mRNA expression profile of serotonin receptor subtypes and distribution of serotonergic terminations in marmoset brain

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| Abbreviations          | Description                                          |
|------------------------|------------------------------------------------------|
| ABA                    | Allen Brain Atlas                                    |
| BLa                    | Basolateral amygdala                                 |
| BMa                    | Basomedial amygdala                                  |
| CA1                    | The hippocampal region, CA fields 1                  |
| CA2                    | The hippocampal region, CA fields 2                  |
| CA3                    | The hippocampal region, CA fields 3                  |
| CB+                    | Calbindin positive neurons                           |
| CG                     | Cingulate cortex                                     |
| CL                     | Central lateral nucleus of thalamus                  |
| CNS                    | Central nervous system                               |
| Co                     | Cortical nucleus of amygdala                         |
| DAB                    | Diaminobenzidine                                     |
| DG                     | Dentate gyrus                                        |
| DIG                    | Digoxygenin                                           |
| DNP                    | Dinitrophenyl hapten                                 |
| DRN                    | Dorsal raphe nucleus                                 |
| EDTA                   | Ethylenediaminetetraacetic acid                      |
| eGP                    | External globus pallidus                             |
| Er                     | Entorhinal cortex                                    |
| GABA                   | Gamma-Aminobutyric acid                              |
| GAD67                  | Glutamate decarboxylase                              |
| HDC                    | Histidine decarboxylase                              |
| iGP                    | Internal globus pallidus                             |
| InG                    | Intermediate gray of superior colliculus             |
| ISH                    | In-situ Hybridization                                |
| ITG                    | Inferotemporal gyrus                                 |
| La                     | Lateral amygdaloid nuclei of amygdala                |
| LD                     | Lateral dorsal                                       |
| LG                     | Lateral geniculate nucleus                           |
| LH                     | Lateral hypothalamus                                 |
| LM                     | Lateral mammillary nucleus                           |
| LS                     | Lateral septum                                       |
| M1                     | Primary motor cortex                                 |
| MD                     | Mediodorsal                                           |
| Abbreviation | Description                                           |
|-------------|-------------------------------------------------------|
| Me          | Medial amygdaloid nuclei of amygdala                  |
| MG          | Medial geniculate body                                |
| MM          | Medial mammillary nucleus                             |
| MO          | Somatomotor area of mouse                             |
| MRN         | Median raphe nucleus                                  |
| MS          | Medial septum                                         |
| MT          | Area V5                                               |
| Op          | Optical nerve layer of superior colliculus            |
| P14         | Postnatal 14 days                                     |
| P56         | Postnatal 56 days                                     |
| PBST        | Phosphate Buffered Saline with Tween                 |
| PFC         | Prefrontal cortex                                     |
| PS          | Presubiculum                                          |
| RH          | Retro-hypothalamus                                    |
| RT          | Reticular nucleus                                     |
| S           | subiculum                                             |
| S1          | Primary somatosensory area                            |
| SC          | Superior colliculus                                   |
| SERT        | Serotonin transporter                                 |
| Slm         | Stratum lacunosum moleculare                          |
| SNC         | Substantia nigra pars compacta                        |
| SNr         | Substantia nigra pars reticulate                      |
| SS          | Somatosensory area of mouse                           |
| SuG         | Superficial gray of superior colliculus               |
| TBS         | Tris-buffered saline                                  |
| TE          | Temporal area                                         |
| TM          | Tuberomamillary nucleus                               |
| TNT         | Tris-NaCl-Tween buffer                                |
| V1          | Area V1                                               |
| V2          | Area V2                                               |
| VglutT1     | Vesicular glutamate transporter 1                     |
| VIS         | Visual area of mouse                                  |
| VL          | Ventral lateral thalamic nuclei                       |
| VPL         | Ventral posterior lateral thalamic nuclei             |
| VPM         | Ventral posterior medial thalamic nuclei              |
| VTM         | Ventral tuberomamillary nucleus                       |
Abbreviations

Zo : zonal layer of superior colliculus
5HT : 5-hydroxytryptamine (serotonin)
5HT1A : Serotonin receptor 1A
5HT1B : Serotonin receptor 1B
5HT1D : Serotonin receptor 1D
5HT1E : Serotonin receptor 1E
5HT1F : Serotonin receptor 1F
5HT2A : Serotonin receptor 2A
5HT2C : Serotonin receptor 2C
5HT3A : Serotonin receptor 3A
5HT3B : Serotonin receptor 3B
5HT4 : Serotonin receptor 4
5HT5A : Serotonin receptor 5A
5HT6 : Serotonin receptor 6
5HT7 : Serotonin receptor 7
5HTR : Serotonin receptor
Summary

Serotonin is a monoamine neurotransmitter. The majority of serotonin is produced in the intestine and only a minor population (about 10%) in the brain, but there have been a number of reports that demonstrate critical modulatory roles of serotonin in the brain. Although the entire gene map atlas in the mouse brain has been constructed (http://mouse.brain-map.org), such map for the primate has not been available yet.

To better understand serotonin function in the primate brain, I examined the mRNA expression patterns of all 13 members of the serotonin receptor (5HTR) family, by in situ hybridization and the distribution of serotonergic terminations by serotonin transporter (SERT) protein immunohistochemical analysis in adult common marmoset (callithrix jucchus) and compared the data with that of adult B6 mice and available gene map atlas for human cortex from allen institute. For the cortex the expression levels were semi-quantitatively analyzed using image-J image analysis software.

Ten of the 13 5HTRs showed significant mRNA expressions in the marmoset brain. My study shows several new features of the organization of serotonergic systems in the marmoset brain. (1) The thalamus expressed only a limited number of receptor subtypes compared with the cortex, hippocampus, and other subcortical regions. (2) In the cortex, there were layer-selective and area-selective mRNA expressions of 5HTRs. (3) Visual cortex (V1) showed a conspicuous area specific expression of 5HT1A in layer IVCβ and 5HT1F in layer IV, although the expression of 5HT1A was not reported in previous studies of macaque V1. (4) Highly localized mRNA expressions of 5HT1F in
presubiculum and lateral mammillary nucleus (LM) of hypothalamus and that of 5HT3A in CA field of hippocampus were observed. (5) There was a conspicuous overlap of the mRNA expressions of receptor subtypes known to have somatodendritic localization of receptor proteins with dense serotonergic terminations in the V1, the central lateral nucleus of the thalamus (CL), the presubiculum (PS), and the medial mammillary nucleus of the hypothalamus (MM). This suggests a high correlation between serotonin availability and receptor expression at these locations. (6) 5HTRs showed similarities of mRNA expression patterns in the V1 of marmoset and human. (7) There was a conspicuous difference in mRNA expression pattern between the marmoset and mouse cortices whereas the patterns of both species were much similar in the hippocampus.

The present study highlights several functional implications of serotonin system in the marmoset brain. (1) 5HT1A might be recruited for direct whereas 5HT4 might be recruited for indirect (feed forward) inhibition of layer II pyramidal neurons depending upon the known synaptic and extrasynaptic transmission from Median raphe (MRN) and dorsal raphe nucleus (DRN), respectively. (2) Based on the diversity of receptor expression in thalamus and cortex it seems that the serotonergic system has less pronounced effect in thalamic function of gating the information whereas more pronounced function in cortical function of integrating the information. (3) Dense serotonergic projection and expression of 5HTR subtypes with excitatory cellular effects in medial hypothalamus suggests that serotonin mainly has facilitatory function in these region. (4) The expression of most 5HTR subtypes in the pyramidal layer of hippocampus suggests the recruitment of serotonin receptors for the modulation of glutamatergic transmission in the hippocampus. (5) The prominent innervation of
serotonin in layer 1 and Stratum lacunosum moleculare (Slm) of hippocampus, where the apical tuft of pyramidal cells are located had no expression of any 5HTR subtype, suggesting that serotonin projection helps in a dendritic integration of pyramidal neurons to control gain in these regions.

In conclusion, the mRNA expression pattern of 5HTRs in the marmoset as compared with those in the mouse shows some significant differences in the cortex, which suggests certain primate specific roles of 5HTRs and the usefulness of the marmoset as a primate model in further studies of serotonergic modulations in higher brain functions that are specific to primates.
Introduction

Serotonin is an important neurotransmitter with multiple neuromodulatory functions in the central nervous system (CNS) (Millan et al., 2008; Lesch and Waider, 2012). Its receptors consist of 13 genetically, pharmacologically, and functionally distinct subtypes belonging to seven subfamilies (Alexander et al., 2011). All serotonergic receptors (5HTRs) are metabotropic G-coupled proteins except for 5HT3A, which is ionotropic. Serotonergic innervations in mammalian CNS originate from the median and dorsal raphe nuclei of the mesencephalon (Moore et al., 1978; Bowker et al., 1983). Previous studies demonstrate that the termination patterns in mammalian subcortical regions are very similar across species [for thalamus see Lavoie and Parent (1991) for basal ganglia see Lavoie and Parent (1990) and Wallman et al.(2011)]. The difference in serotonin-dependent modulation among species therefore depends largely on the receptor type present in each locus.

To date, the distribution of serotonin and its receptors has been examined by immunohistochemical analysis, receptor ligand autoradiography, and in situ hybridization (ISH) in rodents (Mengod et al., 1996), nonhuman primates (Lidow et al., 1989; Hornung et al., 1990; Wilson and Molliver, 1991), and humans (Burnet et al., 1995; Raghanti et al., 2008). The detailed mRNA expression profiles of all the serotonin receptor genes in mice (Lein et al., 2007) and for some brain areas in human (Shen et al., 2012) are now publicly available in the Allen Brain Atlas (ABA) (ABA, 2009; ABA, 2012). Previous study has shown that 5HT1B and 5HT2A are abundant in the visual cortex of macaque monkeys but not in rodents (Watakabe et al., 2009). This species difference demonstrates the
importance of exploring the expression profiles of serotonin and its receptors in primates. In view of the heterogeneity of serotonin receptor subtypes, I wanted to obtain an integrated view of serotonergic modulation in primates by compiling the expression profiles of all the subtypes along with the termination pattern of serotonergic projections in the primate, which may contribute to an understanding of serotonin function in the primate brain.

For this purpose, I chose the common marmoset (*Callithrix jucchus*), a species of small New World monkey, that has attracted the interest of many biomedical researchers because of small size and ease of breeding (Mansfield, 2003). Moreover, the marmoset is the only nonhuman primate that can be used for generating germline-transmitted transgenic lines (Sasaki et al., 2009). In this study, I examined the mRNA expression profiles of all the known serotonin receptor subtypes by 1) ISH of *5HTR* s and 2) the serotonergic projection pattern by immunohistochemical analysis of the serotonin transporter (SERT) in various brain regions of the marmoset. Here, I discuss the differences and similarities of ISH patterns between some of the mouse and marmoset brain areas and publically available human data set by ABA (Shen et al., 2012).

