Published in final edited form as:

Brain Behav Evol. 2009 ; 73(1): 16–25. doi:10.1159/000202987.

Distribution of 2-[¹²⁵I]iodomelatonin Binding in the Brain of Mexican Free-Tailed Bats (*Tadarida brasiliensis*)

Christine Schwartz^a, Paul Bartell^b, Vincent Cassone^c, and Michael Smotherman^a

^a Department of Biology, Texas A&M University, College Station, Tex

^b Department of Poultry Science, Pennsylvania State University, State College, Pa

^c Department of Biology, University of Kentucky, Lexington, Ky., USA

Abstract

The neurohormone melatonin is an important signal for both time of day and time of year in many seasonally breeding animals. High densities of melatonin receptors have been found in the suprachiasmatic nucleus, median eminence, and the pituitary gland in almost all mammals investigated so far, and lower densities of melatonin receptors have also been localized to other brain regions varying in a species-specific fashion. Because species-specific differences in receptor distributions have been correlated with differences in behavior and ecology, a comparative study of how melatonin receptors are distributed in vertebrate brains can be useful to the understanding of the functional organization of neural circuits controlling daily and seasonal behaviors. In this study, we localized and characterized melatonin binding sites in the brain of the Mexican free-tailed bat (*Tadarida brasiliensis*) using in vitro autoradiography with 2-[¹²⁵I]iodomelatonin. *Tadarida brasiliensis* is a nocturnal insectivorous mammal that seasonally migrates, reproduces once a year, and exhibits documented sexual dimorphisms in seasonal reproductive behaviors, most notably in courtship vocalizations. Prominent 2-[¹²⁵I]iodomelatonin binding was found in the median eminence, suprachiasmatic nuclei, and hippocampus, similar to that observed in other mammals. High densities of binding were also localized to structures of the basal ganglia, including the caudate nucleus, putamen, and nucleus accumbens, a feature commonly observed in songbirds but not in mammals. Saturation analysis indicated that the observed binding sites had an affinity for melatonin typical of the binding properties for the Mel_{1a} receptor subtype. We conclude that melatonin receptor distributions in the Mexican free-tailed bat brain appear to show similarities with the reproductive and circadian systems of other mammals and the basal ganglia of songbirds.

Keywords

Bats; Basal ganglia; Hippocampus; Melatonin; Suprachiasmatic nucleus

Introduction

Melatonin, an indoleamine neurohormone produced and released by the pineal gland, is an important regulator of vertebrate circadian rhythms and seasonal processes [Cassone and Menaker, 1984; Wiechmann, 1986; Lincoln, 2006]. Melatonin levels are low during the day and high at night, making it an effective conveyer of both time of day and time of year, exerting its circadian and circannual actions through discrete populations of melatonin receptors. In

many seasonally breeding mammals, melatonin durations relay information about photic cues to other parts of the brain and body to ensure that reproduction and associated behaviors occur at the optimal time. Reproductive behaviors can be induced in pinealectomized hamsters if given melatonin infusions mimicking longer days of spring [Maywood et al., 1990; Goldman, 1991]. Similarly, pinealectomized sheep, short day breeders, can be reproductively induced if given melatonin infusions mimicking shorter days [Karsch et al., 1988]. Lesions of melatonin binding sites in the mediobasal hypothalamus of male Syrian hamsters blocks normal testis regression and the associated drops in gonadotropic hormone levels that normally occur with exposure to short days, suggesting that melatonin acts on the reproductive axis through this region [Maywood and Hastings, 1995]. Melatonin also appears to be involved in songbird seasonal reproduction, playing a role in the seasonal regulation of song nuclei volume. In starlings, melatonin implants attenuated the long day increase in the volume of the song nucleus HVC and reduced the volume of another song nucleus, Area X [Bentley et al., 1999]. Thus, melatonin appears to act through receptors in discrete brain areas to control seasonal behaviors. When examining seasonal behaviors where the neuroanatomy is not understood, mapping of melatonin receptor distributions could provide useful insight into the neural networks underlying these behaviors.

2-[¹²⁵I]iodomelatonin (IMEL) binding assays [Vakkuri et al., 1984] are a reliable method for determining the distribution of melatonin receptors in many vertebrates. In mammals, melatonin binding sites are found in the hypothalamus and pituitary, specifically the median eminence (ME) and pars tuberalis (PT), regions known to directly regulate reproduction, and also in the suprachiasmatic nuclei (SCN), the site of the mammalian circadian clock and recipient of the monosynaptic tract providing light input from the retina, important for environmental entrainment [Cassone, 1990]. Additionally, melatonin binding sites are found relatively infrequently in a species-specific manner in other brain regions, including parts of the cortex, thalamus, and hypothalamus [Duncan et al., 1986; Weaver et al., 1989; Stankov et al., 1991b]. In birds, melatonin binding sites are found in the visual system (including visual SCN) and in the song control systems of passerines [Rivkees et al., 1989; Gahr and Kosar, 1996; Whitfield-Rucker and Cassone, 1996; Bentley and Ball, 2000]. A description of melatonin receptor distribution in the brain areas of *Tadarida* could help to identify areas involved in the seasonal processes of breeding and song control as they do in other species.

Mexican free-tailed bats, *Tadarida brasiliensis*, provide a unique opportunity to comparatively study the role of melatonin because these animals display a variety of seasonal behaviors normally found separately in either mammals or birds, such as migration [Fleming and Eby, 2003] and courtship singing behavior [Bohn et al., 2008]. In this study, we use in vitro autoradiography to localize and characterize melatonin-binding sites in the Mexican free-tailed bat brain. We hypothesize that IMEL binding sites will be found in the ME/PT and also in the SCN, as sites in these regions are found in most mammals. Furthermore, we anticipate that if other IMEL binding sites were present in the bat brain, these sites could be directly involved in migration or seasonal reproductive behaviors, including courtship song production. We also look for sexual dimorphisms in IMEL binding in this study, due to sexual dimorphisms present in reproductive behavior.

