (Figure 2C) and the optic tract (Figure 2D–E). Some structures showed higher density than the SCN (density 7–17 fmol/$mg_{protein}$): the pars tuberalis (Figure 2C), olivary nucleus (Figure 2F), the vestibular nucleus (Figure 2F) and the diagonal Band of Broca (Figure 2A). Other areas were labelled with lower density than the SCN (4 and 1 fmol/$mg_{protein}$): the globus pallidus (Figure 2B), the

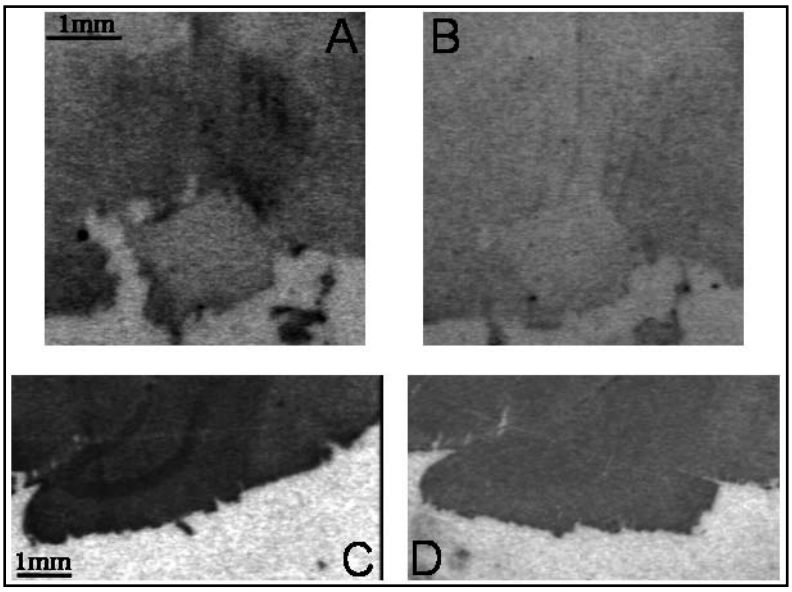

Figure 1. Binding of 2-[^{125}I]-MLT in SCN and hippocampus of the tree shrew brain. Binding of the 2-[^{125}I]-MLT in the suprachiasmatic nucleus (A & B) and hippocampus (C & D). A & C: total binding, 2-[^{125}I]-MLT 280 pM. B & D: non specific binding, 2-[^{125}I]-MLT 280pM and MLT 10 µM

interpedoncular nucleus (Figure 2E), the accessory occulomotor nucleus (Figure 2D–E.), the periventricular grey nucleus (not shown), the cerebellar lobule 1 (Figure 2F), the hippocampus (Figure 1C–D; Figure 2D) and the substantia nigra (Figure 2D), the choroids plexus (Figure 2C), the posterior thalamic nucleus and the subiculum (Figure 2D), the anterior thalamic nucleus (not shown), the pineal gland (Figure 2D.) and the pontine nucleus (Figure 2E). Finally, binding close to detection threshold (1 fmol/mg$_{protein)}$ was detected in some areas: corpus callosum and the cortex (Figure 2A–C) and the corona radiata (Figure 2A).

The concentrations of 2-[^{125}I]-MLT bound in each of these areas are represented in Figure 3.

DISCUSSION

In this study we have demonstrated a high density (4.22 fmol/mg) of melatonergic (MT_1/MT_2) receptors by using 2-[^{125}I]-MLT in the tree shrew SCN but also in the pars tuberalis. Other structures were also labelled with higher and lower densities.

Two important characteristics of binding of the 2-[^{125}I]-MLT must be taken into account. Firstly, it has been observed in a wide variety of cerebral and peripheral structures [21] with a large variation in these structures between species except for the pars tuberalis where binding is found in all species studied. For instance, the SCN displays binding in some species (rats [22,23], mouse [24,25], human [26,27]) whereas no binding was observed in others (sheep [28], red deer [24,29]). Secondly, the density of high-affinity 2-[^{125}I]-MLT binding is generally low (1–10 fmol/mg$_{protein)}$ [30], with the exception of the *pars tuberalis* (up to 40–60 fmol/mg$_{protein}$ in rat and sheep pars tuberalis [31,32]). In tree shrews, 2-[^{125}I]-MLT labelling was the highest one in the pars tuberalis. In the SCN, labelling was diffuse (Figure 1A–B, Figure 2B), in sharp contrast to what has been observed in rat brain in which 2-[^{125}I]-MLT labelling appears as two points located above the optic chiasm, with density closed to 5 fmol/mg$_{protein}$ [31]. This difference is related to different shapes of the SCN in these species: in rats, the SCN is a dense and compact structure whereas in the tree shrew, it is not as strictly delimited [33].

