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# The olfactory bulb and the number of its glomeruli in the common marmoset (*Callithrix jaccus*)

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ABSTRACT

The olfactory system has been well studied in mammals such as mice and rats. However, few studies have focused on characterizing this system in diurnal primates that rely on their sense of smell to a lesser extent due to their ecological environment. In the present study, we determined the histological organization of the olfactory bulb in the common marmoset (*Callithrix jaccus*). We then constructed 3-dimensional models of the glomeruli of the olfactory bulb, and estimated the number of glomeruli. Olfactory glomeruli are the functional units of olfactory processing, and have been investigated in detail using mice. There are approximately 1800 glomeruli in a mouse hemibulb, and olfactory sensory neurons expressing one selected olfactory receptor converge onto one or two glomeruli. Because mice have about 1000 olfactory receptor genes, it is proposed that the number of glomeruli in mammals is nearly double that of olfactory receptor genes. The common marmoset carries only about 400 intact olfactory receptor genes. The present study revealed that the number of glomeruli in a marmoset hemibulb was approximately 1500–1800. This result suggests that the number of glomeruli is not positively correlated with the number of intact olfactory receptor genes in mammals.



## 1. Introduction

The morphology of a species' sensory system, especially in peripheral organs and primary brain regions, is modified by its ecologic situation. The olfactory system is the most important sensory system in nocturnal mammals, such as mice and rats, and this system is well characterized. In contrast, many diurnal primates do not rely as extensively on the olfactory system. The common marmoset (*Callithrix jaccus*) is a diurnal, small New World monkey that carries 393 olfactory receptor (OR) genes, which are very similar in number to that of humans (396 genes), and much more than that of macaques (326 genes) (Bendesky and Bargmann, 2011). The extent to which marmosets can sense odors is not clear, but scent marking and urine spraying can typically be observed in marmosets in the laboratory setting (Smith, 2006). Therefore, it is speculated from their irritant body odors that olfaction serves a critical survival function in marmosets.

In the mammalian olfactory bulb, a glomerulus is a specialized structure for specific odor information processing. It consists of axons of olfactory sensory neurons, dendrites of olfactory bulb neurons, and glial components (Shepherd and Greer, 1998). The glomeruli are devoid of cell somata. The spherical neuropil that characterizes each glomerulus is distinguished from other glomeruli by surrounding interneurons such as periglomerular (PG) cells and astrocytes. According to studies in mice, one glomerulus receives information from one OR (Mombaerts et al., 1996; Ressler et al., 1994; Zou et al., 2009). Moreover, one OR expressing axons converge onto 1 or 2 glomeruli (Mori et al., 1999; Zou et al., 2009). Since there are about 1800 glomeruli in mice (Royet et al., 1988), the number of glomeruli in mammals is considered to be nearly double the number of OR genes. It is presently unclear whether this phenomenon generalizes to other mammals.

In this study, we first examined the histological organization of the marmoset olfactory bulb, as information regarding this is minimal. We then developed a method for counting glomeruli and calculated the number of glomeruli in the common marmoset. If

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glomerular convergence is similar to that of the mouse, it is conceivable that the number of glomeruli is around 800 in marmosets.

## 2. Materials and methods

### 2.1. Animals

Six (5 male, 1 female) 1.6- to 4-year-old adult common marmosets were used for the histology study. These animals were kept in a breeding colony at Tokyo Metropolitan Institute of Medical Science, and all animals were cared for and used according to the Guidelines for The Care and Use of Animals of Tokyo Metropolitan Institute of Medical Science.

### 2.2. Histology

We prepared histological sections as previously described (Tokuno et al., 2009), with some modifications. Briefly, animals were anesthetized with ketamine hydrochloride (10–30 mg/kg body weight, intramuscular) and sodium pentobarbital (40–60 mg/kg body weight, intraperitoneal). They were perfused with 200 ml physiological saline and fixed with 500 ml 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB, pH 7.3). The brains were removed and post-fixed with 4% PFA in 0.1 M PB at 4 °C overnight.

The brains were sequentially immersed in 20% glycerol in 0.1 PB at 4 °C until fully dipped down, in 1.5% gelatin 20% glycerol in 0.1 M PB (glycerol buffer) at 37 °C for 1 day, and in 10% gelatin in glycerol buffer at 37 °C for 4 days. Following this, the brains were embedded in 10% gelatin in glycerol buffer. Before slicing, the gelatin blocks containing the brains were fixed again with 4% PFA in glycerol buffer at 4 °C for 3 days. Then, the blocks were rinsed in glycerol buffer for more than 3 days. The blocks were serially sliced (30 μm in thickness) on a freezing microtome.