Materials and Methods

Animals

Seven male and seven female Mexican free-tailed bats (*Tadarida brasiliensis*) were obtained in May 2007 from a natural roosting site on the College Station campus of Texas A&M University. Additionally, six males in torpor were obtained from the same site in January 2008. All animals were killed by anesthetic overdose (sodium pentobarbital) between 10 am and noon after their capture. Their brains were quickly removed, frozen in isopentane, and stored

at -80°C . All experimental procedures were completed in accordance with NIH guidelines for experiments involving vertebrate animals and were approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC).

In vitro Autoradiography

Serial coronal sections (20 μm) of the entire brain were cryosectioned, thaw mounted on Histobond microscope slides, and stored at -80°C until use. Adjacent brain sections were placed on separate sets of slides so that every fourth slice was in the same set. Two of the slide sets were used for these experiments and the remaining two were used in separate experiments not presented here. We used slight modifications of previously described protocols for determining the distribution and characterization of melatonin binding sites [Duncan et al., 1986; Dubocovich and Takahashi, 1987; Lu, 1994]. Briefly, the two slide sets were incubated in 0.5 M Tris buffer with 0.1% bovine serum albumin at 21°C for 1 h. One slide set was then incubated in 100 pM IMEL (PerkinElmer) in Tris buffer and the second set was incubated in 100 pM IMEL in Tris buffer with 1 μM melatonin for 2 h at 21°C . For the saturation experiment, the concentrations of IMEL used for this step ranged from 1 pM to 1.2 nM and three different animals were used for each concentration. The slide sets incubated with 1 μM melatonin served as controls to determine nonspecific binding. All experimental and control slide sets were then washed in Tris buffer at 4°C for 30 min and air dried. The slides used for determining the distribution of melatonin binding sites were dried and apposed to BioMax Maximum Resolution autoradiographic film (Kodak) with a calibrated ^{14}C standard for six weeks at -80°C . After developing the film, optical density measurements from five sections on the experimental slide set were averaged for each brain area for each animal using NIH Image J [Rasband, 1997]. The slides used for saturation studies were dried and apposed to phosphor imaging plates (Fujifilm) with a calibrated ^{14}C standard and incubated for three days at 21°C . The plates were then developed using a phosphorimager (Fujifilm BAS-5000). The optical density of the caudate putamen was measured in five sections from the experimental slide set for each animal and averaged for each concentration in the same manner as above. All sections were subsequently stained with cresyl violet to aid in brain structure identification. Areas exhibiting IMEL binding were also measured on control brains to provide nonspecific binding control data. A standard curve was generated using the optical densities from the calibrated standards. 2- ^{125}I iodomelatonin binding (nCi/mg protein) from each brain region was calculated using the values from the standard curve. Nonspecific binding was subtracted from total binding to determine specific binding for each brain region. Differences in IMEL binding between sexes for each brain region were assessed using t tests. A nonlinear regression was performed on the data collected from the saturation experiment to determine K_d and B_{max} .

Results

Distribution of IMEL-Binding Sites

We used a custom brain atlas developed in our lab for *Tadarida brasiliensis* to identify the brain areas reported in this paper. The basal ganglia are large, easily recognizable structures located beneath the external capsule, anterior to the thalamus. The caudate nucleus and putamen, hereafter referred to collectively as the caudate putamen, are one structure in rodents and look very similar in bats, except that the internal capsule more pronouncedly bisects the two structures, similar to the pattern in primates. The positions of the globus pallidus, ventral pallidum, and nucleus accumbens were inferred by cytoarchitectonic features as determined using cresyl violet and cytochrome oxidase stains, and the positions of these structures were similar to those described in rats [Paxinos and Watson, 1998]. Similarly, we identified the SCN, ME, and PT based on their relative sizes and positions in the brain. The hippocampus, including dentate gyrus, was also identified based on location and cytoarchitectonics of the

structures. A comprehensive review of the available neuroanatomical resources from other bat species also helped identify the major brain regions reported in this paper [Prasada Rao and Kanwal, 2004; Maseko and Manger, 2007].

The entire *Tadarida* brain, from the most rostral tip of the olfactory bulbs to the caudal-most extension of the brainstem nucleus ambiguus, was evaluated to determine where IMEL binding sites were present. The threshold for binding was determined by adjusting background level for each image according to the nonspecific binding levels obtained from the control slide sets exposed to melatonin. Any area with binding higher than the nonspecific binding levels was analyzed. We found binding sites, in order from highest to lowest amount of binding, in the ME/PT, SCN, caudate putamen, nucleus accumbens, and the granular dentate gyrus of the hippocampus in all bats studied (fig. 1). The nucleus accumbens was the first region with binding to appear in the rostral to caudal direction, followed by the caudate putamen. Binding in these areas persisted for the entire duration of the structures and was relatively uniform throughout each structure. The highest receptor densities were found in the hypothalamus, in the ME and SCN. We include the PT of the pituitary along with the ME as one structure for our analysis because although it is likely that IMEL binding sites are present there due to their prevalence in many other mammals [Stankov et al., 1992; Nonno et al., 1995a; Williams et al., 1995, 1996], these small structures are located so close together and the free-tailed bat is such a small animal that we were unable to differentiate between them on the autoradiographic film, an issue seen in many of the small mammals examined [Weaver et al., 1989, 1990]. In the hippocampus, we quantified the most caudal portion of the granular dentate gyrus. The levels of binding in the rostral portions of the granular dentate gyrus were low and quite variable or even absent in some of the bats surveyed. Binding in the caudal regions was more consistent and dense, so we chose to focus our analysis there.