The presence of melatonergic receptors in the SCN of the tree shrew brain supports the possible role of these receptors in the desynchronisation of circadian rhythms observed during psychosocial stress. This provides evidence for the existence of a morphological basis for chronobiotic properties of agomelatine through the melatonergic receptors in the suprachiasmatic nucleus. Indeed, a resynchronisation of circadian rhythms with agomelatine requires the presence of a functionally intact SCN, but not of the pineal gland [34]. Moreover, the anxiolytic activity of agomelatine in the social defeat requires the integrity of the SCN [11].

Our study also identifies melatonergic binding sites in brain areas others than the SCN. Among them, some areas were well described in other species such as the *pars tuberalis*, thalamic and hypothalamic nuclei, others have been poorly described such as nuclei of the cerebellum, choroids plexus and pineal gland. Interestingly, we found high affinity melatonergic binding sites in areas implicated in some physiological functions potentially associated to depression syndromes. These areas could, in addition to the SCN, be the basis for the antidepressant effects of agomelatine, particularly the cerebellum, the hippocampus and dopaminergic structures (caudate putamen and nucleus accumbens). In the human, the presence of melatonergic binding sites was described

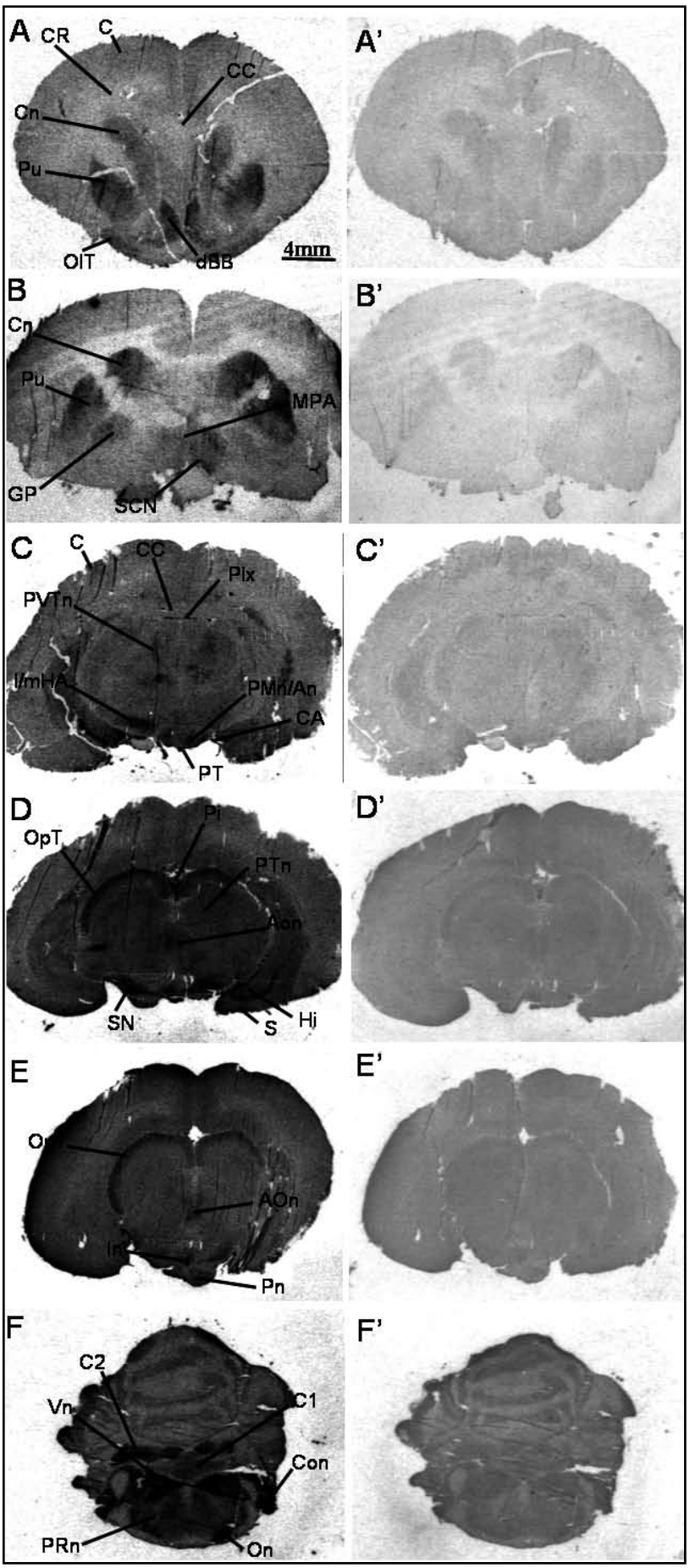

Figure 2. Binding of the 2-[^{125}I]-MLT in some areas of the tree shrew brain. A, B, C, D, E and F illustrate total binding. A', B', C', D', E' and F' illustrate non specific binding (MLT 10µM). A: Diagonal band of Broca (dBB), caudate nuclei (Cn), putamen (Pu), olfactory tubercle (OlT), cortex (C), corona radiate (CR), corpus callosum (CC); B: caudate nucleus (Cn), putamen (Pu), globus pallidus (GP), suprachiasmatic nucleus (SCN); C: pars tuberalis (PT), lateral/medial hypothalamic area (l/mHA), paraventricular thalamic nucleus (PVTn), premammillary nuclei/arcuate nucleus (PMn/An), cerebral arteries (CA), choroid plexus (Plx), corpus callosum (CC), cortex (C); D: pineal gland (Pi), hippocampus (Hi), subiculum (S), substantia nigra (SN), posterior thalamus nuclei (PTn), accessory occulomotor nuclei (AOn), optic tract (OpT); E: optic tract (OpT), accessory occulomotor nucleus (AOn), interpedoncular nucleus (In), pontine nucleus (Pn); F: vestibular nucleus (Vn), cochlear nucleus (Con), pontine reticular nucleus (PRn), olivary nucleus (On), cerebellar lobule 1 (C1) and 2 (C2)