For glomerulus counting, each post-fixed olfactory bulb was embedded in paraffin. The coronal sections (5 μm in thickness) were serially cut on a rotary microtome.

#### 2.2.1. Nissl staining

The sections were stained with cresyl violet for Nissl staining using an automated slide stainer (Tiss-Tek Prism JOD, Sakura Finetek USA, CA, USA).

#### 2.2.2. Acetylcholinesterase (AChE) histochemical staining

For AChE histochemistry, the sections were incubated in 0.1 M acetate buffer containing acetylthiocholine iodine, promethazine-HCl, $CuSO_4$, and glycine at 37 °C for 60 min. Then, the sections were incubated in 1.25% sodium sulfite, 1% silver nitrate, and 5% sodium thiosulfate aqueous solutions for visualization of the reaction product (Hardy et al., 1976).

#### 2.2.3. NeuN immunostaining

The sections were incubated with anti-NeuN antibody (1B7, mouse monoclonal, Abcam, UK, 1:1000) at 4 °C for three days, and then incubated with a biotinylated anti-mouse IgG raised in goat as a secondary antibody (Vector laboratories, USA, 1:250) at room temperature for 2 h. For visualization, the sections were stained using an ABC elite kit (Vector laboratories, 1:200) at room temperature for 1.5 h.

#### 2.2.4. Solochrome staining

The sections were defatted and stained in solochrome cyanin solution containing 0.2% solochrome cyanin RS and 10% iron alum (Page, 1965) at room temperature for 20 min. Then, the sections were washed in running tap water for 3–5 min. The sections were differentiated in freshly prepared 0.25% ammonium hydroxide for a few seconds (Clark, 1979). Finally, the sections were washed in running tap water until the background color became light blue.

### 2.3. Data preparation

All stained sections were scanned with a Mirax Scan (Zeiss, Germany), and the obtained digital images were modified using Photoshop CS5 (Adobe, USA).

### 2.4. Counting of glomeruli

For glomeruli counting, the solochrome-stained complete serial sections were used. The digital images were placed into the lower layer on Photoshop. Next, the glomerular structures shown as uniform dark blue circles by solochrome-staining were determined, and these areas were manually extracted from each image. The layered Photoshop images were converted into a movie file (MPEG-4). The 3D reconstructions of glomerular arrangement were reconstructed using the MPEG-4 files with free imaging software OsiriX (Neuton Graphics Inc., Japan). We then counted glomeruli using the following methods. Based on the 3D data, glomerular models were reconstructed using a 3D printer (Micro Factory Corp., Japan) in five hemibulbs. The mean weight of one glomerulus was calculated by measuring approximately 40–120 glomeruli dispersed from each model. We divided the total weight of the printed model by the mean weight of one glomerulus to estimate the total number of glomeruli.

## 3. Results

### 3.1. Anatomy of the olfactory bulb

The olfactory bulb of the marmoset is 3–4 mm in length and about 2 mm in width. It is an elliptic organ anterior to the thin olfactory tract (Fig. 1A). We have already published a web-accessible digital brain atlas of the common marmoset at http://marmoset-brain.org. Because histological data about the olfactory bulb was absent in this digital atlas, we provide those of the common marmoset using mainly Nissl stained sections (Fig. 1B and C). At the light microscopic level, the laminar organization of the marmoset olfactory bulb was comparable to that of many other mammalian species (Kosaka and Kosaka, 2004; Kosaka et al., 2005; Smith et al., 1991). Unlike mice or rats (Paxinos and Watson, 2014; Watson and Paxinos, 2010), the olfactory ventricle and rostral migratory stream (RMS) exhibiting dense cells were not distinct in the marmoset olfactory bulb (Fig. 1B and C), as shown in a previous report (Sawamoto et al., 2011). The anterior olfactory area is located in the ventrolateral region of the caudal olfactory bulb in mice and rats. In the marmoset, this area was found in the cerebral cortex, rather than the olfactory bulb (Paxinos et al., 2012). Moreover, the accessory olfactory bulb (AOB) was not in the dorsocaudal region of the bulb, as it is in rodents. Rather, it was seen more posteriorly in the lateral olfactory tract (Fig. 1B and C). There were a small number of neurons in this tiny AOB. The AOB seemed to be comprised of a mixture of large projection neurons and small interneurons in a band area. We were unable to discriminate between the mitral cell layer and the granule cell layer in the AOB. The glomerular structure of the AOB was also unclear.

### 3.2. Organization of the main olfactory bulb (MOB)

Fig. 2 shows the marmoset MOB at higher magnification. These histological data are available now in the digital atlas of the marmoset brain at http://marmoset-brain.org. Five distinctive laminar structures could be identified in the MOB: olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral

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