In our final analysis, we quantified the relative amount of binding in each brain region for both males and females (table 1). Two of the seven females and one of the seven males only provided data for some of the brain regions because of cryosectioning differences that resulted in the loss of some brain sections. The brains of males and females were analyzed separately to assess any sexual dimorphism in IMEL binding, but where no significant differences were found, the brain regions are presented together. No significant differences between sexes were found for the ME/PT region ($0.136 \pm 0.0780 \mu\text{Ci/g}$, $n = 13$), but binding in the SCN of males ($0.0619 \pm 0.0247 \mu\text{Ci/g}$, $n = 7$) was significantly higher ($p = 0.048$, $t = 2.206$ with 12 d.f.) than in females ($0.0383 \pm 0.0136 \mu\text{Ci/g}$, $n = 7$). IMEL binding in the caudate putamen and nucleus accumbens of the basal ganglia (0.0382 ± 0.0237 and $0.0280 \pm 0.0174 \mu\text{Ci/g}$ respectively, $n = 13$) and in the dentate gyrus of the hippocampus ($0.0173 \pm 0.00771 \mu\text{Ci/g}$, $n = 11$) were not significantly different between sexes. In addition, in one animal we observed binding in the frontal cortex, including the anterior cingulate cortex. Although we can only report actual binding values for this one individual ($0.0193 \mu\text{Ci/g}$), it is possible that IMEL binding sites occur here in the other animals as well, but in levels too low to quantify with autoradiographic techniques.

In addition, six male bats taken out of torpor were also analyzed to determine if there were any seasonal changes in melatonin receptor distribution or density. 2-[^{125}I]iodomelatonin binding sites were found in the exact same areas of the brain in these torpid bats and there was no significant difference in IMEL binding density between the male bats collected in the summer and the torpid male bats collected in the winter.

Characterization of IMEL-Binding Sites

Saturation experiments were performed to characterize the binding affinity and maximum number of melatonin receptors found in the basal ganglia. The caudate putamen was chosen because its relatively large size allowed for analysis of several sections for each animal. Nonlinear regression analysis on data from our saturation experiments revealed a high affinity

binding site (equilibrium dissociation constant (K_d) is 22.3 ± 15.1 pM) in the caudate putamen of *Tadarida brasiliensis* (fig. 2). Because the value obtained was in the picomolar range, the binding sites can be characterized as high affinity. Specific binding plateaued at approximately 200 pM, demonstrating that binding is saturable and the total number of binding sites (B_{max}) was 0.1992 ± 0.0285 fmol/mg protein (fig. 2).

Discussion

This study provides a report of melatonin receptor localization and characterization in the Mexican free-tailed bat, *Tadarida brasiliensis*. 2-[125 I]iodomelatonin binding sites were found in the caudate putamen, nucleus accumbens, ME/PT, SCN, and hippocampus (fig. 1; table 1). Saturation experiments revealed a saturable high affinity binding site (fig. 2).

Melatonin Binding Sites in Typical Mammalian Regions

2-[125 I]iodomelatonin binding sites were found in the bat brain in areas previously reported in other mammals: the SCN and ME/PT. The mammalian SCN is the circadian pacemaker and receives monosynaptic input from the retina, allowing the animal to entrain to the photic environment [Cassone and Menaker, 1984; Cassone, 1990]. Melatonin production by the pineal is controlled by the SCN via a polysynaptic pathway and the presence of melatonin receptors in the SCN provides the mechanism for melatonin to feedback to the SCN and inhibit its activity. Melatonin receptors in the ME/PT region mediate the effects of melatonin on reproductive behavior and physiology [Cassone, 1990, 1998]. Lesions of melatonin binding sites in the mediobasal hypothalamus of male Syrian hamsters blocked the normal testis regression and gonadotropic hormone level drop associated with exposure to short days, suggesting that melatonin acts on the reproductive axis through these sites [Maywood and Hastings, 1995]. In the same study, secretion of the reproductive hormone prolactin was unaffected by the mediobasal hypothalamic lesions, but it was speculated that prolactin levels are instead controlled by melatonin binding sites in the pituitary, specifically pars tuberalis. The presence of melatonin receptors in the bat SCN and ME/PT appears to be consistent with the assumption that these areas control reproductive behaviors in bats via mechanisms common to most mammals.

Sexual Dimorphism in 2-[125 I]iodomelatonin Binding

The SCN was the only area to show sexual dimorphism in IMEL binding in *Tadarida*, with higher binding observed in males. Our findings represent the first case of sexual dimorphism in IMEL binding in a mammalian brain region. The only other reported instances of sexual dimorphism in IMEL binding are in the song control system of birds and parts of the visual system and preoptic area in quail, which all displayed higher binding in males [Gahr and Kosar, 1996; Whitfield-Rucker and Cassone, 1996; Aste et al., 2001]. The reasons for these differences are unclear, but could potentially be the result of sexually dimorphic neurotransmitters, specifically GABA, which, when given at physiological doses, increases IMEL binding in several brain regions of the quail, including the preoptic area [Canonaco et al., 1994]. In addition, sexually dimorphic GABA-like immunoreactivity was found in the song control system of zebra finches [Grisham and Arnold, 1994]. Both of these examples are from the bird literature, but it seems reasonable that something similar could be occurring in bats. The sexual dimorphism in IMEL binding in the bat SCN might contribute to differences seen in male and female reproductive behaviors, but it must be presumed that changes in SCN physiology would generally affect all circadian behaviors, due to its role as pacemaker of the mammalian circadian system.