Serotonergic terminations were particularly pronounced in the primary visual cortex (V1), the central lateral (CL) nucleus of the thalamus, the presubiculum, and the mammillary nucleus (MM) of the hypothalamus, where terminations overlapped with the abundant expressions of selected *5HTR* subtypes. Overall, when compared with mice, the serotonin receptor expression patterns in the marmoset brain were largely different in cortex but similar in hippocampus. The thalamus, which gates sensory information (Min,
2010; Monckton and McCormick, 2002), showed less receptor diversity than the cortex and hippocampus, which integrate sensory information.
Results

I examined the mRNA expression patterns of all thirteen known serotonin receptor subtypes. I found significant expressions of ten of them; I was unable to detect the expressions of 5HT1D, 5HT3B, and 5HT5A mRNAs in the marmoset brains examined. 5HT3A mRNA was exclusively expressed in the CA fields of the hippocampus. 5HT1F mRNA was expressed only in layer VI of V1, the presubiculum, and the lateral mammillary body (LM) of the hypothalamus. In general, the expression patterns of all the genes differed in both the intensity and density of ISH signals throughout the marmoset brain. Most of the examined nuclei showed overlapping expressions of multiple 5HTR subtypes. In the cerebral cortex, most subtypes of 5HTR were expressed, whereas I found only limited 5HTR subtypes in the thalamus. The termination pattern obtained by SERT immunohistochemical analysis in my study was similar to those obtained in previous studies of marmosets (Hornung et al., 1990; Hornung and Celio, 1992) and squirrel monkeys (Lavoie and Parent, 1991). Below, I first describe the patterns of expression of 5HTR mRNAs, across cortical areas. I then describe their expression patterns in the hippocampus, thalamus, superior colliculus, hypothalamus, amygdala, striatum, and substantia nigra. I also compared anti-SERT immunoreactivity with 5HTR mRNA expression profiles.

1. Serotonin receptor mRNA expression in cortical areas

To examine the expression profiles in the association and sensory areas of different lobes of the cortex in the rostrocaudal axis, I examined areas 46 and 6, the primary motor cortex
(M1), the primary somatosensory cortex (S1), the inferotemporal gyrus (ITG), area V5 (MT), the temporal cortex (TE), the primary visual cortex (V1), and the secondary visual cortex (V2). Besides these six-layered areas, I also examined the cingulate (CG) cortex and entorhinal cortex (Er) of four-layered areas. In these cortical areas, nine of the ten serotonin receptor genes (i.e. excluding 5HT3A) were expressed. I noted that several 5HTR subtypes exhibited gradients in expression profiles in the sensory and association areas. The most conspicuous example was the V1-V2 border (Figure 3A-F), which has the most differentiated architecture of the primate cortex. 5HT2A, a gene abundantly expressed in the middle layer, also showed a marked difference in mRNA expression level between S1 and M1 (Figure 1, c5, d5).

Despite such differences in mRNA expression level between areas, a few 5HTR subtypes exhibited similarities in their laminar expressions across areas when compared with their expression in the upper, middle, and lower layers. In addition, a few 5HTRs showed sporadic expression across the cortex. 5HT1A, 5HT6, 5HT1E, and 5HT4 were all generally expressed in the upper layers irrespective of the area (Figures 1 and 2, see a1, 2, 3, 4 to k1, 2, 3, 4). This group of genes shared several similar characteristic features in their expression profiles. Compared with 5HT1A and 5HT6, both 5HT1E and 5HT4 were less abundant in layer II. To test my hypothesis of dense expression in excitatory neurons and sparse expression in inhibitory neurons I performed the double hybridization of 5HT1A, 5HT1E, 5HT4, and 5HT6 using excitatory (VgluT1) and inhibitory (GAD67) neuronal markers in V1. Indeed, my results indicated the presence of 5HT1A and 5HT6 in excitatory neurons and that of 5HT4 in inhibitory neurons (Figure 4). I was unable to obtain signals for 5HT1E using either of the markers. In the frontal (areas 46 and 6) and
temporal (ITG and TE) association areas, 5HT1A and 5HT6 were expressed from layers II through V, but their mRNA expression levels in layer IV of ITG and TE were much lower. In contrast to the widespread expression in the association areas, in early sensory areas, such as S1, V1, and V2, their expression was mostly limited to layer II. The area difference was conspicuous for 5HT1A and 5HT6 but not for 5HT1E and 5HT4.

5HT2A mRNA was expressed at various levels from layers III to V throughout the neocortical areas. Its expression was more abundant in lower tiers of layer III and relatively sparse in layers IV and V. 5HT2C was expressed sparsely in layers II and V. Although 5HT2A and 5HT2C expressions overlapped in layer V, they generally exhibited opposite patterns of layer and area distributions: 5HT2A was highly expressed in V1 whereas 5HT2C showed a gradient in expression from being rostrally high to caudally low and was almost undetectable in V1 and V2. In the entorhinal cortex, both the genes were expressed complementarily; unlike in other areas, 5HT2A was present in layer II and lower layers V and VI (Figure 2, k5), whereas 5HT2C was expressed in layers I and III (Figure 2, k6) where 5HT2A was little expressed. I performed double hybridization of 5HT2A with GAD67 and VgluT1 neuronal markers in V1. Because the expression of 5HT2C was scant in V1, I performed its double hybridization in sections from the frontal cortex and observed layer V encompassing all areas of the frontal cortex covered in the section. 5HT2A was mainly expressed in VgluT1-positive excitatory neurons (Figure 5), and almost all the cells expressing 5HT2C were positive for GAD67 inhibitory neurons (Figure 5).
The expression levels of 5HT1B, 5HT1F, and 5HT7 mRNAs were low throughout the neocortical areas. However, 5HT1B mRNA was abundantly expressed in V1 (Figures 2, h7 and 3D) and significantly in V2 (Figure 2, i7); a higher intensity of 5HT1F mRNA signals was observed in layer VI of V1 (Figure 2, h9 and Figure 3E) and 5HT7 mRNA was expressed at a moderately high level in layer IV of area ITG (Figure 2, f8). Note that the increase in the expression level of 5HT7 overlapped with the enhanced serotonergic terminations at ITG (Figure 3G). 5HT1B was also sparsely expressed in layer V of M1 (Figure 1, c7) and CG (Figure 2, j7). In the entorhinal cortex, 5HT1B and 5HT7 showed similar expression patterns, that is, highly expressed in layer II and moderately expressed in lower layers.

2. Marmoset V1 is characterized by serotonergic projections and expression of a group of 5HTR subtypes

5HT1B and 5HT2A showed high expression levels selectively in V1 and 5HT1A and 5HT1F were specifically expressed in V1 (Figure 3). The high expression levels of 5HT1B and 5HT2A in V1 were previously reported in macaques (Watakabe et al., 2009), and marmosets (Takahata et al., 2012). In the present study, I found a relatively low level thin band like pattern of expression of 5HT1A in layer IV Cβ (Figure 3C), which differed from that of macaques and the expression level of 5HT1F was moderate to high in layer VI (Figure 3E), which was observed to be very low in macaques. When examined by double ISH with excitatory VgluT1 or inhibitory GAD67 neuronal marker probes, both 5HT1A and 5HT1F were found exclusively expressed in excitatory neurons (Figure 4B-C). I also observed that serotonergic projections were dense in layers IV and VI.
(Figures 3B and 7A), where these four subtypes were expressed. The expressions of 
5HT1A, 5HT1B, and 5HT2A overlapped with highly dense serotonergic terminations in 
layer IV and that of 5HT1F overlapped with moderately dense terminations in layer VI 
(Figure 3B). The expressions of the four genes and the serotonin terminations formed 
sharp boundaries between V1 and V2 (Figure 3A-F).

3. Serotonin receptor mRNA expressions in hippocampus

The hippocampal region consists of the dentate gyrus (DG), CA fields, and subiculum (S) 
(Figure 10). It was densely innervated by serotonergic terminals in the areas with no 
receptor expression and stratum lacunosum moleculare (Slm) (Figure 10K). Interestingly, 
the expressions of 5HTR mRNAs in the hippocampus were highly subregion-specific. 
5HT1A, 5HT6, 5HT1E, and 5HT4 mRNAs, which are expressed in the cortical upper 
layer, were all abundantly expressed in the DG and pyramidal cell layer from CA3 to 
CA1. Among them, 5HT1A mRNA showed particularly prominent expression 
throughout these structures, whereas the other 5HTR mRNAs exhibited relatively weak 
expression in CA3.

In contrast to this group of genes, 5HT2A and 5HT2C mRNAs as well as 5HT3A 
mRNA exhibited characteristically scattered expressions in the polymorph layer of DG 
(5HT2A) and CA fields (5HT2C and 5HT3A) (Figure 10E, F and J). Note that these three 
mRNAs showed very low expression levels in granule cells, no higher than the 
expression level of the sense probe, which showed nonspecific faint background staining 
in DG. Such scattered expression suggests that they are expressed in inhibitory neurons.
Indeed, by double ISH I confirmed that the $5HT2C$ and $5HT3A$ mRNAs in the hippocampus were expressed in a subset of $GAD67$-positive inhibitory neurons (data not shown). The observation that the expression distribution and density differed among $5HT2A$, $5HT2C$, and $5HT3A$ mRNAs (Figure 6) suggests that they are expressed in different types of cell.

Despite dense projection by serotonergic terminals, $5HT1F$ was the only subtype expressed in the presubiculum above a moderate level. Other receptor types were distributed sparsely and expressed only at low levels (Figure 6).

4. Serotonin receptor mRNA expression in thalamus, hypothalamus, and amygdala

Regarding subcortical regions, I examined the thalamus, hypothalamus, amygdala, caudate, septum, ventral striatum, and superior colliculus. Overall, the repertoires of $5HTR$ subtypes expressed were quite limited in the thalamus, and as in V1 of the cortex, many regions showed conspicuous overlap between mRNA expression and serotonergic termination as described below.

I examined the expression patterns in a few conspicuous nuclei (as described below) belonging to various groups of the thalamus. Overall, in terms of the number of receptor types expressed, the thalamus showed the least receptor diversity (see Table 4). I did not observe the expressions of $5HT1E$, $5HT1F$, $5HT3A$, and $5HT4$ in any subnuclei at levels above the background level. The serotonergic terminations into the thalamus were
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heterogeneous and showed laterally low and medially high gradations (see Figure 7B and E). Both the medial geniculate nucleus (MG) (Figure 8K) and the lateral geniculate nucleus (LG) (Figure 9K) had moderate and heterogeneous serotonergic terminations.

5HT1A showed a high level of mRNA expression in the central lateral nucleus (CL) (Figure 11A), which overlaps with the dense serotonergic termination in CL (Figure 11K, also see Figure 7B-C). In sharp contrast, 5HT1B showed little expression in CL but was expressed at high levels from nuclei lateral dorsal (LD), ventral lateral (VL), and mediodorsal (MD) cortices to CL, where the 5HT1A mRNA expression levels were very low to low. 5HT2A and 5HT2C were both sparsely expressed in CL and were little expressed from nuclei medial and lateral cortices to CL (Figure 11E-F). 5HT2C was also expressed near the midline thalamic nuclei where the serotonergic projections were dense (Figure 7D-E). 5HT6 and 5HT7 were expressed in CL, VL, LA, and MD from very low-to-low and from low to moderately high levels, respectively.

