Cell Tissue Res (1994) 278:97–106

Cell & Tissue Research

An attempt to correlate brain areas containing melatonin-binding sites with rhythmic functions: a study in five hibernator species

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Received: 15 October 1993 / Accepted: 11 February 1994

Abstract. High affinity melatonin-binding sites have been described, by means of autoradiography with 2-^{125}I-melatonin as the ligand, in more than 60 brain areas of about 20 mammalian species, with dramatic variations in the nature and number of labelled structures among the different species studied. As melatonin is involved in the synchronization of biological rhythms, we have tried to correlate the brain areas containing melatonin-binding sites with some rhythmic functions typical of given species. Therefore, we have studied the location of melatonin-binding sites in the complete brain of five long-day breeders with hibernation cycles, viz. one insectivore and four rodents. With the exception of the suprachiasmatic nuclei and the pars tuberalis of the pituitary, both of which contain binding sites in all five species, few reactive structures are common, even among species from the same family, e.g. the edible dormouse and the garden dormouse.

Key words: Melatonin receptors – Pituitary gland, pars tuberalis – Hibernators – Autoradiography – Melatonin – 2-Iodomelatonin – *Jaculus orientalis*, *Eliomys quercinus*, *Glis glis*, *Cricetus cricetus* (Rodentia), *Erinaceus europaeus* (Insectivora)

Introduction

Melatonin is a pineal hormone secreted only during the dark phase of the light/dark cycle (Rollag and Niswender 1976). It is involved in the synchronization of biological rhythms at the seasonal (Goldman and Darrow 1983; Karsch et al. 1984; Pévet 1988) and at the circadian (Cassone et al. 1986 a, b; Kirsch et al. 1993) levels. The sites of action of this hormone were unknown until the recent introduction of a new ligand, 2-^{125}I-melatonin, which has allowed the localization of high affinity (pM range) binding sites in the pars tuberalis (PT) of the adenohypophysis and in the suprachiasmatic nuclei (SCN) of the rat (Vanecek et al. 1987). Since this pioneer study, melatonin-binding sites have been localized in mammals in many different brain areas (reviews in Stankov et al. 1991 b, 1993; Weaver et al. 1991; Bittman 1993) (Table 1). The striking point is the large variability in the number and location of these areas among the various species studied. This great variability can perhaps be explained by the many different biological rhythms in which melatonin is involved, and which are expressed depending on the species. Therefore, in order to understand these discrepant results, comparative studies have been performed on the location of melatonin-binding sites in five different species with common annual rhythmic physiological functions: reproduction and hibernation.

In this work, we have studied the location of melatonin-binding sites and their density in the complete brain of one insectivorous species, the hedgehog (*Erinaceus europaeus*) and four rodent species: the European hamster (*Cricetus cricetus*), the jerboa (*Jaculus orientalis*), the garden dormouse (*Eliomys quercinus*) and the edible dormouse (*Glis glis*). The brain areas containing melatonin-binding sites have been compared with those previously described in mammals, and their function has been discussed.

Materials and methods

Animals

The hedgehogs (3 males) were obtained from the breeding colony of the Centre d'Etudes Biologiques de Chizé (CNRS, Villiers-en-Bois, France). The other species were caught in the field: jerboas (2 males, 2 females) in the middle Atlas mountain, Morocco; European hamsters (3 males, 1 female) in the vicinity of Strasbourg, France; edible dormice (5 males) and garden dormice (2 females) in the vicinity of Niort, France. All animals (adults, sexually active) were kept out of doors in individual cages under their natural photoperiod and temperature (European hamsters, edible dormice and garden dormice

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Table 1. Identified structures containing 2-^{125}I-melatonin-specific binding on autoradiograms in adult mammalian brain and pituitary. Superscripts refer to references given in the footnote to this table