2-[¹²⁵I]iodomelatonin Binding in the Telencephalon

The hippocampus is important for short-term memory and spatial navigation. 2-[¹²⁵I]iodomelatonin binding in the hippocampus was previously reported in other mammals, including primates, rabbits, cattle, and red deer [Stankov et al., 1991a, 1993; Nonno et al., 1995b; Williams et al., 1996]. In bats the hippocampus could be important for migration, as seasonally migrating bats are consistent in their routes and final destinations [Fleming and Eby, 2003]. Several species of birds exhibit seasonal changes in hippocampal volume associated with behaviors requiring enhanced spatial memory, such as food hoarding and brood parasitism [Smulders et al., 1995, 2000; Clayton et al., 1997], however, melatonin receptors have not been found in the bird hippocampus [Cassone et al., 1995], and evidence suggests that photoperiod is only indirectly responsible for changes in hippocampal volume [MacDougall-Shackleton et al., 2003]. Seasonal changes in hippocampal volume have not been found in mammals with similar food hoarding behaviors, such as the food-caching gray squirrel, even though the behavior is seasonal [Lavenex et al., 2000]. There is no evidence of seasonal or circadian changes in memory capacity or hippocampal functioning that might illustrate a potential role for melatonin in the mammalian hippocampus. It is possible that in mammals certain seasonal behaviors such as migration and reproduction might be supported by melatonin receptors in the hippocampus facilitating increased memory capacity without concurrent changes in hippocampal volume. We found no difference in IMEL binding between males and females, indicating that the role of melatonin receptors in the free-tailed bat hippocampus is most likely unrelated to sexually dimorphic reproductive behaviors.

Three areas of the basal ganglia of the Mexican free-tailed bat brain were found to contain putative melatonin receptors: the caudate nucleus, putamen, and nucleus accumbens. 2-[¹²⁵I]iodomelatonin binding in the mammalian putamen was previously reported only in red deer [Williams et al., 1996]. 2-[¹²⁵I]iodomelatonin binding in the nucleus accumbens, a ventral component of the basal ganglia, was previously observed in mice [Weaver et al., 1990]. Melatonin injections into the nucleus accumbens of rats changed their locomotor activity [Gaffori and Van Ree, 1985; Paredes et al., 1999], but the presence of melatonin receptors in the nucleus accumbens has not been confirmed in the rat. What is particularly striking about the melatonin receptor profile in the bat basal ganglia is that putative melatonin receptors were observed throughout the three regions of the basal ganglia at relatively high densities, which has not been observed in other mammals. This distribution is more reminiscent of the high densities of IMEL binding observed in homologous regions of the songbird brain [Gahr and Kosar, 1996; Whitfield-Rucker and Cassone, 1996; Bentley et al., 1999], which might be correlated with similarities in the seasonal migratory, foraging, and reproductive behaviors displayed by birds and bats.

2-[¹²⁵I]iodomelatonin binding in the bat basal ganglia is interesting because the basal ganglia are generally involved in the coordination of complex behaviors, including the orchestration of complex motor sequences [Aldridge and Berridge, 1998; Graybiel, 2000, 2001; Berridge et al., 2005; Grillner et al., 2005]. The caudate nucleus is credited with planning complex behaviors, and the putamen is believed to act as a substrate for the integration of multisensory, memory and contextual cues for the ongoing control of behaviors. This control is achieved by a striatal-thalamo-cortical feedback loop through the basal ganglia that selectively activates or modulates subsequent motor patterns- [Graybiel, 2000; Hikosaka et al., 2000; Aim, 2004; Bear et al., 2006]. Extrapyramidal inputs from the motor cortex converge with sensory and memory cues in the basal ganglia to regulate the activity of excitatory thalamic neurons projecting back onto the motor cortex via inhibitory outputs to the globus pallidus. We did not find IMEL binding in the primary output centers of the basal ganglia, the globus pallidus and the substantia nigra [Graybiel, 2000; Grillner et al., 2005]. Disruption of basal ganglia circuits seriously impacts motor coordination in mammals, but less dramatic pharmacological manipulations of

synaptic activity can profoundly influence the initiation and drive to complete specialized complex behaviors [Berridge and Aldridge, 2000a, b; Berridge et al, 2005]. Melatonin receptors in the *Tadarida* basal ganglia could contribute to the regulation of seasonally specific behaviors such as migration, foraging patterns, prey preferences, and reproductive behaviors, including separation of the sexes, the formation of maternity roosts, and male courtship behaviors such as singing and territorial displays [Altringham, 1996].

In songbirds, IMEL binding is found in Area X, the avian homologue of the basal ganglia [Gahr and Kosar, 1996; Whitfield-Rucker and Cassone, 1996; Bentley and Ball, 2000]. Area X is the part of the song control system involved in learning and development of song [Brenowitz et al., 1997]. In a manner analogous to its general function in mammals, the avian basal ganglia is believed to contribute to the development of the characteristic sequence stereotypy of oscine courtship songs [Doupe et al., 2004, 2005]. Through the anterior forebrain pathway, the bird basal ganglia contribute to both the variability of the song during development [Brainard and Doupe, 2000; Kao and Brainard, 2006] and its stereotypy [Scharff and Nottebohm, 1991]. We do not know if the basal ganglia in the free-tailed bat are involved in the production of the courtship song, but current models of how non-human mammalian vocalizations are generated in the brain do not include the basal ganglia. Humans, however, do rely upon basal ganglia functions for normal speech production [Aim, 2004].

In addition to the areas containing IMEL binding sites in all bats, we also found binding in the frontal cortex, including the anterior cingulate cortex, of one male animal in quantifiable levels. It is possible that binding sites were present here in the other animals, but in levels too low to quantify with autoradiographic techniques. Frontal cortex binding was previously reported in primates and rabbits [Stankov et al., 1991a, 1993]. Similar to these previous findings, the IMEL binding sites of the *Tadarida* frontal cortex were not restricted to a specific cortical layer, but rather appeared to be relatively uniform through all layers. The significance of the IMEL binding in the frontal cortex is unclear.