The overall expression patterns of all the 5HTR subtypes were similar in the posterior nuclei including the medial, lateral, and inferior pulviner (Figure 6), medial geniculate nucleus (Figures 6 and 8), and ventral posterior nuclei including the ventral posterior lateral (VPL), and ventral posterior medial (VPM) nuclei (Figures 6 and 9). In the lateral geniculate nucleus (LG), 5HT1A and 5HT6 were expressed at very low levels, 5HT7 at a low level (Figure 6), and 5HT1B at a high level (Figures 6 and 9). Finally, in the reticular nucleus (RT), 5HT1B, 5HT2A, and 5HT2C were expressed at moderately high levels and 5HT1A from very low-to-low levels (Figure 6).
Within the hypothalamic nuclei, the mammillary nucleus exhibited conspicuous heterogeneity of $5HTR$ mRNA expressions (Figure 12). Such heterogeneity corresponded to the density of serotonergic projections (Figure 12K). The medial part of the mammillary nucleus (MM) received denser serotonergic projections than the retro-hypothalamus (RH), lateral hypothalamus (LH) and lateral mammillary (LM) nucleus which lie dorsal, lateral and ventro lateral to MM, respectively (Figure 12 reference) The distribution of $5HTR$ mRNAs was specific in these regions, which conspicuously overlapped with the serotonergic projections: $5HT2A$ and $5HT7$ mRNAs were densely expressed in MM but were absent in RH, LH and LM (Figure 12E and J), and $5HT6$ mRNA was also more highly expressed in MM, although it was expressed in both RH and MM. In contrast, I observed a moderately high expression level of $5HT1A$ mRNA, very low to low expression levels of $5HT1B$ mRNA, and a high expression level of $5HT2C$ mRNA in RH, LH and LM but not in MM. $5HT1E$, $5HT3A$, and $5HT4$ mRNAs were expressed at insignificant levels.

There was some ambiguity in assigning the localization of $5HT1F$ mRNA expression, which was at a high level exclusively in the nucleus lateral to MM, which could be either LM or the ventral tuberomamillary nucleus (VTM) (Figure 12D). VTM, which is part of tuberomamillary nucleus (TM), shows the densest population of histaminergic neurons and can be identified using histidine $HDC$ as a marker (Ericson et al., 1987; Sakai et al., 2010). $5HT1F$ if present in histaminergic neurons can directly modulate the regulation of these neurons. To examine this possibility and locate $5HT1F$ expression, I performed ISH of $5HT1F$ and $HDC$ in adjacent sections (Figure 13). My
Result shows that 5HT1F and HDC were expressed in a complementary manner, suggesting that 5HT1F is expressed exclusively in LM.

The amygdala consists of several subnuclei connected with each other (Figure 14). 5HT1F and 5HT3A showed no detectable signals above the background in the amygdala. ISH signals of other 5HTR subtypes were generally observed in most parts of the amygdala, although signals were heterogeneous and not as pronounced as those in the mammillary nucleus. 5HT1A, 5HT4, 5HT6, and 5HT7 mRNA showed high expression levels in the cortical amygdaloid nucleus (Co), where there were dense serotonergic projections. 5HT1A mRNA was highly expressed in the basolateral (BLa), basomedial (BMa) and Co and not expressed in the La. 5HT2A mRNA was expressed only in La and not in Bla, BMa, or Co. 5HT2C was expressed densely in the medial amygdaloid nucleus (Me), and the expression became very sparse towards La. 5HT1B mRNA was faintly expressed and 5HT1E mRNA was homogeneously expressed at low to moderately high levels across all the nuclei. 5HT4 and 5HT7 mRNAs were generally expressed towards the medial part, mostly in Co. The 5HT6 mRNA expression levels were high in Co and low in other nuclei.

5. Serotonin receptor mRNA expressions in superior colliculus

The 5HTR subtypes expressed in the superior colliculus (SC) (Figure 15) were similar to those in MD, the adjacent substructure of the thalamus. In SC, I did not find any significant expression of 5HT1F, 5HT3A, or 5HT4. All the other 5HTR subtypes were sparsely expressed at various levels. The serotonergic projections in SC were moderately
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dense and appeared to overlap with 5HT6 expression in the zonal layer (Zo). 5HT1A was mostly expressed in superficial layers including the zonal layer, superficial gray (SuG) layer, and optical nerve layer (Op), and its expression levels ranged from moderately high to high depending on the cell type. 5HT2A and 5HT1B were expressed at very low and low levels, respectively, in Zo and SuG. 5HT1E was exclusively expressed in Zo at a low level. 5HT2C was expressed across the superior colliculus at a moderately high level; its expression was generally dense in Zo and SuG. 5HT6 was expressed at a moderately high level in two tiers, densely in Zo and SuG, and sparsely in the intermediate gray (InG) layer. Finally, 5HT7 was expressed at a low level in InG.

6. Serotonin receptor mRNA expressions in caudate and septum

Owing to the spatial proximity of caudate and septum (Figure 16), the description of 5HTRs mRNA patterns are clubbed together.

In the caudate, medial septum (MS), and lateral septum (LS) (from right to left in Figure 16), the serotonergic projections varied and showed no apparent overlap with 5HT expression. In the caudate, 5HT1F and 5HT3A were not expressed. 5HT1E and 5HT7 were faintly expressed. The mRNA expression levels were low for 5HT1A, moderately high for 5HT1B, and moderately high-to-high for 5HT6 and 5HT4. 5HT2C at moderately high mRNA expression levels was densely expressed towards the medial part (Figure 6) and more scattered towards the lateral part of the caudate (Figure 16F).

In the septum, 5HT1F and 5HT3A were not expressed. 5HT1A showed sparse but significant expression in both the medial septum (MS) and lateral septum (LS). 5HT1B
showed a moderately high mRNA expression level, $5HT1E$ and $5HT7$ were expressed at low levels, and $5HT6$ was faintly expressed in the lateral septum. $5HT4$ was generally expressed at moderately high-to-high levels in the medial septum. $5HT2A$ was exclusively expressed in the medial septum at a moderately high level, and complimentarily $5HT2C$ was expressed at a moderately high level in the lateral septum (indicated by arrow heads in Figure 16E-F)

7. **Serotonin receptor mRNA expressions in ventral striatum**

I examined the $5HTR$ expression patterns in the internal globus pallidus (iGP), and external globus pallidus (eGP), substantia nigra pars reticulate (SNr), and substantia nigra pars compacta (SNc), representing the ventral striatum. The serotonergic projections in these regions were again heterogeneous. In SNc, the projection density increased near the inferior regions where the expression was generally denser. In the globus pallidus (Figure 6), a small repository of $5HTR$ subtypes was expressed and I did not detect signals above the background level for $5HT1B$, $5HT1F$, $5HT4$, $5HT3A$, or $5HT7$ in both nuclei. All the $5HTR$ subtypes were sparsely expressed in these nuclei. The mRNA expression levels were very low for $5HT1A$ and low for $5HT1E$, $5HT2A$, and $5HT6$ in both the iGP and eGP. Interestingly, $5HT2C$ was expressed in the iGP and eGP at high and very low levels, respectively (Figure 6).

In the substantia nigra (Figures 17 and 6), $5HT1F$ and $5HT3A$ were not expressed. In SNc, $5HT2C$ and $5HT4$ mRNAs were expressed sparsely whereas mRNAs of other $5HTR$s were expressed densely. The levels of expression were very low for $5HT2A$, low
for 5HT1A and 5HT1B and moderately high for 5HT1E, 5HT2C, 5HT4, 5HT6, and 5HT7.

In SNr all the 5HTRs were expressed sparsely at very low levels except 5HT2C, which was expressed sparsely but at a high level (Figure 17).

8. Serotonin receptor mRNA expressions in mouse brain

To compare the expression pattern between mouse and marmoset I selected presubiculum, CA fields and dentate gyrus of the hippocampal formation and visual (VIS), somatosensory (SS) and somatomotor (MO) of the mouse cortex.

In the hippocampus except for 5HT2C and 5HT3A, the expression of all the 5HTRs was limited only to the pyramidal layer (Figures 10 and 18). The serotonergic projections were dense at S1m and as like in marmoset, the presubiculum was having enriched expression of 5HT1F overlapping with the dense serotonergic projections. Again as like in marmoset, 5HT2A showed specific expression in the polymorph layer of DG, and 5HT1A showed high overall expression all through out the hippocampal formation.

In cortex, besides the conspicuous differences in the overall mRNA expression levels of 5HTRs (Figure 20), which were low in mice, there are some notable differences between the mouse and marmoset expression profiles observed in the cortex. 5HT1E found in the marmosets (Figure 1) was not detected in the mice (ABA, 2009), and the enriched and specific expressions of 5HT1A, 5HT1B, 5HT1F and 5HT2A found in V1 of the marmosets (Figure 3) were also not observed in the mice (Figure 19). 5HT4 observed in inhibitory neurons of the marmosets was scarcely expressed in the mouse cortex (Figure 19). 5HT3A is expressed in cerebral cortex of macaques (Jakab and
Goldman-Rakic, 2000) but was not observed in my study of the marmosets. In mice it was expressed mainly in upper layers including layer I (Figure 19), where there was no expression of any 5HTRs in the marmoset. Among the other expression patterns that were exclusively observed in the mice are as follows: the expression of 5HT1D in layer 6b of SS (Figure 19, c2), the sparse expression of 5HT1B in layer 4 of SS (Figure 19, b2), abundant expression of 5HT1F in MO (Figure 19, d3).
Figure legends

Figures 1 and 2. ISH expression profiles of 5HTRs in cortex. area 46, area 6, primary motor cortex (M1), primary somatosensory cortex (S1), V5 (MT), inferotemporal gyrus (ITG), temporal cortex (TE), primary visual cortex (V1), secondary visual cortex (V2), cingulate cortex (CG), and entorhinal cortex (Er). Layers identified by Nissl staining (not shown) are indicated on the left. Note that all images of a given gene are grouped together and presented at the same contrast level. Scale bar, 100 μm.

Figure 3. Sections showing specific staining at V1 and V1-V2 border (A-F) and ITG (G-H). (A) Nissl staining and architecture of V1-V2. (B) Immunohistochemical staining with anti-SERT antibodies. Note that the projection density is particularly high in layers IV and VI. (C-F) Expression profiles of 5HT1B, 5HT2A, 5HT1A, and 5HT1F. The arrow heads indicate the border between V1 and V2. (G-H) show the overlap of increased expression of 5HT7 in ITG with serotonergic projections at layer IV. The precise layers of expression of the genes studied here can be seen in Figure 2. Each image has been adjusted at a contrast level that shows the clearest border. Scale bars for A to F, 200 μm and for G and H, 100 μm.

Figure 4. Double ISH of 5HTRs (red, DIG) with GAD67 and VgluT1 neuronal markers (green, FITC). 5HT1A, 5HT1F, 5HT4, and 5HT6 with GAD67 and VgluT1 neuronal markers in marmoset V1. 5HT1A in layers II (row A) and IVcβ (row B), 5HT6
in layer II (row E), and 5HT1F in layer VI (row C) were not expressed in GAD67-positive inhibitory cells but were expressed in VgluT1-positive excitatory cells. 5HT4 in layer II (row D) was expressed in GAD67-positive inhibitory cells but not in VgluT1-positive excitatory cells. The arrow heads indicate the positive signals and coexpressions. Scale bar, 50 μm.