Structures	Species
Hypothalamus	
Suprachiasmatic nucleus	Rat[9,11,12,14,20,25,27,30], Syrian hamster[27,32], Siberian hamster[8,27], white footed mouse[28], guinea pig[20], rabbit[21], goat[6], human[19,29], vervet monkey[22], baboon[22], rhesus monkey[29]
Dorsomedial nucleus	White footed mouse[28], Syrian hamster[32], sheep[1]
Ventromedial nucleus	Syrian hamster[32], rabbit[21], sheep[1,13]
Median preoptic nucleus	Syrian hamster[32], rabbit[21], sheep[1]
Anterior hypothalamic area	White footed mouse[28], sheep[1,13]
Septohypothalamic area	Sheep[13]
Septohypothalamic nucleus	White footed mouse[28], sheep[1]
Median preoptic area	Syrian hamster[32], sheep[1,13]
Tuberal median area	Sheep[1]
Supramammillary nucleus	Sheep[1]
Paraventricular nucleus	Sheep[1]
Preoptic area	Sheep[1,13], goat[6]
Lateral hypothalamic area	Sheep[1]
Supraoptic nucleus	Guinea pig[20]
Thalamus	
Reuniens nucleus	White footed mouse[28], Siberian hamster[8,27]
Paraventricular nucleus	White footed mouse[28], Syrian hamster[27,32] Siberian hamster[8,27], sheep[1], rat[27], guinea pig[20]
Paratenial nucleus	Guinea pig[20]
Ventromedial nucleus	Sheep[1]
Anteroventral nucleus	Rat[16,27]
Anterodorsal nucleus	Rat[16]
Dorsolateral nucleus	Rabbit[21]
Lateral habenular nucleus	Syrian hamster[27,32]
Nucleus of the stria medullaris	Siberian hamster[27]
Ventral lateral geniculate nucleus	White footed mouse[28]
Medial geniculate nucleus	White footed mouse[28]
Subparafascicular nucleus	White footed mouse[28]
Basal forebrain and septal zones	
Lateral septum: ventral part	Sheep[1,4]
intermediate part	Sheep[1,4]
Medio-lateral septum	Sheep[1,4]
Septum	Sheep[13]
Islands of Calleja	White footed mouse[28]
Bed nucleus of the stria terminalis	White footed mouse[28], sheep[1]
Band of Broca (medial edge)	Sheep[13]
Amygdala, hippocampus, cortical and subcortical structures	
Amygdala	Sheep[1]
Amygdala-striatum border zone	White footed mouse[28]
Intercalated nucleus, amygdala	White footed mouse[28]
Frontal cortex	White footed mouse[28]
Orbitofrontal cortex	White footed mouse[28]
Parietal cortex	White footed mouse[28], rabbit[21]
Insular cortex	White footed mouse[28]
Temporal cortex	White footed mouse[28]
Entorhinal cortex	White footed mouse[28], sheep[1,13]
Central cerebral cortex	Goat[6], sheep[1]
Indusium griseum	Rabbit[21]
Parasubiculum	White footed mouse[28]
Hippocampus	Goat[6], sheep[1,13]
Subiculum	Sheep[1,13], rat[27]
Presubiculum	Sheep[1], rabbit[21]
Dentate gyrus	Sheep[1], rabbit[21]
Cerebellum	Sheep[13]
Stria terminalis	Sheep[13]

Table 1. Continued

Structures	Species
Brainstem, other central structures and pituitary	
Pars tuberalis	Rat[10,11,12,20,25,27,30,31], Syrian hamster[24,27,32] Siberian hamster[8,27], European hamster[10], ferret[26], rabbit[21], sheep[1,4,5,13,17,18,20], goat[6], western spotted skunk[7], white footed mouse[28], golden-mantled ground squirrel[23], mink[2,3], vervet monkey[22], baboon[22], rhesus monkey[29]
Pars distalis	Ferret[26], sheep[13]
Anterior hypophysis	Syrian hamster[24,27,32], rat[27]
Ventral raphe	Sheep[1]
Inferior colliculus	Sheep[1]
Deep pineal	Syrian hamster[32]
Inferior olivary complex	White footed mouse[28]
Area postrema	White footed mouse[28], rabbit[21], rat[15,27]
Choroid plexus	White footed mouse[28]
Spinal tract of the trigeminal nucleus	White footed mouse[28]
Basis of ventral tegmental area	Syrian hamster[32]
Olfactory bulb	Rabbit[21]
Interpeduncular nucleus	Syrian hamster[32]
Trigeminal nucleus	Sheep[13]
Substantia gelatinosa	Sheep[13]

[1] Bittman and Weaver 1990; [2] Boissin-Agasse et al. 1992; [3] Bonnefond et al. 1993; [4] De Reviers et al. 1989; [5] De Reviers et al. 1991; [6] Deveson et al. 1992; [7] Duncan and Mead 1992; [8] Duncan et al. 1989; [9] Gauer et al. 1992a; [10] Gauer et al. 1992b; [11] Gauer et al. 1993a; [12] Gauer et al. 1993b; [13] Helliwell and Williams 1992; [14] Laitinen and Saavedra 1990; [15] Laitinen et al. 1990; [16] Lindroos et al. 1992; [17] Morgan et al. 1989; [18] Pelletier et al. 1990; [19] Reppert et al. 1988; [20] Stankov and Fraschini 1993; [21] Stankov et al. 1991a; [22] Stankov et al. 1993; [23] Stanton et al. 1991; [24] Vanecek and Jansky 1989; [25] Vanecek et al. 1987; [26] Weaver and Reppert 1990; [27] Weaver et al. 1989; [28] Weaver et al. 1990; [29] Weaver et al. 1993; [30] Williams 1989; [31] Williams and Morgan, 1988; [32] Williams et al. 1989

Table 2. 2-^{125}I-melatonin specific binding in the brain and pituitary of the edible dormouse, garden dormouse, jerboa, European hamster and hedgehog. Values are a percentage of the PT value given as 100%