Characterization of 2-[¹²⁵I]iodomelatonin Binding Sites

The K_d and B_{max} values determined for the IMEL binding sites in the caudate putamen of the free-tailed bat (22.3 ± 15.1 pM and 0.1992 ± 0.0285 fmol/mg protein, respectively) were within the same range as reported for rabbit, horse, sheep, and human [Stankov et al., 1991a, b; Yuan et al., 1991]. These values indicate that binding sites in the caudate putamen are most likely to be the Mel_{1a} receptor, which has a K_d of 20–40 pM [Reppert et al., 1996]. Researchers have identified three different melatonin receptors, Mel_{1a} , Mel_{1b} , and Mel_{1c} , and all but Mel_{1c} , have been found in the mammalian brain [Dubocovich, 1995; Reppert et al., 1996; Reppert, 1997]. It is unlikely that the receptors found in the caudate putamen are Mel_{1c} receptors because the K_d range for this receptor is around 160 pM. The melatonin receptors have been cloned and belong to a family of guanine nucleotide binding protein (G protein)-coupled receptors [Ebisawa et al., 1994; Reppert and Weaver, 1995]. This was supported prior to the actual cloning of the genes by showing that IMEL binding could be inhibited by the presence of a non-hydrolyzable GTP analog (GTP γ S), which was subsequently demonstrated in several species [Stankov et al., 1992, 1993; Wiechmann and Wirsig-Weichmann, 1994; Williams et al., 1995, 1996]. Further investigations identifying which melatonin receptors are located in each bat brain region are needed.

Absence of Seasonal Effects on 2-[¹²⁵I]iodomelatonin Binding

It is presumed that seasonal changes in nightly melatonin durations drive seasonal changes in behavior, but it is possible that seasonal changes in receptor numbers might also contribute. In ground squirrels and hamsters it was found that IMEL binding in the PT decreases when the animals were in hibernation [Stanton et al., 1991; Skene et al., 1992], but we found no

significant difference in IMEL binding between bats collected in the summer and the torpid bats collected in the winter. Ground squirrels and hamsters are true hibernators, whereas the free-tailed bats only go through periods of daily torpor. Torpor periods are shorter and the animals wake and raise their body temperature to normal levels more frequently. Because free-tailed bats, which migrate to avoid long periods of cold temperatures, do not go through the lengthy depression of body temperature that these other mammals endure might explain why no differences in IMEL binding density were found to be associated with torpor. Torpor can be induced in bats at any time of year by exposure to cold temperatures regardless of photoperiodic conditions, which suggests that torpor is not regulated by the same seasonal and neuroendocrine cues that initiate hibernation. Seasonal differences in IMEL binding in the PT was also shown in mink, but this was not reported to be shown specifically in hibernating animals [Messenger et al., 1997].

Mexican free-tailed bats have many other seasonal behaviors in addition to torpor, including migration and courtship behaviors, which could potentially be associated with seasonal changes in IMEL binding. For example, in songbirds, there are significant differences in IMEL binding associated with different seasons in the song control system [Whitfield-Rucker and Cassone, 1996; Bentley and Ball, 2000]; however, although *Tadarida brasiliensis* exhibits prominent seasonal behaviors, especially a courtship singing behavior similar to birds, no significant seasonal differences in IMEL binding associated with season were found in any brain region. This suggests that the seasonal control of behaviors in this species is probably not occurring through changes in melatonin receptor numbers, but perhaps through some other mechanism.

Conclusion

The Mexican free-tailed bat, *Tadarida brasiliensis*, is a unique mammalian model for the comparative study of melatonin and melatonin receptors. The bat brain showed IMEL distribution patterns consistent with that observed in other mammals, but also with high densities of receptors distributed in the basal ganglia. The function of melatonin receptors in the mammalian basal ganglia is unknown, but their comparable localization to the song control nuclei in songbirds might provide a clue about their function in bats. Because the free-tailed bats exhibit a suite of behaviors similar to birds, such as flying, singing, and long-distance seasonal migration patterns, our results might shed light on how melatonin regulates seasonal behaviors in the vertebrate brain.

Acknowledgments

We thank Mr. Clint Netherland and the Texas A&M University Athletic Department for access to the bats of Kyle Field. We thank the Texas Parks and Wildlife Department for the collection permits. We thank Kristin Denton and Barbara Earnest for the invaluable assistance and many helpful discussions. This study was funded by Texas A&M University and NIH NIDCD Grant No. DC007962 to MS.

References

- Aldridge JW, Berridge KC. Coding of serial order by neostriatal neurons: a 'natural action' approach to movement sequence. *J Neurosci* 1998;18:2777–2787. [PubMed: 9502834]
- Alm PA. Stuttering and the basal ganglia circuits: a critical review of possible relations. *J Commun Disord* 2004;37:325–369. [PubMed: 15159193]
- Altringham, JD. *Bats: Biology and Behaviour*. New York: Oxford University Press, Inc; 1996.
- Aste N, Cozzi B, Stankov B, Panzica G. Sexual differences and effect of photoperiod on melatonin receptor in the avian brain. *Microsc Res Tech* 2001;55:37–47. [PubMed: 11596148]