**Figure 5.** Double ISH of 5HT2C and 5HT2A (red, DIG) with GAD67 and VgluT1 neuronal markers (green, FITC). 5HT2A with GAD67 in layer III of V1 (row A), 5HT2A with VgluT1 in layer III of V1 (row B), 5HT2C with GAD67 in layer V of frontal cortex (row C) and 5HT2C with VgluT1 in layer V of frontal cortex (row D). The arrows indicate the positive signals and coexpressions. Scale bar, 50 μm. Note that the density of VgluT1 positive excitatory neuron we observed in layer V is less than other layers (row D), which is consistent with the result shown in another report (Gittins and Harrison, 2004).

**Figure 6.** Higher-magnification images of different regions described in the text. The boxed images represent the positive signals with different levels of expression, as mentioned in Table 3. Note that all images of a given gene are grouped together and then adjusted to the same level of contrast. Scale bar, 50 μm.
Figure 7. (A) SERT immunohistochemistry of V1. Note the dense serotonergic projections in layer IV indicated by black arrowheads. (B) More anterior section of thalamus showing characteristic projections at CL and ventricles (black arrowhead). C, D, and E show that the expression and serotonergic projections overlap near the ventricle region (black arrow head). Abbreviations are the same as those in Figure 11 and the main text. Scale bars: (A), 100 µm (B), 200 µm; (C, D, and E), 200 µm.

Figure 8. ISH expression profiles of 5HTRs in medial geniculate nucleus (MG). 5HTR mRNA expressions (A-J) and immunohistochemical staining with anti-SERT antibody (K) in MG of thalamic area. Images are adjusted at contrasts that show the clearest image for each 5HTRs. Scale bar, 100 µm.

Figure 9. ISH expression profiles of 5HTRs in thalamic areas. 5HTR mRNA expressions (A-J) and immunohistochemical staining with anti-SERT antibody (K) in lateral geniculate nucleus (LG), ventral posteromedial nucleus (VPM), and ventral posterolateral (VPL) nucleus of thalamus. Note that only 5HT1B (B) shows a conspicuous expression at moderately high levels in LG. Images are adjusted at contrasts that show the clearest image for each 5HTR. Scale bar, 200 µm.

Figure 10. ISH expression profiles of 5HTRs in hippocampus. 5HTR mRNA expressions (A-J) and immunohistochemical staining with anti-SERT antibody (K) in CA1 and CA3 fields, dentate gyrus (DG), presubiculum (PS), subiculum (S) and stratum
lacunosum moleculare (Slm) of hippocampal formation. Arrows for 5HT1F (I), 5HT2A (E), and SERT (K), show the corresponding similarity of expressions and innervations in the mouse (see Figure 8, C,D,F). Images are adjusted at contrasts that show the clearest image for each 5HTR. Scale bar, 200 µm.

**Figure 11. ISH expression profiles of 5HTRs in thalamus.** 5HTR mRNA expressions (A-J) and immunohistochemical staining with anti-SERT antibody (K) in central lateral (CL), mediodorsal (MD), lateral dorsal (LD), and ventral lateral (VL) thalamic nuclei. The black arrowheads in (A), (E), and (F) show the overlap of 5HT1A, 5HT2A, and 5HT2C expressions with corresponding dense serotonergic projections at CL (also see Figure 7), whereas the white arrowheads in (B) show the corresponding mismatch between 5HT1B expression and projections at CL. Images are adjusted at contrasts that show the clearest image for each 5HTR. Scale bar, 200 µm.

**Figure 12. ISH expression profiles of 5HTRs in hypothalamus.** 5HTR mRNA expressions (A-J) and immunohistochemical staining with anti-SERT antibody (K) in lateral (ML), medial (MM), and ventral tuberomammillary (VTM) nuclei of hypothalamus. We observed the striking complementary relationship between the 5HT2A (E) and 5HT2C (F) expressions and overlap of 5HT2A and 5HT7 expressions with projections at MM. Note that the 5HT1A (A) expression that overlapped with serotonergic innervations in CL (Figure 11A) did not match with the projections at MM.
Images are adjusted at contrasts that show the best image for each 5HTR. Scale bar, 100 µm.

**Figure 13. ISH expression profiles of 5HT1F and HDC in hypothalamus.** 5HT1F mRNA expression in ML(A), HDC mRNA expression in VTM (B), and overlay image of 5HT1F and HDC mRNA expressions (C). Scale bar, 100 µm.

**Figure 14. ISH expression profiles of 5HTRs in amygdala.** 5HTR mRNA expressions (A-J) and immunohistochemical staining with anti-SERT antibody (K) in basomedial (BMa), basolateral (BLa), cortical (Co), lateral (La), and medial (Me) amygdaloid nuclei of amygdala. Note that the arrowheads for 5HT1A (A), 5HT4 (H), 5HT6 (I), and 5HT7 (J) show the overlap of the expressions with serotonergic projections (K) near Co. Images are adjusted at contrasts that show the clearest image for each 5HTR. Scale bar, 200 µm.

**Figure 15. ISH expression profiles of 5HTRs in superior colliculus.** 5HTR mRNA expressions (A-J) and immunohistochemical staining with anti-SERT antibody (K) in zonal layer (Zo), superficial gray (SuG), optic nerve layer (Op), and intermediate gray (InG) of superior colliculus (SC). Images are adjusted at contrasts that show the clearest image for each 5HTR. Scale bar, 100 µm.
**Figure 16.** ISH expression profiles of 5HTRs in caudate and septum. 5HTR mRNA expressions (A-J) and immunohistochemical staining with anti-SERT antibody (K) in the caudate (Cd) nucleus, and medial septum (MS), and lateral septum (LS). Note that the arrowheads for (E) and (F) show the presence and absence of 5HT2A and 5HT2C expression, respectively, in the medial septum. Images are adjusted at contrasts that show the clearest image for each 5HTR. Scale bar, 200 µm.

**Figure 17.** ISH expression profiles of 5HTRs in substantia nigra. 5HTR mRNA expressions (A-J) and immunohistochemical staining with anti-SERT antibody (K) in pars compact (SNc) and pars reticular (SNr) of substantia nigra. Images are adjusted at contrasts that show the clearest image for each 5HTR. Scale bar, 100 µm.

**Figure 18.** ISH expression profiles of 5HTRs in mouse hippocampus. 5HTR mRNA expressions (A-J) and immunohistochemical staining with anti-SERT antibody (K) in CA1 and CA3 fields, dentate gyrus (DG), presubiculum (PS), subiculum (S) and stratum lacunsum moleculare (Slm) of hippocampal formation. Arrows for 5HT1F (I), 5HT2A (E), and SERT (K) show the corresponding similarities for expression and innervations in the marmoset (see Figure 6). Images are adjusted at contrasts that show the clearest image for each 5HTR. Scale bar, 200 µm.

**Figure 19.** ISH expression profiles of 5HTRs in mouse cortex. ISH expression profiles of 5HTRs in as visual (VIS), somatosensory (SS) and somatomotor (MO). Layers
identified by Nissl staining (not shown) are indicated on the left. Arrows for 5HT1D highlights the expression in layer 6b of SS. Note that all images of a given gene are grouped together and presented at the same contrast level. Scale bar, 100 μm.

**Figure 20a, b and c. Laminar profiles of ISH signals quantified by measuring the optical density.** (a) and (b), Optical density of expression in marmoset cortex corresponding to Figure 1 and Figure 2, respectively, of main text. (c), Optical density of expression in mouse cortex corresponding to Figure 19. The numeric figure 0, 100 and 200 correspond to pixel values. As all images of a given gene are grouped together and presented at the same contrast level, the comparison is best for a given gene in different areas.
Figure 1
Figure 5
Figure 6

| MD    | SHT1A | SHT1B | SHT1E | SHT1F | SHT2A | SHT2C | SHT3A | SHT4 | SHT6 | SHT7 |
|-------|-------|-------|-------|-------|-------|-------|-------|------|------|------|
| CL    |       |       |       |       |       |       |       |      |      |      |
| LD    |       |       |       |       |       |       |       |      |      |      |
| Vpul  |       |       |       |       |       |       |       |      |      |      |
| Lpul  |       |       |       |       |       |       |       |      |      |      |
| Ipul  |       |       |       |       |       |       |       |      |      |      |
| VL    |       |       |       |       |       |       |       |      |      |      |
| VPM   |       |       |       |       |       |       |       |      |      |      |
| MG    |       |       |       |       |       |       |       |      |      |      |
| LGN   |       |       |       |       |       |       |       |      |      |      |
| RT    |       |       |       |       |       |       |       |      |      |      |
| CA1   |       |       |       |       |       |       |       |      |      |      |
| CA3   |       |       |       |       |       |       |       |      |      |      |
| S     |       |       |       |       |       |       |       |      |      |      |
Figure 6 (Cont.)

[Image of a grid of micrographs labeled with various regions and 5HT subtypes]
Figure 10
Figure 11
Figure 13
Figure 14
Figure 16
Figure 17
Figure 18
Figure 19
Figure 20a
Figure 20c
Discussion

I report the mRNA localization of all the 10 5HTRs that are expressed, as well as the distribution of serotonin terminations in the marmoset brain. Besides confirming the published results of numerous previous studies, the present study notably demonstrates several new findings about the organization of serotonergic systems. On the basis of my findings I discuss the possible roles of 5HTRs in the marmoset brain, as revealed by my analysis of overall expression patterns.

1 Technical consideration

In my present study I was unable to obtain the results for 5HT1D, 5HT3B and 5HT5A. When checked for their expression patterns in the human data set (ABA, 2012), I was unable to find the expression of 5HT1D and 5HT3B, suggesting that the absence of expression found in my study is not due to artifact. 5HT5A is found in the frontal cortex at low levels in both humans (ABA, 2012) and mice (Goodfellow et al., 2012, Figure S6). On the basis of this finding, I could not exclude the possibility that ISH using my 5HT5A probes might have failed to detect low signals. I also encountered some constant background signals associated with the expression of 5HT1F and 5HT1E, and I was unable to detect signals for 5HT1E when testing for its presence using excitatory or inhibitory, neuronal markers for double hybridization. On the basis of my previous study (Watakabe et al., 2007) I consider that low mRNA expression levels of 5HT1F and 5HT1E might be the reason for the granular background and also both the lower mRNA
expression level and high GC content of 5HT5A (63.41%) might be the reason for the failure to detect ISH signals.

2 Overlap of serotonin receptor mRNA distribution and serotonergic terminations

Serotonergic projections in the marmoset brain were generally associated with serotonin receptor expressions. My data show a marked overlap of the mRNA expressions of most 5HTRs with serotonergic terminations in the visual cortex (Figure 3), the subiculum (Figure 10I), the central lateral nucleus of the thalamus (Figure 11A, E, F, also see Figure 12, B-E), the medial mammillary nucleus (Figure 12E, J), the cortico amygdaloid nucleus of the amygdala (Figure 14), and the midline thalamic nuclei (Figure 7). All the subtypes, except 5HT1B, that showed overlaps have somatodendritic localization of their receptor proteins (Table 5), suggesting a strong correlation between serotonin availability and receptor expression.