Structures	Edible dormouse	Garden dormouse	Jerboa	European hamster	Hedgehog
Pituitary					
Pars tuberalis (PT)	100	100	100	100	100
Basal forebrain and septal zones					
Bulbs					
Internal granular layer of the olfactory bulb (IGr)		70			
External plexiform layer of the olfactory bulb (EPl)		18			
Lateral septal nucleus (LS)	12				
Septohippocampal nucleus (SHi)	13				
Caudate putamen (striatum), ventral part (CPuv)		5			
Bed nucleus of the stria terminalis (BST)	11	3			
Amygdala, hippocampus and cortical structures					
Amygdala					
Central amygdaloid nucleus, lateral division (CeL)	20				
Central amygdaloid nucleus, medial division (CeM)	8				
Medial amygdaloid nucleus (MeA)		12			
Hypothalamus					
Anterior medial preoptic nucleus (AMPO)		5	5		
Suprachiasmatic nucleus (SCN)	9	4	4	1	11
Supraoptic decussation (Sox)		6			
Paraventricular hypothalamic nucleus (PVN)		9	5		
Thalamus					
Paraventricular thalamic nucleus (PVT)			2	2	
Intergeniculate leaflet (IGL)		5			
Brainstem					
Inferior colliculus (IC)			20		
Fasciculus retroflexus (fr)		3			
Substantia nigra, compact part (SNC)		3			

in Strasbourg, hedgehogs in Chizé, and jerboas in Rabat) and provided with food and water *ad libitum*. They were all killed by decapitation between 14.00 and 16.00 h, the hedgehogs on March 31st, jerboas on April 26th, European hamsters on April 27th and June 29th, edible dormice on March 3rd, and garden dormice on June 11th.

In vitro autoradiographic procedure

Brains were rapidly dissected out, frozen in isopentane maintained at −30°C and then kept at this temperature until sectioning. Serial coronal sections (20 µm thick) of the complete brain were cut on a cryostat, thaw-mounted onto gelatin-coated slides and kept at −30°C until used (never longer than 2 weeks). The sections were preincubated in 100 mM TRIS buffer containing 4 mM $CaCl_2$, pH 7.4, for 15 min at 4°C and then were incubated in the same buffer containing 2-^{125}I-melatonin (100–150 pM depending on the experiment; specific activity 1300–2 000 Ci/mmol, synthetized according to the method of Vakkuri et al. 1984, and purified by high pressure liquid chromatography, with or without excess cold melatonin (1 µM) for 60 min at room temperature. After incubation, the sections were washed twice in the assay buffer and then once in distilled water, for 30 s at 4°C under agitation. Sections were air-dried and apposed to hyperfilms (^{3}H-Amersham) for 7 days, together with 20-µm-thick ^{125}I-micro-scale standards (Amersham).

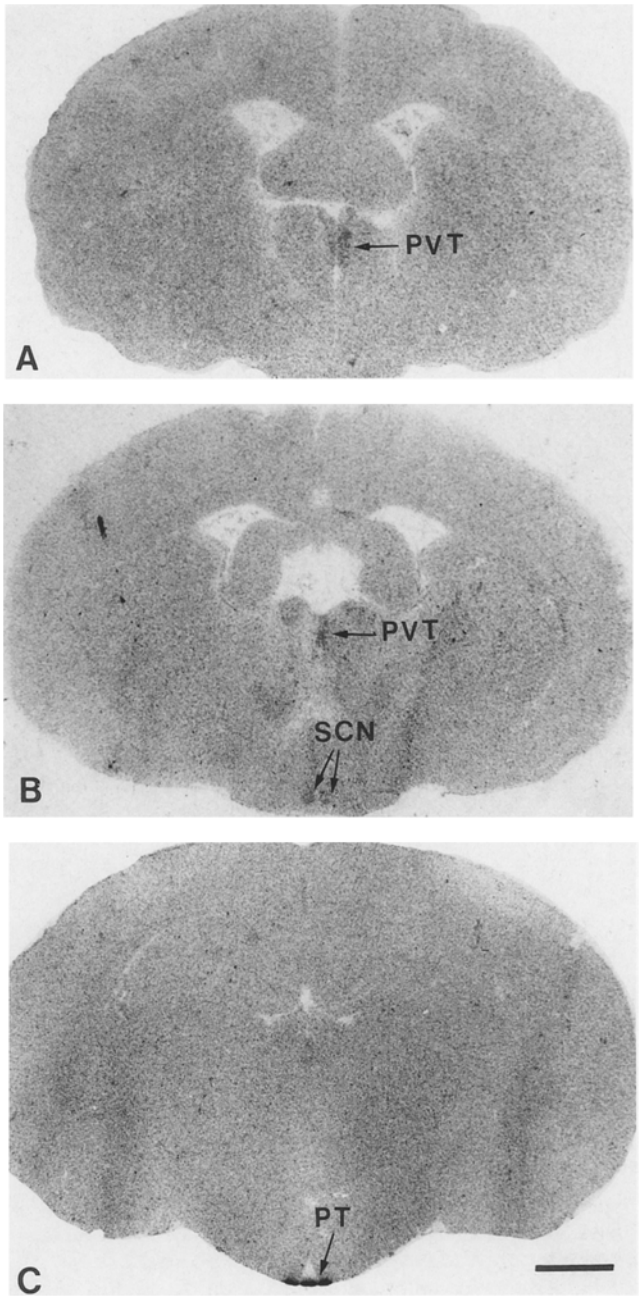
Quantitative analysis of the autoradiograms was performed using the computerized analysis system Biocom (Les Ulis, France), program RAG 200. The optical density of the autoradiograms was converted into fmol/mg polymer using the micro-scale standards, and the resulting data were then converted into fmol/mg protein as described by Nazarali et al. (1989). Specific 2-^{125}I-melatonin binding was determined as the difference between total and non-specific binding (not displaced in the presence of 1 µM cold melatonin).