- Bear, MF.; Paradiso, MA.; Connors, B. Chapter 14: Brain Control of Movement. In: Bear, MF.; Connors, B.; Paradiso, M., editors. *Neuroscience: Exploring the Brain*. Philadelphia: Lippincott, Williams and Wilkins; 2006. p. 451-479.
- Bentley GE, Ball GF. Photoperiod-dependent and -independent regulation of melatonin receptors in the forebrain of songbirds. *J Neuroendocrinol* 2000;12:745–752. [PubMed: 10929086]
- Bentley GE, Van't Hof TJ, Ball GF. Seasonal neuroplasticity in the songbird telencephalon: a role for melatonin. *Proc Natl Acad Sci USA* 1999;96:4674–4679. [PubMed: 10200321]
- Berridge KC, Aldridge JW. Super-stereo-typy I: enhancement of a complex movement sequence by systemic dopamine D1 agonists. *Synapse* 2000a;37:194–204. [PubMed: 10881041]
- Berridge KC, Aldridge JW. Super-stereo-typy II: enhancement of a complex movement sequence by intraventricular dopamine D1 agonists. *Synapse* 2000b;37:205–215. [PubMed: 10881042]
- Berridge KC, Aldridge JW, Houchard KR, Zhuang X. Sequential super-stereo-typy of an instinctive fixed action pattern in hyper-dopaminergic mutant mice: a model of obsessive compulsive disorder and Tourette's. *BMC Biol* 2005;3:4. [PubMed: 15710042]
- Bohn KM, Schmidt-French B, Ma ST, Pollack GD. Syllable acoustics, temporal patterns and call composition vary with behavioral context in Mexican free-tailed bats. *J Acoust Soc Am* 2008;124:1838–1848.
- Brainard MS, Doupe AJ. Interruption of a basal ganglia-forebrain circuit prevents plasticity of learned vocalizations. *Nature* 2000;404:762–766. [PubMed: 10783889]
- Brenowitz EA, Margoliash D, Nordeen KW. An introduction to birdsong and the avian song system. *J Neurobiol* 1997;33:495–500. [PubMed: 9369455]
- Canonaco M, Tavolario R, Facciolo RM, Artero C, Franzoni MF. Gonadal-GABAergic interaction is an important factor in photo-period-induced 2-[¹²⁵I]iodomelatonin binding changes in the Japanese quail brain. *Brain Res Bull* 1994;34:425–435. [PubMed: 8082036]
- Cassone VM. Melatonin: Time in a bottle. *Oxf Rev Reprod Biol* 1990;12:319–367. [PubMed: 2075003]
- Cassone VM. Melatonin's role in vertebrate circadian rhythms. *Chronobiol Int* 1998;15:457–473. [PubMed: 9787936]
- Cassone VM, Menaker M. Is the avian circadian system a neuroendocrine loop? *J Exp Biol* 1984;232:539–549.
- Cassone VM, Brooks DS, Kelm TA. Comparative distribution of 2-[¹²⁵I]iodomelatonin binding in the brains of diurnal birds: out-group analysis with turtles. *Brain Behav Evol* 1995;45:241–256. [PubMed: 7620873]
- Clayton NS, Reboreda JC, Kacelnik A. Seasonal changes of hippocampus volume in parasitic cowbirds. *Behav Process* 1997;41:237–243.
- Doupe AJ, Perkel DJ, Reiner A, Stern EA. Birdbrains could teach basal ganglia research a new song. *Trends Neurosci* 2005;28:353–363. [PubMed: 15935486]
- Doupe AJ, Solis MM, Kimpo R, Boettiger CA. Cellular, circuit, and synaptic mechanisms in song learning. *Ann NY Acad Sci* 2004;1016:495–523. [PubMed: 15313792]
- Dubocovich M. Melatonin receptors: are there multiple subtypes? *Trends Pharmacol Sci* 1995;16:50–56. [PubMed: 7762083]
- Dubocovich ML, Takahashi JS. Use of 2-[¹²⁵I]iodomelatonin to characterize melatonin binding sites in chicken retina. *Proc Natl Acad Sci USA* 1987;84:3916–3920. [PubMed: 3035559]
- Duncan MJ, Takahashi JS, Dubocovich ML. Characterization of 2-[¹²⁵I]iodomelatonin binding sites in hamster brain. *Eur J Pharmacol* 1986;132:333–334. [PubMed: 3816982]
- Ebisawa T, Suresh K, Lerner MR, Reppert SM. Expression cloning of a high-affinity melatonin receptor from *Xenopus* dermal melanophores. *Proc Natl Acad Sci USA* 1994;91:6133–6137. [PubMed: 7517042]
- Fleming, TH.; Eby, P. Ecology of bat migration. In: Kunz, TH.; Fenton, MB., editors. *Bat Ecology*. Chicago, IL: The University of Chicago Press; 2003.
- Gaffori O, Van Ree JM. Serotonin and antidepressant drugs antagonize melatonin-induced behavioural changes after injection into the nucleus accumbens of rats. *Neuropharmacology* 1985;24:237–244. [PubMed: 2859542]