Interestingly, none of the 5HTRs were expressed in layer I where corresponding serotonergic termination were present and were relatively high in density at certain areas (Figure 3). Likewise, both in the mouse and marmoset no serotonergic terminations were found in the pyramidal layer of the hippocampus, where all the 5HTRs are expressed; instead they were more prominent in S1m (Figures 10 and 18). Both layer I of cortex (Shipp, 2007) and S1m (Maccaferri, 2011) of the hippocampus receive the apical tuft of pyramidal cell dendrites. This mismatch suggests that the major target of serotonergic terminations in the supragranular layer of the cortex and hippocampus is the apical
dendritic tuft of neurons, which is known to increase the gain of pyramidal neurons (Larkum et al., 2004).

3 Cortical expressions of 5HTRs and Circuitry implications

In summary, the upper (supragranular), middle, and lower (infragranular) layers showed quite different patterns of 5HTR expressions. This feature of 5HTRs having different mRNA expression patterns in different layers suggests distinct roles of 5HTRs in the primate cortex that presumably affect the function of each layer.

Large varicose serotonergic fibers originating from the median raphe nucleus (MRN) have been reported to project at the supragranular layers in the marmoset (Hornung et al., 1990) and macaque (Wilson and Molliver, 1991). These innervations form synapses with supragranular inhibitory neurons in a basket like pattern in macaques and chimpanzees but not in humans (Raghanti et al., 2008), and in both cats and marmosets such a basket like pattern is observed in calbindin-positive (CB+) interneurons (Hornung and Celio, 1992). In the rat hippocampus also innervation to CB+ inhibitory neurons has been reported (Freund et al., 1990). The interneurons are likely to inhibit the nearby pyramidal cells; as has been demonstrated in many locations of the cortex (Sheldon and Aghajanian, 1990; Ropert and Guy, 1991; Foehring et al., 2002).

I report expression of 5HT4 mRNA in GAD67-positive inhibitory neurons and the expressions of 5HT1A and 5HT6 mainly in VgluT1-positive excitatory neurons in the upper layers of V1 (Figure 4). Thus, 5HT4, which has excitatory cellular effects (Table 5),
might indirectly inhibit neighboring pyramidal neurons and 5HT1A, which has an inhibitory cellular effect, might be recruited to directly inhibit pyramidal neurons. 5HT6, which has an excitatory cellular effect, similarly can be supposed to excite pyramidal neurons.

Direct and indirect inhibition might be recruited separately, depending on the two different populations of terminal axons originating from different raphe nuclei with their unique behavioral consequences. MRN forms a direct synaptic contact with neuronal somata, whereas DRN has a widespread effect through volume or extrasynaptic transmission (Michelsen et al., 2007; Törk, 1990). The MRN innervation forms synaptic contact with CB+ interneurons (as mentioned above), which on the basis of my findings seem to express 5HT4. Interestingly, 5HT4 has also been detected in certain CB+ enteric neurons of rodents (Poole et al., 2006). My observation of 5HT1A expression mainly in excitatory neurons is based on visual inspection in V1, but previous reports have shown that in Layer II of the monkey prefrontal cortex (PFC) 83% of 5HT1A is expressed in VgluT1 positive excitatory neurons and 43% of the remaining inhibitory neurons are found in CB+ interneurons. This suggests that 5HT1A may be recruited by both MRN and DRN in PFC.

The extrasynaptic localization of 5HT1A receptors (Riad et al., 2000) supports the idea of direct inhibition of pyramidal neurons expressing 5HT1A (Figure 4) by volume transmission triggered by DRN. In summary, 5HT4 might be recruited in synaptic-indirect inhibition of pyramidal neurons by the stimuli originating from MRN.
whereas $5HT1A$ might be recruited in extrasynaptic-direct inhibition of pyramidal neurons by the stimuli originating from DRN.

4 Thalamic nuclei projecting to the cortex show less receptor diversity

In thalamic nuclei projecting to cortex, only $5HT1A$, $5HT1B$, $5HT6$, and $5HT7$ were prominently expressed. $5HT1A$ and $5HT1B$ have inhibitory cellular effects (Table 5) whereas $5HT6$ and $5HT7$ have excitatory cellular effects (Table 5). This suggests that the cortically projecting thalamic nuclei, maintain a balance between excitatory and inhibitory effects on inputs and outputs only by recruiting a limited subgroup of $5HTR$s. $5HT2C$ and $5HT2A$ were expressed in addition to these four $5HTR$ subtypes in the CL, which projects to the striatum (Van der Werf et al., 2002), and in the RT, which receives inputs from the cortex (Smith, 2008). Taken together, my data suggest that those regions of the thalamus, which gates afferent information to the cortex, have fewer $5HTR$ subtypes (see Table 4 and Figure 6) and in contrast, the cortex, which integrates sensory information, has more $5HTR$ subtypes. Aligning to my findings, physiological data collected from the ferret thalamus (Monckton and McCormick, 2002) also suggest that serotonin has lesser influence (direct postsynaptic inhibitory) on the primary sensory nuclei than on the intralaminar nuclei.

5 Complementary expression of $5HT2A$ and $5HT2C$

Many studies have suggested independent, reciprocal, opposing and balancing functional features associated with $5HT2A$ and $5HT2C$ receptors (Halberstadt et al., 2009;
Discussion

Winstanley et al., 2004; Popova and Amstislavskaya, 2002; Nonogaki et al., 2006; Aloyo et al., 2009). In the hypothalamo-pituitary-testicular-based system, the neural control of male sexual motivation and arousal involves the facilitative action of 5HT2A and suppressive action of 5HT2C in a reciprocal manner (Popova and Amstislavskaya, 2002).

In the hypothalamus of obese A\textsuperscript{y} mice, 5HT2A and 5HT2C receptors are suggested to have reciprocal roles in the regulation of feeding and energy homeostasis (Nonogaki et al. 2006). The complementary expression of 5HT2A and 5HT2C observed in the hypothalamus in my study (Figure 12E-F) is consistent with the finding of Papova et al., 2002 and Nonogaki et al., 2006 in nonprimates. Besides the hypothalamus, the septum (Figure 16E-F) and entorhinal cortex (Figure 2, k5-k6) also showed complementarity. In V1, there was an enriched expression of 5HT2A in contrast to the scant expression of 5HT2C (Figure 2).

5HT2A is expressed in 86 to 100% of upper layer glutamatergic cells and in 13 to 31% of inhibitory cells in the monkey and human PFC (de Almeida and Mengod, 2007). Similarly, in the marmoset and macaque V1, it is also mostly expressed in the excitatory neurons (Watakabe et al., 2009; Nakagami et al., 2013, Figure 5). In contrast, the expression of 5HT2C was scant and was mostly detected in the inhibitory neurons (Figure 5) of layer V. In rats, 5HT2C is primarily expressed in excitatory neurons in the PFC (Puig et al., 2010). This difference may be species-specific between the marmoset and rat or due to the difference in the equivalent ages of the two animal species used. In rats there is high expression of 5HT2C in layers IV and V until P14, and after P56, the expression level becomes low and is limited to layer V (Li et al., 2004; Jang et al., 2012). Overall, my data
supports the functional complementarity between 5HT2A and 5HT2C suggested in previous pharmacological studies.

6 Sporadic and highly localized expressions of 5HT1F and 5HT3A

5HT1F is only expressed in layer VI of V1 (Figure 3), the presubiculum (Figure 10), and LM of the hypothalamus (Figure 13). In V1 and the presubiculum, its expression overlapped with dense serotonergic terminations, again suggesting a high turnover rate of serotonin at these sites. In mouse V1, a recent study has shown that layer VI works as a major mediator of cortical gain modulation (Olsen et al., 2012). My previous work shows the role of 5HT1B in increasing the signal-to-noise ratio and 5HT2A in gain control in V1 (Watakabe et al., 2009). In this report, I have shown the expression of 5HT1F in excitatory neurons of layer VI. Together, these findings suggest for possible recruitment of the 5HT1F receptor present in layer VI for supporting the visual gain function in marmoset.

The mammillary body, which includes MM and LM (Vann, 2010) (Figure 12), appears to lack interneurons in primates (Veazey et al., 1982), whereas the TM, which surrounds the mammillary body, is composed of inhibitory neurons only. Surprisingly, the members of the 5HT1 family, which have inhibitory cellular effects (Table 5), are not expressed in the mammillary body, except 5HT1F. This suggests that serotonin primarily functions to facilitate the excitation of the mammillary body in MM, as revealed by the dense serotonergic innervations and expression of 5HT2A, 5HT6 and 5HT7 receptors with excitatory cellular effects (Table 5) but hyperpolarizes the ML by recruiting 5HT1F,
thus balancing the overall excitation of the mammillary body. Overall, the sporadic regional localization of 5HT1F receptors in the marmoset brain may be related to the mediation of the gain modulation or balancing functions.

The expression profile of 5HT3A I obtained in the cortex was different from that observed in mice, where it was associated with cortical interneurons. 5HT3A accounts for nearly 30% of all interneurons and is suggested to be involved in shaping the cortical circuit in rodents (Rudy et al. 2011). In addition, Jakab and Goldman-Rakic (Jakab and Goldman-Rakic, 2000) showed the 5HT3A receptor at the cell body of cortical neurons in macaques. There may be species differences in the expression pattern of 5HT3A in the cortex between marmosets and other species. In my present study, I examined 5HT3A expression using several probes of 5HT3A, but except for the probes mentioned in the results (shown in Table 1) I observed high background signal intensities for all probes. The working probe was found expressed only in GABAergic interneurons in the CA fields of the hippocampus (Figure 10J). Therefore, I cannot exclude the possibility that the differences observed in my marmoset study are due to the different isoforms generated by alternate splicing, because two splice variants of 5HT3A are found in humans, which exhibit similar pharmacological and electrophysiological profiles when expressed as homomers (Hannon and Hoyer, 2008)
7 Comparison of 5HTR mRNA expression between different species

5HT1A was expressed in the marmoset, but not in the macaque, in layer IV of V1. The expression is also lacking in human V1 (ABA, 2012). It is tempting to correlate this difference with species-specific physiological differences, such as dichromatic vision, observed in some marmosets (Solomon, 2002; Surridge et al., 2003), compared with the trichromatic vision in humans and macaques (Surridge et al., 2003). Besides this difference, features such as the expression of 5HT1A and 5HT6 in the upper layer, the V1-specific expression of 5HT1B, the enriched expression of 5HT2A in V1, the rostral decrease in the expression of 5HT2C, the low expression level of 5HT7 and the absence of expression of 5HT3A (as discussed above) in the cortex were very much similar to those in humans (ABA, 2012). Besides these similarities, the upper layer expression of 5HT1A, which has been observed in the marmoset (in the present study), macaque and human (de Almeida and Mengod, 2008) is also observed in the rat PFC (Goodfellow et al., 2009), and the expression of 5HT7 mRNA, which is observed prominently in the thalamus and at low levels in the cortex, is also similarly observed in rodents (Gustafson et al., 1996). Together, the expressions of 5HT1A and 5HT7 receptor subtypes in the cortex seem to be conserved between rodents and primates.