The use of different batches of ligand induces variability in the absolute values of binding obtained among experiments. In order to exclude the "ligand quality" parameter and to compare the binding capacity of the different structures, 2-^{125}I-melatonin binding has been converted into a percentage of a reference value (see below).

Results

Only two structures showed specific 2-^{125}I-melatonin binding in all five species studied: the PT and the SCN. In each case, the PT contained the highest density of sites and was therefore used for each species as a reference point (100%) for the binding obtained in all the other structures (Table 2).

In the hedgehog, only the PT and SCN showed displaceable binding. Binding in the SCN represented about 10% of that in the PT (Table 2). The European hamster displayed a low density of binding sites in the SCN and in the paraventricular nucleus of the thalamus (PVT), in comparison with the PT, but this was seen in only 2 animals (Table 2, Fig. 1). The inferior colliculus of the jerboa contained a large number of binding sites and, other than the SCN and PT, two areas of the hypothalamus showed low binding: the anterior medial preoptic nucleus (AMPO) and the paraventricular nucleus of the hypothalamus (PVN) (Table 2, Fig. 2). In the garden dormouse, binding sites were present in the PT and SCN, and in large amounts in the internal granular layer (IGr), external plexiform layer (EPl) of the olfactory bulbs (Table 2, Fig. 3). They could also be found in the PVN and AMPO, the bed nucleus of the stria terminalis (BST), the ventral part of the caudate putamen (striatum) (CPu), the compact portion of the substantia nigra, the intergeniculate leaflet (IGL) and the fasciculus retroflexus, the medial amygdaloid nucleus, and in one animal, in the supraoptic decussation (Fig. 3). Many structures in addition to the SCN and PT contained 2-^{125}I-melatonin binding sites (Table 2) in varying densities in the edible dormouse: the septohippocampal nucleus, lateral septal nucleus, and the central amygdaloid nucleus (medial and lateral divisions) (Fig. 4).



Figs. 1–4. For abbreviations, see Table 2

Fig. 1A–C. Distribution of 2-^{125}I-melatonin binding in the European hamster brain. *Bar*: 180 µm