- Gahr M, Kosar E. Identification, distribution, and developmental changes of a melatonin binding site in the song control system of the zebra finch. *J Comp Neurol* 1996;367:308–338. [PubMed: 8708012]
- Goldman BD. Parameters of the circadian rhythm of pineal melatonin secretion affecting reproductive responses in Siberian hamsters. *Steroids* 1991;56:218–225. [PubMed: 1877061]
- Graybiel AM. The basal ganglia. *Curr Biol* 2000;10:R509–511. [PubMed: 10899013]
- Graybiel AM. Neural networks: neural systems V: basal ganglia. *Am J Psychiat* 2001;158:21. [PubMed: 11136627]
- Grillner S, Hellgren J, Menard A, Saitoh K, Wikstrom MA. Mechanisms for selection of basic motor programs - roles for the striatum and pallidum. *Trends Neurosci* 2005;28:364–370. [PubMed: 15935487]
- Grisham W, Arnold AP. Distribution of GABA-like immunoreactivity in the song system of the zebra finch. *Brain Res* 1994;651:115–122. [PubMed: 7922557]
- Hikosaka O, Takikawa Y, Kawagoe R. Role of the basal ganglia in the control of purposive saccadic eye movements. *Physiol Rev* 2000;80:953–978. [PubMed: 10893428]
- Kao MH, Brainard MS. Lesions of an avian basal ganglia circuit prevent context-dependent changes to song variability. *J Neurophysiol* 2006;96:1441–1455. [PubMed: 16723412]
- Karsch FJ, Malpoux B, Wayne NK, Robinson JE. Characteristics of the melatonin signal that provide the photoperiodic code for timing seasonal reproduction in the ewe. *Reprod Nutr Dev* 1988;28:459–472. [PubMed: 3045928]
- Lavenex P, Steele MA, Jacobs LF. Sex differences, but no seasonal variations in the hippocampus of food-caching squirrels: A stereological study. *J Comp Neurol* 2000;425:152–166. [PubMed: 10940949]
- Lincoln GA. Melatonin entrainment of circannual rhythms. *Chronobiol Int* 2006;23:301–306. [PubMed: 16687303]
- Lu, J. Ph.D. dissertation. Texas A&M University; College Station, TX: 1994. The multi-oscillatory circadian system in the house sparrow, *Passer domesticus*.
- MacDougall-Shackleton SA, Sherry DF, Clark AP, Pinkus R, Hernandez AM. Photoperiodic regulation of food storing and hippocampus volume in black-capped chickadees, *Poecile atricapillus*. *Anim Behav* 2003;65:805–812.
- Maseko BC, Manger PR. Distribution and morphology of cholinergic, catecholaminergic and serotonergic neurons in the brain of Schreiber's long-fingered bat, *Miniopterus schreibersii*. *J Chem Neuroanat* 2007;34:80–94. [PubMed: 17560075]
- Maywood EW, Hastings MH. Lesions of the Iodomelatonin-binding sites of the mediobasal hypothalamus spare the lactotropic, but block the gonadotropic response of male Syrian hamsters to short photoperiod and to melatonin. *Endocrinology* 1995;136:144–153. [PubMed: 7828525]
- Maywood EW, Buttery RC, Vance GHS, Herbert J, Hastings MH. Gonadal responses of the male Syrian hamster to programmed infusions of melatonin are sensitive to signal duration and frequency but not to signal phase nor to lesions of the suprachiasmatic nuclei. *Biol Reprod* 1990;43:174–182. [PubMed: 2378931]
- Messenger S, Caillol M, George D, Martinet L. Seasonal variation of melatonin binding sites in the pars tuberalis of the male mink (*Mustela vison*). *J Neuroendocrinol* 1997;9:523–528. [PubMed: 15305570]
- Nonno R, Capsoni S, Lucini V, Moller M, Fraschini F, Stankov B. Distribution and characterization of the melatonin receptors in the hypothalamus and pituitary gland of three domestic ungulates. *J Pineal Res* 1995a;18:207–216. [PubMed: 8531051]
- Nonno R, Lucini V, Stankov B, Fraschini F. 2-[¹²⁵I]Iodomelatonin binding sites in the bovine hippocampus are not sensitive to guanine nucleotides. *Neurosci Lett* 1995b;194:113–116. [PubMed: 7478190]
- Paredes D, Rada P, Bonilla E, Gonzalez LE, Parada M, Hernandez L. Melatonin acts on the nucleus accumbens to increase acetylcholine release and modify the motor activity pattern of rats. *Brain Res* 1999;850:14–20. [PubMed: 10629744]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. 4. San Diego, CA: Academic Press; 1998.