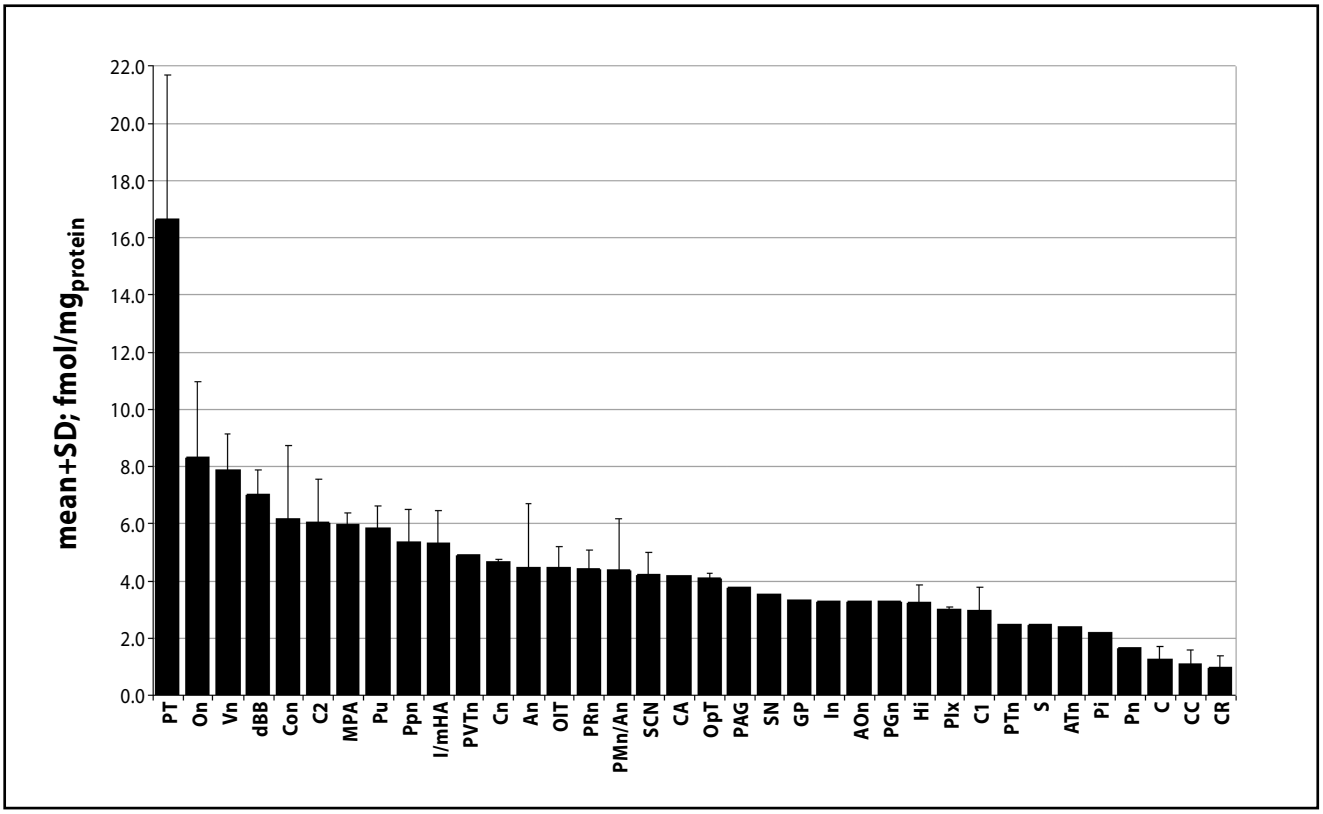

Figure 3. Density of 2-[^{125}I]-MLT binding sites in individual areas of the tree shrew brain. Data are given as mean ± SEM (n=4; 2 to 35 measures per structures). Olivary nucleus (On), *pars tuberalis* (PT), vestibular nucleus (Vn), diagonal band of Broca (dBB), cochlear nucleus (Con), cerebellar lobule 2 (C2), medial preoptic area (MPA), putamen (Pu), prepositus nucleus (Ppn), lateral/medial hypothalamic area (l/mHA), suprachiasmatic nucleus (SCN), nucleus accumbens (An), premammillary nuclei/arcuate nucleus (PMn/An), paraventricular thalamic nucleus (PVTn), caudate nucleus (Cn), pontine reticular nuclei (PRn), olfactory tubercle (OlT), cerebral arteries (CA), optic tract (OpT), globus pallidus (GP), interpedoncular nucleus (In), accessory occulomotor nucleus (AOn), periventricular gray nucleus (PGn), cerebellar lobule 1 (C1), hippocampus (Hi), substantia nigra (SN), choroid plexus (Plx), posterior thalamus nucleus (PTn), subiculum (S), anterior thalamic nucleus (ATn), pineal gland (Pi), pontine nucleus (Pn), corpus callosum (CC), cortex (C) and corona radiate (CR).

in the lobes of the cerebellum [35] with heterogeneous expression of the MT_1 and MT_2 melatonergic receptors [36]. Binding in the cerebellar cortex was also determined in the red deer (*Cervus elaphus*) [29], and in the neonatal pig; in this latter species, binding was described in the trigeminal tract in addition to the cerebellar cortex [37]. In many cases, binding of 2-[^{125}I]-MLT in the cerebellum was found in lobes of the cerebellum, more particularly in the molecular layer of the cerebellum. In the present study, binding was found in the lobule 1 and 2 of the cerebellum, and in some nuclei of the cerebellum (cochlear nucleus, vestibular nucleus, olivary nucleus, pontine reticular nuclei, preapositus nucleus). The role of cerebellar nuclei in depression is poorly described in the literature. In a clinical study, a link between depression and cerebellum, with structural and functional abnormalities in the cerebellum, has been suggested, see for review [38,39]. Consistently with the present study in tree shrew brain, melatonergic binding sites were previously described in the hippocampal formation of numerous species, such as rat [24,30], mouse [24,40], sheep [24], red deer [24,29], rabbit [24,41], hamster [42], and human [43]. In preclinical studies it has been demonstrated that stress, which can induce depression-like symptoms in tree shrews [13], induces alterations in brain metabolism and synaptic plasticity, effects counteracted by antidepressant treatment [44]. Moreover, melatonergic receptors are involved in the process of learning and memory. It appears that melatonin may control the glutamergic system and the hippocampus, and thus may influence memory formation in hippocampus [45]. The impact of melatonin and its receptors on short term memory was described in rats, via the MT_1 melatonergic receptor, while melatonin effect was inhibited by luzindole, a MT_1 and MT_2 antagonist [46]. In addition, in Alzheimer's disease, characterised by cognitive deficits, there is a specific alteration of high affinity melatonin receptors expression in the human hippocampus; indeed, an increase of the MT_1 expression and a decrease of the MT_2 expression was observed [47,48]. Also, MT_2 knockout mice express phenotypes relevant for

neuropsychatric disorders, including Alzheimer's disease, and the implication of the MT_2 receptors in the hippocampal synaptic plasticity and in memory processes has been described [49]. Finally, the present study revealed melatonin binding sites in dopaminergic areas such as the caudate putamen and nucleus accumbens nuclei; in these structures melatonergic binding sites previously described in the rat and human brain [40]. An association between depression and dopaminergic system was described, and led to consider serotonin and dopamine systems as potential targets for antidepressant molecules [50].

In conclusion, the present study demonstrates the presence and regional distribution of melatonin binding sites (MT_1 and MT_2) in the brain of the tree shrew mainly in the SCN and *pars tuberalis* but also in other brain areas such as cerebellum, hippocampus, caudate putamen and nucleus accumbens. The demonstration of melatonin receptors in these brain areas supports their probable involvement in the behavioral, neuroendocrine and circadian rhythm disturbances observed in the psychosocial stress model of depression in the tree shrew and could explain the effects of agomelatine in this model.

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