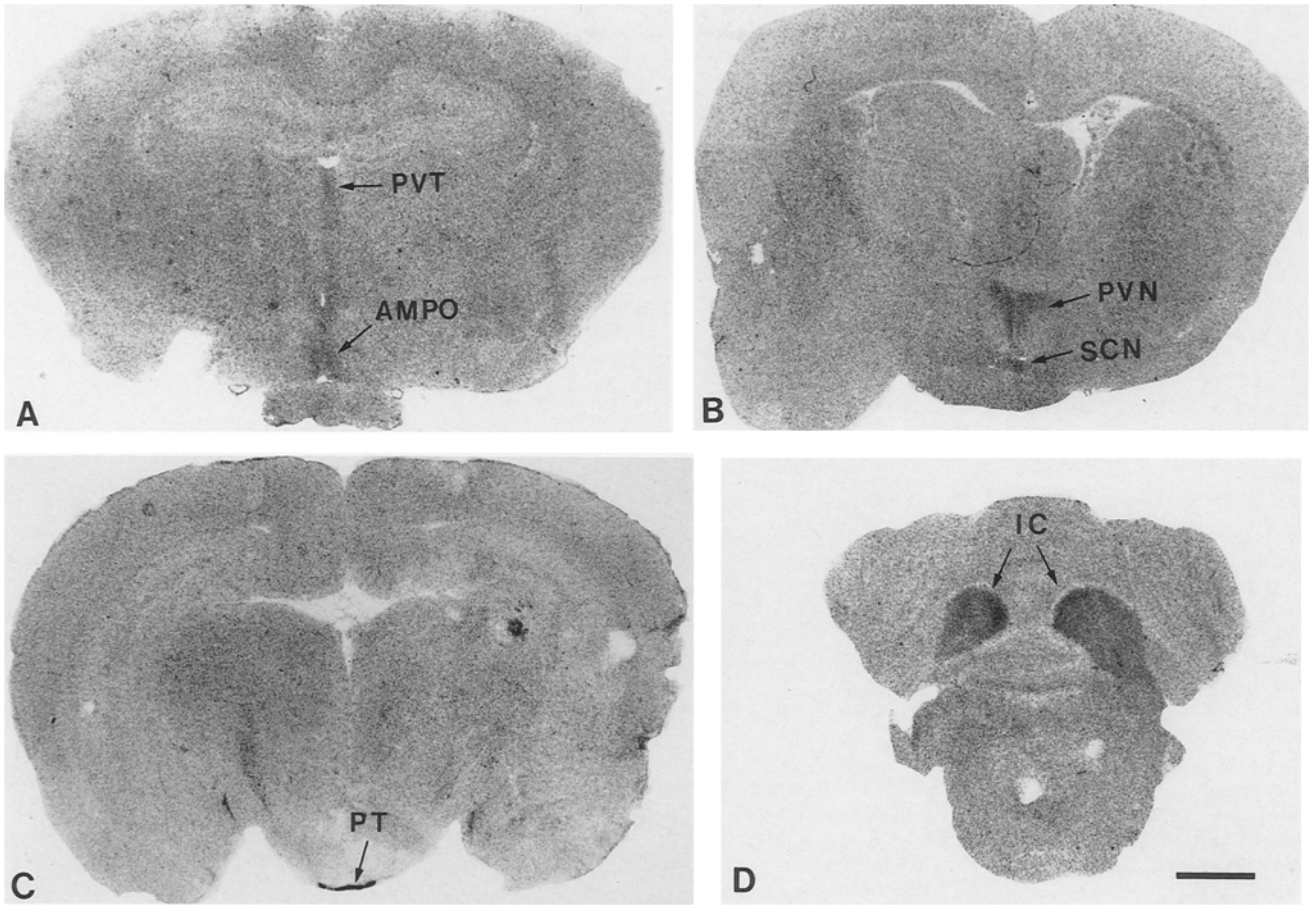


Fig. 2A–D. Distribution of 2-^{125}I-melatonin binding in the jerboa brain. *Bar*: 180 μm

Discussion

The effect of photoperiod on seasonal changes in physiological functions is known to be mediated by the rhythmic secretion of melatonin. However, although our study involves species with similarities in their seasonal physiological functions (reproduction, hibernation), a large variability in the number and location of central areas containing melatonin-binding sites has been observed. Indeed, only two common structures always show melatonin-binding sites in the five different species studied: the PT and SCN. Moreover, a large variability in the melatonin-binding site density of both structures has been observed among the species. The same autoradiographic technique and conditions result in low and barely detectable specific binding in the SCN of the European hamster, whereas in the hedgehog and edible dormouse, the binding is marked and represents about 10% that of the PT; this is also the case in the rat (Gauer et al. 1993 b). Few other areas containing melatonin-binding sites are common, and even then only with respect certain species, e.g. the hypothalamic structures PVN and AMPO in the garden dormouse and jerboa, the thalamic structure PVT in the jerboa and hedgehog, and the BST in the edible dormouse and garden dormouse.

Another great variability has been found in the number of structures displaying melatonin-binding sites: from two in the hedgehog, to 13 in the garden dormouse. These results cannot be accounted for by the fact that the hedgehog, an insectivore, is a more primitive species than rodents. Indeed, the opposite could be expected, since a widespread distribution of melatonin receptors has been reported in non-mammalian species (Rivkees et al. 1989; Stehle 1990; Cassone and Brooks 1991; Martinoli et al. 1991). Moreover, two species, the edible dormouse and the garden dormouse, have been chosen for this study because they are comparable, living in the same biotope and being members of the same family (Gliridae). However, although they both display a widespread distribution of melatonin-binding sites, the labelled areas concerned are totally different (see Table 2). This widespread distribution of melatonin-binding sites in the edible dormouse and the garden dormouse most closely resembles that described previously for the white-footed mouse (Weaver et al. 1990). On the other hand, the restricted distribution of melatonin-binding sites in the European hamster and hedgehog agrees with data from other laboratory rodents, such as rat, hamster and mouse (see Table 1). From the present results obtained in five different species of wild (as opposed to laboratory) animals, it seems that the limited distribution of melatonin-binding sites in laboratory animals cannot be attributed to their history of generations under controlled breeding conditions as proposed by Stankov and Fraschini (1993). No sexual differ-