- Prasada Rao PD, Kanwal JS. Oxytocin and vasopressin immunoreactivity within the forebrain and limbic-related areas in the mustached bat, *Pteronotus parnellii*. *Brain Behav Evol* 2004;63:151–168. [PubMed: 14726624]
- Rasband, WS. ImageJ. Bethesda, MD: US National Institutes of Health; 1997. p <http://rsb.info.nih.gov/ij/>
- Reppert SM. Melatonin receptors: molecular biology of a new family of G protein-coupled receptors. *J Biol Rhythm* 1997;12:528–531.
- Reppert SM, Weaver DR. Melatonin madness. *Cell* 1995;83:1059–1062. [PubMed: 8548792]
- Reppert SM, Weaver DR, Godson C. Melatonin receptors step into the light: cloning and classification of subtypes. *Trends Pharmacol Sci* 1996;17:100–102. [PubMed: 8936344]
- Rivkees SA, Cassone VM, Weaver DR, Reppert SM. Melatonin receptors in the chick brain: Characterization and localization. *Endocrinology* 1989;125:363–368. [PubMed: 2544407]
- Scharff C, Nottebohm F. A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: implications for vocal learning. *J Neurosci* 1991;11:2896–2913. [PubMed: 1880555]
- Skene DJ, Masson-Pevet M, Pevet P. Seasonal changes in melatonin binding sites in the pars tuberalis of male European hamsters and the effect of testosterone manipulation. *Endocrinology* 1992;132:1682–1686. [PubMed: 8462468]
- Smulders TV, Sasson AD, DeVoogd TJ. Seasonal variation in hippocampal volume in a food-storing bird, the black-capped chickadee. *J Neurobiol* 1995;27:15–25. [PubMed: 7643072]
- Smulders TV, Shiflett MW, Sperling AJ, DeVoogd TJ. Seasonal changes in neuron numbers in the hippocampal formation of a food-hoarding bird: The black-capped chickadee. *J Neurobiol* 2000;44:414–422. [PubMed: 10945896]
- Stankov B, Biella G, Panara C, Lucini V, Capsoni S, Fauteck J, Cozzi B, Fraschini F. Melatonin signal transduction and mechanism of action in the central nervous system: using the rabbit cortex as a model. *Endocrinology* 1992;130:2152–2159. [PubMed: 1312448]
- Stankov B, Capsoni S, Lucini V, Fauteck J, Gatti S, Gridelli B, Biella G, Cozzi B, Fraschini F. Autoradiographic localization of putative melatonin receptors in the brains of two Old World primates: *Cercopithecus aethiops* and *Papio ursinus*. *Neuroscience* 1993;52:459–468. [PubMed: 8383820]
- Stankov B, Cozzi B, Lucini V, Capsoni S, Fauteck J, Fumagalli P, Fraschini F. Localization and characterization of melatonin binding sites in the brain of the rabbit (*Oryctolagus cuniculus*) by autoradiography and in vitro ligand-receptor binding. *Neurosci Lett* 1991a;133:68–72. [PubMed: 1665216]
- Stankov B, Cozzi B, Lucini V, Fumagalli P, Scaglione F, Fraschini F. Characterization and mapping of melatonin receptors in the brain of three mammalian species: rabbit, horse and sheep. A comparative in vitro binding study. *Neuroendocrinology* 1991b;53:214–221. [PubMed: 1645852]
- Stanton TL, Siuciak JA, Dubocovich ML, Krause DN. The area of 2-[¹²⁵I] iodomelatonin binding in the pars tuberalis of the ground squirrel is decreased during hibernation. *Brain Res* 1991;557:285–288. [PubMed: 1660755]
- Vakkuri O, Lamso E, Rahkanaa R, Poutsalahti H, Leppaluoto J. Iodinated melatonin: preparation and characterization of the molecular structure by mass and NMR spectroscopy. *Anal Biochem* 1984;142:284–295. [PubMed: 6549370]
- Weaver DR, Carlson LL, Reppert SM. Melatonin receptors and signal transduction in melatonin-sensitive and melatonin-insensitive populations of white-footed mice (*Peromyscus leucopus*). *Brain Res* 1990;506:353–357. [PubMed: 2154292]
- Weaver DR, Rivkees SA, Reppert SM. Localization and characterization of melatonin receptors in rodent brain by in vitro autoradiography. *J Neurosci* 1989;9:2581–2590. [PubMed: 2545841]
- Whitfield-Rucker MG, Cassone VM. Melatonin binding in the house sparrow song control system: sexual dimorphism and the effect of photoperiod. *Horm Behav* 1996;30:528–537. [PubMed: 9047277]
- Wiechmann AF. Melatonin: Parallels in pineal gland and retina. *Exp Eye Res* 1986;42:507–527. [PubMed: 3013666]
- Wiechmann AF, Wirsig-Weichmann CR. Melatonin receptor distribution in the brain and retina of a lizard, *Anolis carolinensis*. *Brain Behav Evol* 1994;43:26–33. [PubMed: 8306189]

- Williams LM, Hannah LT, Hastings MH, Maywood EW. Melatonin receptors in the rat brain and pituitary. *J Pineal Res* 1995;19:173–177. [PubMed: 8789248]
- Williams LM, Hannah LT, Kyle CE, Adam CL. Central melatonin receptors in red deer (*Cervus elaphus*). *Gen Comp Endocrinol* 1996;104:1–6. [PubMed: 8921349]
- Yuan H, Lu Y, Pang SF. Binding characteristics and regional distribution of [¹²⁵I]iodomelatonin binding sites in the brain of the human fetus. *Neurosci Lett* 1991;130:229–232. [PubMed: 1686640]

Fig. 1.

Presence of melatonin receptors in the singing *Tadarida brasiliensis* brain. Representative IMEL binding in the Mexican free-tailed bat. Row **A** indicates the plane of the coronal slice, **B** shows a representative IMEL autoradiograph, **C** provides the accompanying brain atlas, **D** shows the cresyl violet stained sections, and **E** shows nonspecific binding in control brains. 3V = third ventricle; Ac = nucleus accumbens; ACC = anterior cingulate cortex; Aq = aqueduct; Au = auditory cortex; C = caudate nucleus; CA3 = hippocampus Cornu Ammonis 3; cc = corpus callosum; CPu = caudate putamen; ec = external capsule; DG = dentate gyrus; ic = internal capsule; LV = lateral ventricle; ME = median eminence; PAG = periaqueductal gray; Pir = piriform cortex; P = putamen; SCN = suprachiasmatic nucleus.

Fig. 2.

Saturation of IMEL binding to *Tadarida brasiliensis* caudate putamen. The saturation curve is shown in **a**. Specific binding was calculated by subtracting nonspecific binding from total binding. Each point represents three different animals. **b** shows a Scatchard plot illustrating the results of a nonlinear regression analysis indicating a high affinity binding site with $K_d = 22.3 \pm 15.1$ pM and $B_{\max} = 0.1992 \pm 0.0285$ fmol/mg protein.

Table 12-[¹²⁵I]iodomelatonin binding in male versus female *Tadarida brasiliensis* brains

Brain region	Males	Females	P	t(d.f)
Nucleus accumbens (NAc)	0.0213 ± 0.0100 (n = 7)	0.0358 ± 0.0222 (n = 6)	0.147	-1.562(11)
Caudate putamen (CPu)	0.0297±0.0176(n = 7)	0.0481 ± 0.0281 (n = 6)	0.179	1.435(11)
Suprachiasmatic nuclei (SCN)	0.0619±0.0247(n = 7)	0.0383 ± 0.0136 (n = 7)	0.048	2.206 (12)
Median eminence/pars tuberalis (ME/PT)	0.187±0.0909(n = 7)	0.0932 ± 0.0701 (n = 6)	0.181	1.724(11)
Dentate gyrus (DG)	0.0148 ± 0.00336 (n = 6)	0.0204 ± 0.0106 (n = 5)	0.253	-1.222 (9)