In the hippocampus there was a surprising similarity in the expression patterns observed between marmosets and mice. In both species, except for 5HT2C and 5HT3A, the expression of all the 5HTRs was limited only to the pyramidal layer (Figures 10 and 18), suggesting that majority of serotonin receptors are recruited for the modulation of glutamatergic transmission in the hippocampus. The serotonergic projections, in both the
species (as discussed above) were dense at Slm (Figures 10 and 18K). The overlap between serotonergic terminations and 5HT1F observed in the presubiculum, the specific expression of 5HT2A in the polymorph layer of DG, and high overall expression level of 5HT1A observed in the marmoset study was very similar to that in mice (Figure 10 and 18). In the thalamus, again the number of receptor subtypes expressed was smaller than that in the cortex (ABA, 2009).

Besides the conspicuous differences in the overall mRNA expression levels of 5HTRs (Figures 20), which were low in mice, there are some notable differences between the mouse and marmoset expression profiles observed in the cortex. 5HT1E found in the marmosets (Figure 1) was not detected in the mice (ABA, 2009), and the enriched and specific expressions of 5HT1A, 5HT1B, 5HT1F and 5HT2A found in V1 of the marmosets (Figure 3) were also not observed in the mice (Figure 19). 5HT4 observed in inhibitory neurons of the marmosets was scarcely expressed in the mouse cortex (Figure 19). 5HT3A is expressed in cerebral cortex of macaques (Jakab and Goldman-Rakic, 2000) but was not observed in my study of the marmosets. In mice it was expressed mainly in upper layers including layer I (Figure 19), where there was no expression of any 5HTRs in the marmoset. Among the other expression patterns that were exclusively observed in the mice are as follows: the expression of 5HT1D in layer 6b of SS (Figure 19, c2), the sparse expression of 5HT1B in layer 4 of SS (Figure 19, b2), abundant expression of 5HT1F in MO (Figure 19, d3).

Taken together, the mRNA expression pattern of 5HTRs in the marmoset as compared with those in the mouse shows some significant differences in the cortex,
which suggests certain primate specific roles of 5HTRs and the usefulness of the marmoset as a primate model in further studies of serotonergic modulations in higher brain functions that are specific to primates.
Materials and Methods

1 Ethics statement

All the experiments were conducted in accordance with the guidelines of the National Institutes of Health, and the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, and were approved by the Animal Care and Use Committee in the National Institutes of Natural Sciences. I made all efforts to minimize the number of animals used and their suffering.

2 Experimental animals, tissue preparation, and sectioning

Five brains of the adult common marmoset (*Callithrix jucchus*) (Two male: 2 years 6 months, and 3 years 5 months; Three female: ages-1 year 9 month, 2 years, and, 2 years 1 month) were used for confirmation of the mRNA expression patterns and their reproducibility. To avoid any chance of ambiguity owing to technical issues, the data presented in this work are collected from the 6 years 2 months old, female marmoset monkey. I observed no individual difference in mRNA expression patterns. For tissue fixation, the animal was deeply anesthetised with Nembutal (100 mg/kg body weight, intraperitoneally) and perfused intracardially with saline (0.9% NaCl) and then with 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were postfixed for 5 hours at room temperature and then cryoprotected with 30% sucrose in 0.1 M phosphate buffer at 4°C. The two hemispheres were sectioned separately, and approximately 600 coronal sections of 40 µm thickness encompassing the regions from the frontal cortex to the tectum were prepared from each hemisphere. All thirteen serotonin receptor genes (Table
1) were examined for their expression patterns using an ISH technique. Two sets of tissue sections were immunohistochemically stained for SERT and nissl stained for laminar identification. For mice, data was collected from 3 male (46 weeks) and 2 female (42 and 35 weeks) B6 mice. The presubiculum, which showed expression of 5HT1F (see results), could be best visualized by the sagittal sections of the mice brain, therefore I prepared sagittal sections of the mice brain. Because the visual (VIS), somatosensory (SS) and somatomotor (MO) areas cover the major part of the mouse brain and have analogous areas in the marmoset brain, these areas were selected for comparison between the mouse and marmoset brains.

3 ISH

Both the sense and antisense digoxigenin (DIG)-labeled riboprobes used in this study were prepared from plasmids containing PCR-amplified fragments of marmoset 5HTRs, histidine decarboxylase (HDC) and GAD67 genes. For VgluT1, riboprobes previously used for monkey ISH were used (Komatsu et al., 2005). To confirm the specificity of the antisense probes, the sense probes were used as the control in all the experiments. Details of the probes designed for the marmoset are shown in Table 1 and those for the mouse are shown in Table 2. Single and double-colored ISH were performed using the methods described in previous papers of (Watakabe et al., 2007, 2009; Takaji et al., 2009). Briefly, free-floating sections were treated with proteinase K (5 µg/mL) for 30 min at 37°C, acetylated, then incubated in a hybridization buffer (5X SSC, 2% blocking regent [Roche Diagnostics, Basel, Switzerland], 50% formamide, 0.1% N-lauroylsarcosine, 0.1% SDS) containing 0.5 µg/mL DIG-labeled riboprobes at 65°C.
Materials and Methods

for 5HT3A receptor gene and 60°C for the others. The sections were sequentially treated in 2XSSC/50% formamide/0.1% N-lauroylsarcosine for 15 min at 60°C twice, 30 min at 37°C in RNase buffer (10 mM Tris-HCl [pH 8.0], 1 mM ethylenediaminetetraacetic acid [EDTA], 500 mM NaCl) containing 20 µg/mL RNase A (Sigma Aldrich, Saint Louis, MI), 15 min at 37°C in 2XSSC/0.1% N-lauroylsarcosine twice, and 15 min at 37°C in 20X SSC/0.1% N-lauroylsarcosine twice. The hybridization probe was detected with an alkaline-phosphatase conjugated anti-DIG antibody using DIG nucleic acid detection kit (Roche Diagnostics).

For double-colored ISH, the sections were cut to 15 or 20 µm thickness. The hybridization and washing were carried out as described above, except that both DIG- and fluorescein-labeled probes were used for the hybridization. After blocking in 1% blocking buffer (Roche Diagnostics) for 1 hour, the probes were detected in two different ways. For the detection of fluorescein probes, the sections were incubated with an anti-fluorescein antibody conjugated with horseradish peroxidase (Jackson ImmunoResearch Laboratories, West Grove, PA:#200-032-037, 1:4000 in the blocking buffer) for 3 hours at room temperature. After washing in TNT buffer (0.1 M Tris-HCl [pH 7.5], 0.15 M NaCl, 0.1% Tween20) 3 times for 15 min, the sections were treated with 1:100 diluted TSA-Plus reagents (Perkin Elmer, Boston, MA) for 30 min following the manufacturer’s instruction, and the fluorescein signals were converted to dinitrophenol (DNP) signals. After washing with TNT buffer 3 times for 10 min, the sections were incubated overnight at 4°C with an anti-DNP antibody conjugated with Alexa 488 (1:500, Molecular Probes, Life Technologies Corporation, Carlsbad, CA) in 1% blocking buffer for the fluorescence detection of the DNP signals. At this point, an
anti-DIG antibody conjugated with alkaline phosphatase (1:1000, Roche Diagnostics) was also incubated for the detection of the DIG probes. The sections were washed 3 times in TNT buffer, once in TS 8.0 (0.1 M Tris-HCl [pH 8.0], 0.1 M NaCl, 50 mM MgCl₂), and the alkaline phosphatase activity was detected using HNPP fluorescence detection kit (Roche Diagnostics) following the manufacturer’s instruction. This substrate was incubated for 30 min and the incubation was stopped in PBS containing 10 mM EDTA.

4 SERT immunohistochemistry

Immunohistochemical analysis was conducted essentially in accordance with the protocol previously reported (Sakata et al., 2002). Briefly, I used antisera raised against SERT (1:12000) as primary antibodies and biotinylated goat anti-rabbit IgG (1:1000) as secondary antibodies (all supplied by Immunostar, Inc., USA). The free-floating sections were incubated consecutively in PBS containing 1% H₂O₂ for 10 min at room temperature, and then in PBS with 0.2% Triton X-100 (PBST) and 5% normal goat serum (serum of the species of the secondary antibody) for 60 min at room temperature. This was followed by overnight incubation in a buffer containing 1% normal goat serum and the primary antibody at 4 °C. After incubation with the biotinylated secondary antiserum for 2 hours at room temperature, the sections were processed with an avidin-biotinylated horseradish peroxidase complex (1: 200; Vectastain ABC Elite kit, Vector Laboratories, Burlingame, CA, USA) in PBST at room temperature for 1 hour and the immunoreaction was visualized by staining with nickel-enhanced colouring solution (0.2 mg/mL diaminobenzidine: DAB, 0.03% H₂O₂, 0.03% nickel chloride in
TBS).

5 Data quantification

Representative areas and regions were identified by referring to the stereotaxic atlas of the marmoset brain (Palazzi and Bordier, 2008; Yuasa et al., 2010; Paxinos et al., 2011) and Nissl staining. The intensity of hybridization signals of different genes varied across different areas of the brain. I present the intensity of the signals as mRNA expression level rated as very low (+), low (++), moderately high (+++), or high (++++) by visual inspection (Tables 2, 3). To show the weak signals, the images were adjusted to different contrast levels. In some instances, this enhanced the noise from the adjacent white matter. The true signals based on size and color can be clearly differentiated from the noise. Because DIG based ISH provides cellular resolution, I also distinguished dense and disperse expression profiles for relevant regions. To provide a more objective comparison of the laminar distribution of expression between the mouse and marmoset cortices, I analyzed the optical densities of ISH signals using imageJ image analysis software (Abramoff et al., 2004) (Figures 20 a, b, c). After making the contrast level the same for all images of the same gene, individual images were inverted and optical density was measured using the straight-line tool that sampled all layers of the cortex. To subtract the background noise, the optical density of either layer I or white matter (the region where there was no expression above background level) was taken as the control.
Table Legends

Table 1. Summary of ISH probes for 13 serotonin receptor genes, HDC and GAD67 in the marmoset. Note that owing to unavailability of the marmoset-specific 5HT1E sequence in the public database, the 5HT1E primers were designed using the macaque 5HT1E sequence. The hybridization temperature for 5HT3A was 65°C and that for others was 60°C. The amplicon includes the primer sequence. F indicates forward and R indicates reverse.

Table 2. Summary of ISH probes for 11 serotonin receptors in mice. The hybridization temperature for all the 5HTRs is 60°C. The amplicon includes the primer sequence. F indicates forward and R indicates reverse.

Tables 3 and 4. Arbitrary values assigned for different levels of expression in cortical and subcortical brain areas: ++++, high; +++, moderately high; ++, low; and +, very low levels of expression. +/- was assigned to areas of uncertain level of expression. The superscripts ‘S’ and ‘VS’ denote sparse and very sparse expressions, respectively. The numbers from 1 to 6 denote the layers of the cortex. The abbreviations of the cortical areas are the same as those mentioned in the main text.