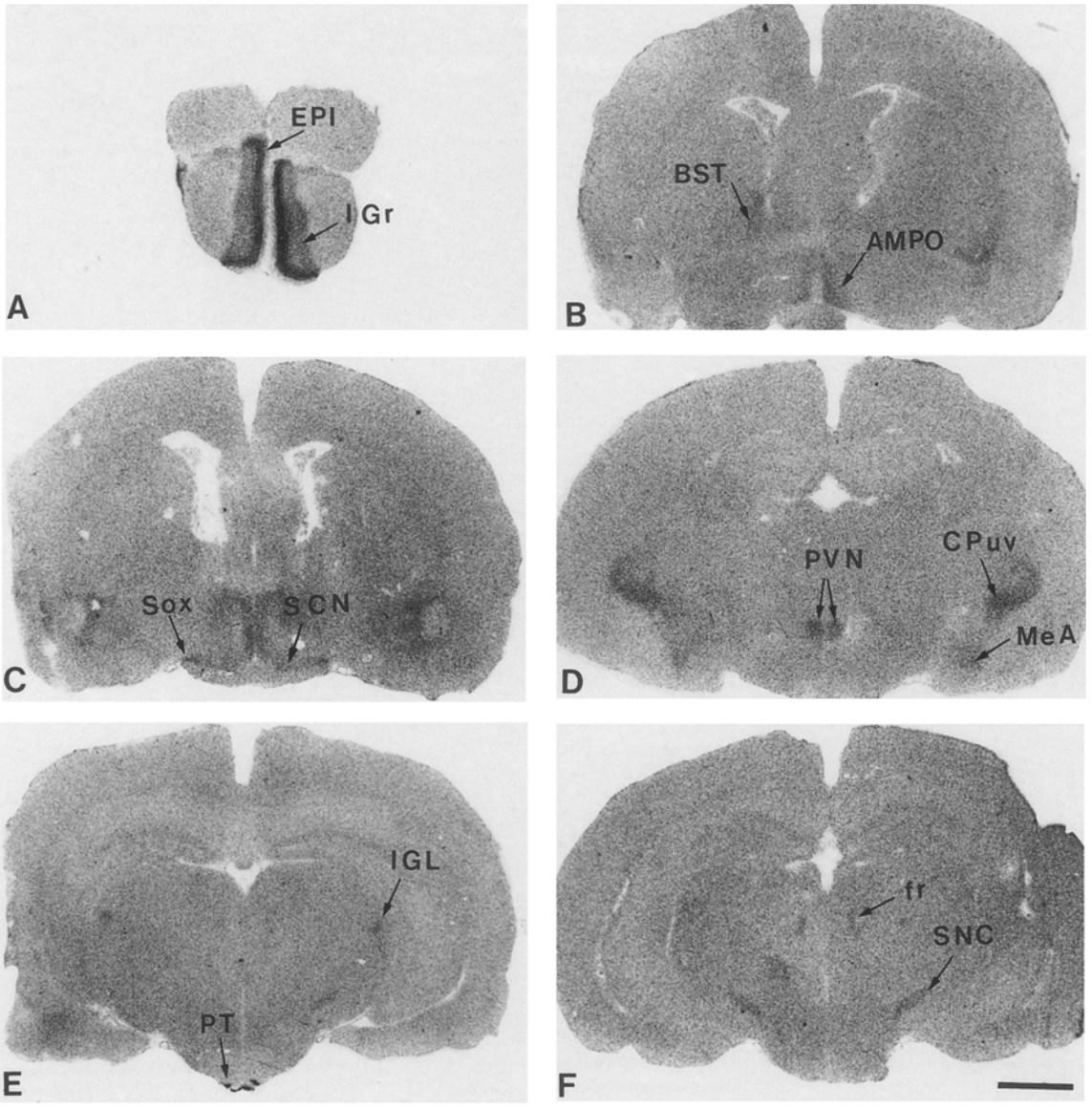


Fig. 3A–F. Distribution of 2-^{125}I-melatonin binding in the garden dormouse brain. *Bar*: 160 μm

ences have been observed in the location of melatonin-binding sites in the two species for which both males and females have been studied (jerboa and European hamster).

Nature of binding sites

Although we have not performed a pharmacological study of the melatonin-binding sites, it is clear that they are of high affinity. Indeed, they have been visualized using low concentrations of 2-^{125}I-melatonin (between 100 and 150 pM) and, in former studies, they have been characterized in the PT of the European hamster and in the PT and SCN of the hedgehog, their affinity being around 100 pM (Gauer et al. 1992 b, 1993 c). Moreover, they have been shown to be coupled to a G-protein in the PT of the European hamster (Skene et al. 1992).

Causes of the large variability in results

The enormous variability observed from one species to another in the number and location of areas containing melatonin-binding sites, and the large variability in the number of binding sites observed in a given structure (e.g. the SCN) could have two main causes, viz. the existence of different melatonin receptor subtypes and the method used to detect the binding sites. Regarding the method, the poor resolution of the autoradiograms could play a role. Indeed, the binding is detected only if the number of receptors present in a given structure is high. If the density of receptors is too low or if the receptors are localized on special cell types that are not anatomically clustered, then relevant sites could be overlooked. One way to solve this problem would be to work at the cell level, using cyto-autoradiographic techniques. Progress in this direction has been hampered by the fact that, until recently

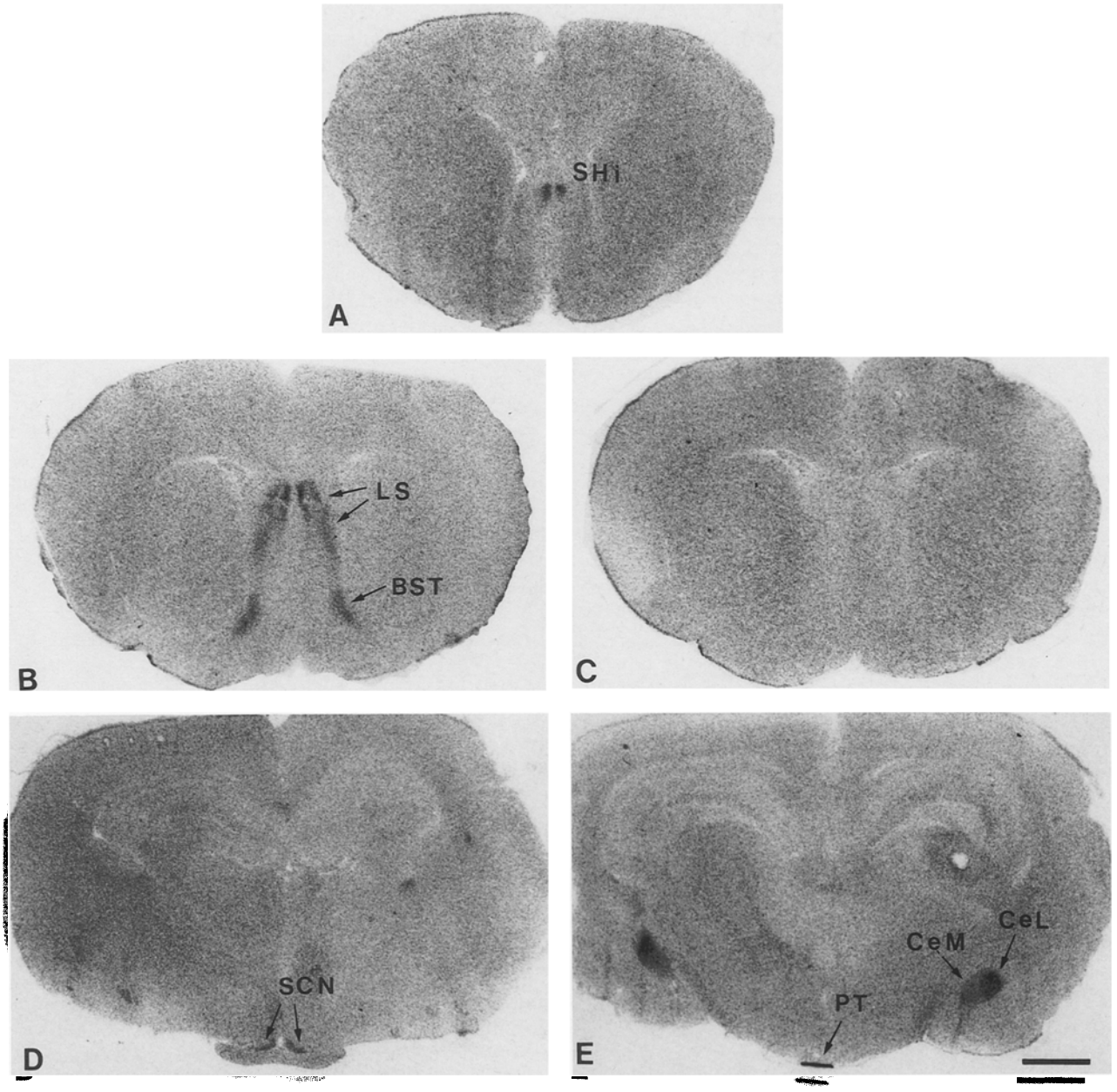


Fig. 4A–E. Distribution of 2-^{125}I-melatonin binding in the edible dormouse brain. **C** Non-specific binding (determined in the presence of 1 μM melatonin) in a section adjacent to that shown in **B**. *Bar*: 160 μm

(Masson-Pévet et al. 1993), it has not been possible to detect melatonin receptors on fixed tissues, i.e. on structurally well-preserved material. In situ hybridization (a highly sensitive technique) will also solve this problem, once the gene coding for melatonin receptors is cloned.

The potential existence of different melatonin receptor subtypes could also explain the variability in the results. Looking at the many different roles ascribed to melatonin (Reiter 1991) and the many different sites containing melatonin receptors, it seems probable that different subtypes of melatonin receptors exist, as is the case for most receptors. The only possibility for discriminating between them would be the use of various different specific agonists and/or antagonists. Although the search for such molecules is extremely active and seems promising (Yous et al. 1992; Duranti et al. 1992), no autoradiographic localization of melatonin-binding sites using a ligand other than 2-^{125}I-melatonin has ever been published.

Possible role of melatonin-binding sites

The assignement of a role for melatonin-binding sites within specific brain areas is limited by two factors: (1) our understanding of the effects of melatonin and of the concerned brain areas themselves; (2) the variations observed among species (our results and Table 1). However, the different structures shown in this study to contain melatonin binding sites will now be examined, bearing in

mind that melatonin possesses the principal characteristics of a time-keeping hormone involved in the regulation of biological rhythms (Redman et al. 1983; Goldman and Darrow 1983; Tamarkin et al. 1985; Pévet 1988; Armstrong 1989).

SCN. The fundamental role of the SCN, which contains the biological clock in mammals (Ralph et al. 1990), is the generation of many daily rhythms and their synchronization with lighting conditions (Rusak and Zucker 1979; Meijer and Rietveld 1989). Melatonin has been shown (1) to resynchronize daily motor activity in free running rats (Redman et al. 1983; Armstrong 1989) and hamsters (Kirsch et al. 1993), but only when the SCN is intact (Cassone et al. 1986 a); (2) to affect SCN metabolism (see review in Cassone 1991); (3) to induce neurophysiological responses of rat SCN neurons in culture (Stehle et al. 1989). The presence of high-affinity melatonin receptors within the SCN of most mammalian species studied so far (present results and Table 1) probably represents the substrate for the effects of melatonin on SCN function and circadian rhythmicity.