Table 5. G-protein involved, signaling pathways, postsynaptic potential, and species-specific cellular and regional localization for each 5HTR. ↓ and ↑ represent decrease and increase, respectively. NA: Not available.
Table 1. Summary of ISH probes (marmoset)

| Gene   | Primer F            | Primer R            | Amplicon Size | GC%  | NCBI Accession |
|--------|---------------------|---------------------|---------------|------|----------------|
| 5HT1A  | F: TCCGACGTGACCTCGGTGCCCTAC  
        | R: AGTTCTCTGTCCTCCCCGATTCTCC | 703bp | 61.02 | XM_002744919 |
| 5HT1B  | F: TATTGGCGCTCATACCTTG  
        | R: TAGCCTGACCGCCAGGAAGAG      | 408bp | 60.54 | XM_002746745 |
| 5HT1D  | F: ATCCCTGAAAGCCACGAAAC  
        | R: GACCAAAAGACACCACGAAAGAA   | 917bp | 56.92 | XM_002750410 |
| 5HT1E  | F: TCACTCAGGAGAACAGTGTGGCCGTCC  
        | R: TMGATACCAACAGCAAAGTGTGTCCA     | 636bp | 51.10 | XM_001090686 |
| 5HT1F  | F: CTGGACCGCTACGGCTCCGATCC  
        | R: CGATAGGTCTTGAGTACCCAGAC      | 987bp | 42.93 | XM_002761291 |
| 5HT2A  | F: CTGGACCAGCTACTGCTGCTGGCCATCC  
        | R: TGTACACCAGAGGATTGAGTGAATCCC    | 653bp | 48.55 | XM_002742676 |
| 5HT2C  | F: CCACACTTAGATATTGTGGCCATCC  
        | R: TGTACACCAGAGGATTGAGTGAATCCC    | 754bp | 44.97 | XM_002763170 |
| 5HT3A  | F: AGTACTGGACTGATGAGTTTC  
        | R: CAGAGCCATGCACACCACAAAAA      | 683bp | 51.83 | XM_002754423 |
| 5HT3B  | F: GGGAAATTCTAGCCACAGATACG  
        | R: CCGACACTGGTGCTGCACACCAC      | 785bp | 47.13 | XM_002754430 |
| 5HT4   | F: AGAACGTGATGCTGCTGCTGCTCAGGTTA  
        | R: GGACAGTGTGATGCTGCACCCACAAG   | 816bp | 49.26 | XM_002744348 |
| 5HT5A  | F: TGCTGGATGCTGCTGCTGCTGCTGCTCAGGTTA  
        | R: ATGAGGATGCCCACCATGACGAGG   | 604bp | 63.41 | XM_002751806 |
| 5HT6   | F: CAACTTCTTTCCTGTCGCTGCTC  
        | R: GCTTGAAAGTCCCGCATGAAAAGG   | 803bp | 65.88 | XM_002750377 |
| 5HT7   | F: GGCAGAATGGAATGGAGTATGACG  
        | R: AGAGCCTCCTGCTGCTGCTGCTGCTCAGGTTA   | 655bp | 50.84 | XM_002756389 |
| HDC    | F: TGATGGAGCTCGAGGTGCTGAGTCAG  
        | R: TGGTCCCATTAGTTGTCACAGAC    | 741bp | 55.47 | XM_002753473 |
| GAD67  | F: GCTTCTGGCAAGGACACCACCAAC  
        | R: CTTCTGTTGGCTTGCAAGAGAAGA  | 858bp | 49.10%| XM_002749363 |
| Gene  | Primer F | Primer R | Amplicon Size | GC%  | NCBI Accession |
|-------|----------|----------|---------------|------|---------------|
| 5HT1A | F: GCTACCAAGTGATCACCTCTCT | R: TGACACATCGATCACCTCCAGG | 787bp  | 60.48 | NM_008308 |
|       |          |          |               |      |               |
| 5HT1B | F: GGCTACATTTACCAGGACTCCA | R: TTGGTTCACGTACAGGAGAC | 759bp  | 57.81 | NM_010482 |
|       |          |          |               |      |               |
| 5HT1D | F: TCACAGGTTGGAAGCCAAAGGA | R: TGATAAGCTGTGCCGTGTTGAA | 830bp  | 56.02 | NM_008309 |
|       |          |          |               |      |               |
| 5HT1F | F: ACAGTGTGAGCTGCCACACCAC | R: AGTCCGGTGATGGATCGGACAA | 837bp  | 45.40 | NM_008310 |
|       |          |          |               |      |               |
| 5HT2A | F: GCTGCAGAATGCGCAACTAT | R: AGTGTTGACTAAAATACTGC | 928bp  | 49.89 | NM_172812 |
|       |          |          |               |      |               |
| 5HT2C | F: CGTAATCCATTGAGCATAGCC | R: CTCCTCCAGACAAAGCAGTG | 762bp  | 46.33 | NM_008312 |
|       |          |          |               |      |               |
| 5HT3A | F: AGTACTGGAGCTGAGTGTTTC | R: CAGAGCCATGCAACACACAA | 683bp  | 51.47 | NM_013561 |
|       |          |          |               |      |               |
| 5HT4  | F: AGAAGGTCTGCTGCTCACGTT | R: GGACAGTGTAGTCTATGAAAGG | 816bp  | 51.10 | NM_008313 |
|       |          |          |               |      |               |
| 5HT5A | F: TGCTGGGTGCTGGCTACCATCCT | R: ATGAGGATGCCCACCAGTGGG | 700bp  | 58.00 | NM_008314 |
|       |          |          |               |      |               |
| 5HT6  | F: GCATGAACTGGGCAAAGCTCGA | R: GAAACAAAGTGGATGCTGCCGTA | 813bp  | 62.24 | NM_021358 |
|       |          |          |               |      |               |
| 5HT7  | F: GGCAGAATGGGAAATGTATGGC | R: GAGAGCTCCGGTGATTGCC | 655bp  | 52.06 | NM_008315 |
### Tables 3. Arbitrary values assigned for different levels of expression in cortical areas:

|       | 5HT1A | 5HT1B | 5HT1E | 5HT1F | 5HT2A | 5HT2C | 5HT3A | 5HT4 | 5HT6 | 5HT7 |
|-------|-------|-------|-------|-------|-------|-------|-------|------|------|------|
| Area46 | 1:    | -     | -     | -     | -     | -     | -     | -    | -    | -    |
|       | 2:    | ++++  | +/-   | ++    | -     | +/+   | +++   | +/   | +++  | +/-  |
|       | 3:    | +++   | +/-   | +++s  | -     | ++++  | +    | -    | +++  | +/-  |
|       | 4:    | +++   | +/-   | +++s  | -     | ++++  | +/   | -    | +++  | +/-  |
|       | 5:    | +++   | +     | +++s  | -     | ++++  | +++vs| +/   | +++  | +/-  |
|       | 6:    | ++    | -     | +++s  | -     | ++++  | +/   | -    | +++  | +/-  |
| Area6 | 1:    | -     | -     | -     | -     | -     | -     | -    | -    | -    |
|       | 2:    | ++++  | +/-   | ++    | -     | +/+   | +++   | +/   | +++  | +/-  |
|       | 3:    | +++   | +/-   | +     | -     | ++++  | +    | -    | +++  | +/-  |
|       | 4:    | +++   | +/-   | +     | -     | ++++  | ++   | -    | +++  | +/-  |
|       | 5:    | +++   | +     | +     | -     | ++++  | +/   | -    | +++  | +/-  |
|       | 6:    | +     | -     | ++    | -     | ++++  | +/   | -    | +++  | +/-  |
| M1    | 1:    | -     | -     | -     | -     | -     | -     | -    | -    | -    |
|       | 2:    | ++++  | +/-   | ++    | -     | +/+   | +++   | +/   | +++  | +/-  |
|       | 3:    | ++    | +/-   | +     | -     | ++++  | +    | -    | +++  | +/-  |
|       | 4:    | +     | +/-   | +     | -     | ++++  | ++   | -    | +++  | +/-  |
|       | 5:    | +     | +/-   | +     | -     | ++++  | +/   | -    | +++  | +/-  |
|       | 6:    | +     | +/-   | +     | -     | ++++  | +/   | -    | +++  | +/-  |
| S1    | 1:    | -     | -     | -     | -     | -     | -     | -    | -    | -    |
|       | 2:    | ++++  | +/-   | +++   | -     | +/+   | +++   | +/   | +++  | +/-  |
|       | 3:    | ++    | +/-   | +     | -     | ++++  | +    | -    | +++  | +/-  |
|       | 4:    | +     | +/-   | +     | -     | ++++  | ++   | -    | +++  | +/-  |
|       | 5:    | +     | +/-   | +     | -     | ++++  | +/   | -    | +++  | +/-  |
|       | 6:    | +     | +/-   | +     | -     | ++++  | +/   | -    | +++  | +/-  |
| MT    | 1:    | -     | -     | -     | -     | -     | -     | -    | -    | -    |
|       | 2:    | ++++  | +/-   | +++   | -     | +     | +++   | +/   | +++  | +/-  |
|       | 3:    | +++   | +/-   | +++s  | -     | ++    | -    | -    | +++  | +/-  |
|       | 4:    | +     | +/-   | +     | -     | ++++  | +    | -    | +++  | +/-  |
|       | 5:    | +     | +/-   | +     | -     | ++++  | +++vs| +/   | +++  | +/-  |
|       | 6:    | ++    | +     | +/   | -     | ++++  | +++vs| +/   | +++  | +/-  |
| ITG   | 1:    | -     | -     | -     | -     | -     | -     | -    | -    | -    |
|       | 2:    | ++++  | +/-   | +++   | -     | +     | +++   | +/   | +++  | +/-  |
|       | 3:    | +++   | +/-   | +++s  | -     | ++    | -    | -    | +++  | +/-  |
|       | 4:    | +     | +/-   | +     | -     | ++++  | +    | -    | +++  | +/-  |
|       | 5:    | +     | +/-   | +     | -     | ++++  | +++vs| +/   | +++  | +/-  |
|       | 6:    | +     | +     | +/   | -     | ++++  | +++vs| +/   | +++  | +/-  |
| TE    | 1:    | -     | -     | -     | -     | -     | -     | -    | -    | -    |
|       | 2:    | ++++  | +/-   | ++    | -     | +     | +++   | +/   | +++  | +/-  |
|       | 3:    | +++   | +/-   | +++s  | -     | ++    | -    | -    | +++  | +/-  |
|       | 4:    | +     | +/-   | +     | -     | ++++  | +    | -    | +++  | +/-  |
|       | 5:    | +     | +     | +     | -     | ++++  | +++vs| +/   | +++  | +/-  |
|       | 6:    | +++   | +     | +/   | -     | ++++  | +++vs| +/   | +++  | +/-  |
|      | 5HT1A | 5HT1B | 5HT1E | 5HT1F | 5HT2A | 5HT2C | 5HT3A | 5HT4 | 5HT6 | 5HT7 |
|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| V1   |       |       |       |       |       |       |       |       |       |       |
| 1:   | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| 2:   | ++++  | +++   | +++   | +++   | ++    | -     | +++   | +++   | +++   | +/-   |
| 3:   | +++   | +++   | ++    | +++   | -     | +++   | -     | +++   | +++   | +/-   |
| 4:   | ++    | ++++  | +/-   | >++++  | -     | -     | +     | +/-   | +/-   | +/-   |
| 5:   | +/-   | -     | +++   | -     | +     | +++   | vs    | +++   | ++    | +/-   |
| 6:   | +/-   | -     | +++   | +     | -     | -     | +/-   | +/-   | +++   | +/-   |
| V2   |       |       |       |       |       |       |       |       |       |       |
| 1:   | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| 2:   | ++++  | ++    | ++    | ++    | ++    | ++    | vs    | +++   | +++   | +/-   |
| 3:   | ++    | +     | +s    | +++   | -     | +++   | -     | +++   | +++   | +/-   |
| 4:   | +     | +     | +s    | +     | -     | +     | -     | +     | +/-   | +/-   |
| 5:   | +/-   | -     | +s    | ++    | +++   | +++   | vs    | ++    | ++    | +/-   |
| 6:   | +/-   | -     | +++   | +     | -     | -     | +/-   | +/-   | +++   | +/-   |
| CG   |       |       |       |       |       |       |       |       |       |       |
| 1:   | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| 2:   | ++++  | ++    | +     | ++++  | +/-   | -     | +++   | +++   | +++   | +/-   |
| 3:   | +++   | +     | +     | +++   | -     | +++   | -     | +++   | +++   | +/-   |
| 5:   | ++++  | +     | ++++  | -     | +++   | -     | +++   | +++   | +++   | +     |
| 6:   | ++++  | ++    | +/-   | -     | +++   | -     | +/-   | +/-   | +++   | +     |
| ER   |       |       |       |       |       |       |       |       |       |       |
| 1:   | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| 2:   | ++++  | ++    | +     | ++++  | +/-   | -     | +++   | +++   | +++   | +++   |
| 3:   | ++++  | +     | +     | ++++  | -     | +++   | -     | +++   | +++   | +++   |
| 5:   | ++++  | +     | ++++  | -     | +++   | -     | +++   | +++   | +++   | +     |
| 6:   | ++++  | ++    | +/-   | -     | +++   | -     | +/-   | +/-   | +++   | +     |
### Tables 4. Arbitrary values assigned for different levels of expression in subcortical areas:

| Area                              | 5HT1A | 5HT1B | 5HT1E | 5HT1F | 5HT2A | 5HT2C | 5HT3A | 5HT4 | 5HT6 | 5HT7 | Fig Ref |
|-----------------------------------|-------|-------|-------|-------|-------|-------|-------|------|------|------|---------|
| Thalamus                          |       |       |       |       |       |       |       |      |      |      |         |
| Ventral Anterior (VA)             | +++   | ++    | -     | -     | -     | -     | -     | ++   | +++  | 7    | S2,S4   |
| Mediodorsal nucleus (MD)          | +     | +++   | -     | -     | +/-   | +     | -     | +    | ++   | ++   | 7       |
| Central lateral nucleus (CL)      | +++   | +/-   | ++    | -     | +++   | +++   | -     | -    | ++   | ++   | 7       |
| Ventral lateral group             |       |       |       |       |       |       |       |      |      |      |         |
| lateral dorsal nucleus (LD)       | +     | +++   | -     | -     | -     | -     | -     | +++  | +++  | 7    |         |
| Ventral lateral nucleus (VL)      | +     | +++   | -     | -     | -     | -     | -     | -    | ++   | +++  | 7       |
| Ventral posterior group           |       |       |       |       |       |       |       |      |      |      |         |
| Ventral posterior lateral (VPL)   | +     | +++   | -     | -     | -     | -     | -     | -    | +    | +++  | S2,S4   |
| Ventral posterior medial (VPM)    | +     | +++   | -     | -     | -     | -     | -     | -    | +    | +++  | S2,S4   |
| Posterior group                   |       |       |       |       |       |       |       |      |      |      |         |
| Medial geniculate body (MG)       | +     | +++   | +/-   | -     | -     | -     | -     | -    | ++   | ++   | S3      |
| Lateral geniculate body (LG)      | +     | ++++  | -     | -     | -     | -     | -     | -    | +    | ++   | S4      |
| Pulvinar                          | ++    | ++++  | -     | -     | -     | -     | -     | -    | ++   | +++  | S2      |
| Thalamic reticular nucleus (Rt)   | +     | +++   | -     | -     | +++   | +++   | -     | -    | -    | -    | S2      |
| Hippocampus                       |       |       |       |       |       |       |       |      |      |      |         |
| CA1                               | ++++  | ++    | +++   | -     | +/-   | ++++* | ++++* | ++   | +++  | ++   | 6,S2    |
| CA2                               | ++++  | +++   | ++++  | +     | +/-   | ++++* | ++++* | +++  | ++++ | ++   | 6,S2    |
| CA3                               | ++++  | ++    | ++++  | -     | +/-   | ++++* | ++++* | +++  | ++++ | ++   | 6,S2    |
| Dentate gyrus                     | ++++  | ++    | ++++  | -     | ++++  | -     | -     | +++  | ++   | +/-  | 6,S2    |
| Subicular complex                 | ++    | ++    | +++   | +     | +++*  | -     | -     | ++   | ++   | ++   | 6,S2    |
| Amygdala                          |       |       |       |       |       |       |       |      |      |      |         |
| Basolateral (BLa)                 | +++   | +     | ++    | -     | +     | ++++* | -     | +    | +    | +    | 10      |
| Basomedial (BMa)                  | +++   | +     | ++    | -     | +     | ++++* | -     | +    | +    | +    | 10      |
| Cortical amygdaloid (Co)          | +++   | +     | ++    | -     | +     | ++++* | -     | +    | +    | +    | 10      |
| Medial amygdaloid (Me)            | +++   | +     | ++    | -     | +     | ++++  | -     | +    | +    | +    | 10      |
| Lateral amygdaloid (La)           | ++    | +     | ++    | -     | ++++  | ++++* | -     | +    | +    | +/-  | 10      |
| Area                                      | 5HT1A | 5HT1B | 5HT1E | 5HT1F | 5HT2A | 5HT2C | 5HT3A | 5HT4 | 5HT6 | 5HT7 | Fig Ref |
|-------------------------------------------|-------|-------|-------|-------|-------|-------|-------|------|------|------|---------|
| **Hypothalamus**                          |       |       |       |       |       |       |       |      |      |      |         |
| Medial mammillary nucleus (MM)            | +/-   | -     | +/-   | -     | +++   | -     | -     | +    | ++   | +++  | 8, S2   |
| Lateral mammillary nucleus (ML)           | +++   | ++    | -     | -     | +++   | -     | -     | ++   | -    | -    | 8, S2   |
| Ventral tuberomammillary (VTM)            | -     | -     | -     | +++   | -     | -     | -     | +/-. | +++  | 8, S2 |         |
| **Dorsal striatum**                       |       |       |       |       |       |       |       |      |      |      |         |
| Putamen                                   | ++    | ++    | +     | -     | +     | +++   | -     | +++  | +++  | +    | 12      |
| Caudate nucleus                           | ++    | ++    | +     | -     | +     | +++   | -     | +++  | +++  | +    | 12      |
| Medial septum                             | +++   | +/-.  | -     | -     | +++   | -     | -     | +/-. | +/-. | +/-. | 12      |
| Lateral septum                            | +++   | +++   | ++    | -     | +++   | -     | +     | +    | +    | ++   | 12      |
| **Ventral striatum**                      |       |       |       |       |       |       |       |      |      |      |         |
| Globus pallidus internal (IGP)            | +     | -     | ++    | -     | ++    | +++   | -     | +    | ++   | -    | S2      |
| Globus pallidus external (EGP)            | +     | -     | ++    | -     | ++    | +     | -     | +    | +    | -    | S2      |
| Substantia nigra reticulata (SNr)         | ++    | ++    | +++   | -     | +/-   | +++   | -     | +++  | +++  | +++  | 13      |
| Substantia nigra compacta (SNc)           | ++    | ++    | +++   | -     | ++    | +++   | -     | +++  | +++  | +++  | 13      |
| **Midbrain tectum**                       |       |       |       |       |       |       |       |      |      |      |         |
| Superior colliculi (SC)                   | ++++  | ++    | -     | -     | +     | +++   | -     | +++  | ++   | 11    |         |
| Receptor | Major G-Protein, Reference | Signal, Reference | Potential (I/E), Reference | Cellular localization: Region Species, Reference |
|----------|----------------------------|------------------|--------------------------|-------------------------------------------------|
| 5HT1A    | G<sub>i</sub><sub>/G<sub>o</sub></sub>[1] | ↓ cellular levels of cAMP, I, [2] |  | **Somatodendritic**: hippocampus, cortex, and others **Rat, Monkey** [3,4]  |
| 5HT1B    | G<sub>i</sub><sub>/G<sub>o</sub></sub>[1] | ↓ cellular levels of cAMP, I, [2] |  | **Preterminal Axon**: globus pallidus and substantia nigra **Rat**, suprachiasmatic Nucleus **Mouse** [10] |
| 5HT1E    | G<sub>i</sub><sub>/G<sub>o</sub></sub>[1] | ↓ cellular levels of cAMP, I, [2] |  | NA |
| 5HT1F    | G<sub>i</sub><sub>/G<sub>o</sub></sub>[1] | ↓ cellular levels of cAMP, I, [2] |  | **Somatodendritic**: clustrum, thalamus, amygdala, cortex **Guinea Pig** [6] |
| 5HT2A    | G<sub>i</sub><sub>/G<sub>q</sub></sub>[1] | ↑ IP<sub>3</sub> and cytosolic [Ca<sup>2+</sup>], E, [2] |  | **Somatodendritic**: cortex, hippocampus, septum, basal ganglia, amygdala **Rat** [4,7] and **Axonal**: cortex **Monkey** [8] |
| 5HT2C    | G<sub>i</sub><sub>/G<sub>q</sub></sub>[1] | ↑ IP<sub>3</sub> and cytosolic [Ca<sup>2+</sup>], E, [2] |  | **Somatodendritic**: cortex, amygdala, hippocampus, thalamus **Rat, Human** [9] |
| 5HT3A    | ligand-gated channel | depolarizing membrane, E |  | **Somatodendritic** and **Axonal**: cortex, hippocampus, amygdala **Rat** [9] |
| 5HT4     | G<sub>q</sub>[1] | ↑ cellular levels of cAMP, E, [2] |  | **Somatodendritic** and **Axonal**: basal ganglia and hippocampus **Rat** [9] |
| 5HT6     | G<sub>q</sub>[1] | ↑ cellular levels of cAMP, E, [2] |  | **Somatodendritic**: cortex, striatum, hippocampus, and others **Rat** [5,9] |
| 5HT7     | G<sub>q</sub>[1] | ↑ cellular levels of cAMP, E, [2] |  | **Somatodendritic** and **Axonal**: suprachiasmatic nucleus **Mouse** [9,10] |
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