Whether the SCN is a necessary site of melatonin action in the regulation of seasonal functions is not yet clear. However, different experimental results support the idea that they are not involved, at least not directly. For example, the gonadal response of male Syrian hamsters infused with melatonin is not prevented by SCN destruction. Moreover, melatonin receptors show no seasonal variations in the SCN in the hedgehog (Gauer et al. 1993 c). On the other hand, the medial preoptic area has been shown to be implicated in the mediation of melatonin action on the gonadal response (Devries et al. 1989) and luteinising hormone-releasing hormone (LHRH) perikarya are present in the preoptic area of the Syrian hamster (Jennes and Stumpf 1980). These observations suggest that the preoptic area and/or anterior hypothalamic area, rather than the SCN, are involved in mediating melatonin effects on reproduction. In this hypothesis, melatonin receptors present in the AMPO of the garden dormouse and the jerboa could be the substrate for this effect. Moreover, high affinity melatonin receptors have been found in these areas in other species (see Table 1).

PT. The PT has been suggested as being the site in which melatonin mediates its photoperiodic effects on the neuroendocrine reproductive system (Tamarkin et al. 1985; De Reviers et al. 1989; Vanecek and Jansky 1989; Weaver and Reppert 1990). Moreover, acute treatment with melatonin has been shown to regulate LH release from PT tissue (Nakazawa et al. 1991). This hypothesis is strengthened by the observation that the density of melatonin receptors in the PT varies with the seasons in the ground squirrel (Stanton et al. 1991), the European hamster (Skene et al. 1993), the hedgehog (Gauer et al. 1993 c) and short photoperiod versus long photoperiod Syrian hamsters (Vanecek and Jansky 1989). Moreover, the density of melatonin receptors in the hedgehog changes in parallel with two different seasonal functions, sexual activity on the one hand and hypothermia versus euthermia during the hibernation period on the other (Gauer et al. 1993 c). The presence of melatonin receptors in the PT of mammals (these results and Table 1) could represent the substrate for the effect of melatonin on synchronizing seasonal functions.

Other hypothalamic areas. In addition to the SCN and PT, a number of other hypothalamic structures are labelled in the present (Table 2) and previous studies (Table 1). Melatonin-binding sites in the preoptic and anterior hypothalamic areas have been speculated to be involved in mediating melatonin effects on reproduction and hibernation (Stankov et al. 1991 b). In the present study, two species (the garden dormouse and jerboa) also expressed melatonin-binding sites in the PVN. Previously, melatonin-binding sites in the PVN have only been reported for the sheep (Bittman and Weaver 1990). Taking into account the well-known role of the PVN as an integrative site for neuroendocrine and automatic functions, this provides another structure via which melatonin could influence the internal milieu of the animal. However, more information about the specific cell types involved is needed to improve insights into the possible roles of melatonin.

Extra-hypothalamic areas. The phylogenetic trend towards a reduction in the number of brain areas expressing melatonin-binding sites in higher vertebrates is especially clear in the extra-hypothalamic areas. In non-mammalian vertebrates, a wide array of cortical structures display specific 2-^{125}I-melatonin binding, viz. in birds (Rivkees et al. 1989; Stehle 1990; Cassone and Brooks 1991; Brooks and Cassone 1992), fishes (Martinoli et al. 1991), amphibians and reptiles (Wiechmann and Wirsig-Wiechmann 1992, 1993), most of them being associated with visual processing structures. In the present study, none of the five different species of hibernators show clear expression of melatonin-binding sites in the cortex. Nevertheless, a number of the subcortical structures labelled are involved in the processing of sensory information. Both the inferior colliculi (jerboa, sheep) and the IGL (garden dormouse, white-footed mouse) receive a direct projection from the retina. Moreover, the olfactory bulb and amygdala are involved in the processing of sensory (i.e. olfactory) information. Different sub-areas of the amygdala complex are labelled in both the edible dormouse and garden dormouse, whereas the highest number of melatonin-binding sites outside the PT is found in the olfactory bulbs of the garden dormouse. Previously, melatonin-binding sites in the olfactory bulb have been described in the rabbit, whereas putative melatonin receptors in the amygdala have been reported for the sheep and white-footed mouse (Table 1).

In the present study, some species exhibit melatonin-binding sites in the septal zone, i.e. in the edible dormouse and garden dormouse, as previously described for white-footed mouse and sheep. Both lateral septum and BST, where most of the melatonin-binding sites are found, are known as important relay stations for hippocampal information on its way to the hypothalamus. The presence of melatonin-binding sites in well-known relay stations such as the septum and the thala-

mus indicates that melatonin may be able to influence not only incoming sensory information, but also the internal flow of information depending on the time of day.

Acknowledgements. The authors wish to thank Drs. F. Gauer and V. Simonneaux for their critical comments, Mr. D. Bonn for taking care of the animals, and Miss F. Murro for typing the manuscript.

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