A Quantitative Study of the Purkinje Cells in the Cerebellum and the Inferior Olivary Neurons in the Chicken

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ABSTRACT. A single olivocerebellar fiber branches off several climbing fibers. One Purkinje cell receives input from only one climbing fiber. Therefore, synapses with several Purkinje cells, so that there are more Purkinje cells than the inferior olivary neurons. We aimed to elucidate the numerical ratio of the inferior olivary neurons to Purkinje cells in the chicken. The total numbers were 353,834 ± 5,274 in the Purkinje cells per the cerebellum and 21,553 ± 904 in the inferior olivary neurons of both sides. The numerical ratio of inferior olivary neurons to Purkinje cells was 1:16. The ratio of those neurons in mammals is about 1:4–17, so that the ratio in the chicken is within the range of mammals.

KEY WORDS: chicken, inferior olivary neurons, Purkinje cell.

The mammalian inferior olivary complex (IOC) consists basically of the medial and dorsal accessory olivary nuclei (MAO and DAO, respectively), and the principal olivary nucleus (PO). The accessory nuclei are older than the PO, and the MAO is the oldest in a phylogenetic viewpoint [12]. The avian IOC consists of a large dorsal and a small ventral lamellae. It has been believed on the basis of the morphological appearance that the ventral lamella corresponds to the PO, and the lateral and medial parts of the dorsal lamella are the homologue of the DAO and MAO, respectively [12]. Recently, it is suggested that the chicken dorsal lamella represents the DAO, PO, and the rostral and a small part of the caudal MAO, while the ventral lamella represents most of the caudal MAO on the basis of the olivocerebellar projection [10].

Purkinje cells (PCs) are known to receive double inputs from the mossy and climbing fibers. An olivocerebellar fiber gives off several climbing fibers, but each Purkinje cell receives only one climbing fiber [6]. The IOC is the sole source of the climbing fibers in birds and mammals [3, 9]. Although there are several quantitative studies on the PCs and the IOC neurons, there are a few studies on a numerical correlation between PC and IOC neurons by the same researcher. Our goal of this study is to elucidate the numerical ratio of the IOC neurons to PCs in the chicken.

Seven chickens (*Gallus domesticus*) (four males and three females) ranging from 1.5 to 2 months of age (300 to 500 g in body weight) were used in this study. The animals were deeply anesthetized with sodium pentobarbital (Nembutal). After intravenous administration of heparin (270 IU), the animals were perfused with 800 ml of 0.75% saline, followed by 800 ml of 10% formalin through the left ventricle. Both solutions were delivered at 70 ml/min [9]. After two to three hr of perfusion, the brain was removed. The specimens were dehydrated, embedded in celloidin, and were serially cut at 30 µm, and stained with toluidine blue or thionin. The medulla was sectioned transversely, while the cerebellum was sectioned sagittally. Cell counts were done by projecting the microscopic sections onto an Olympus Video Micro Meter [Model VM-31] at final magnification × 350. In four chickens, the IOC neurons of both sides were counted in every fifth section. The procedure was repeated on three separate occasions. The total number of the IOC neurons was then estimated by multiplying the number of neurons counted by 2 (2 is half of the number of sections non-counted between the section counted and the next counted section) [7]. All visible neurons with and without a nucleolus were included in the counts. To check the reliability of this method, we counted only the neurons with a nucleolus in two successive sections of one case, and then compared for both methods. The two methods gave no significant differences in the total cell number.

PCs with a nucleolus were counted in every tenth section in the other three chickens. The total number of PCs in the cerebellum was obtained by multiplying the number of PCs counted by 10 [15].

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<th>Table 1. Counts of inferior olive neurons in the chicken</th>
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<td><strong>Dorsal Lamella</strong></td>
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CV, coefficient of variation; L & M, the lateral and medial parts of the dorsal lamella; SDEV, standard deviations.
The left and right IOC included 10,733 and 10,820 neurons, respectively, and therefore, there were 21,553 IOC neurons on average in both sides (Table 1). There were no significant differences between the left and right sides and even in sexual difference. The number of IOC neurons of the dorsal lamella was about three times greater than of the ventral one, representing 74.4% of the total number of the IOC neurons, and the lateral part (48.2%) contained more neurons than the medial part (26.2%). In the previous study, the dorsal lamella is markedly larger than the ventral one, the lateral part is the largest component of the dorsal lamella [20], and the dorsal and ventral lamellae contain about 75% and 25% of the total number of IOC neurons, respectively [13]. Thus, our present results agree with those of the previous studies.

The avian cerebellum consists exclusively of the vermis on the basis of gross anatomy. However, the lateral parts of lobules VI–VIII have been considered to correspond to the cerebellar hemispheres, which receive the most abundant input from the pontine nuclei [3, 5, 21]. In the mammalian IOC, the MAO, DAO and PO project generally into the vermal and paravermal zones and the hemisphere, respectively. Thus, it is expected that in the chicken IOC the MAO is largest, and PO is smallest. Contrary to our expectation, the DAO (namely, the dorsal lamella) in the classical interpretations was largest, and the MAO was similar in neuronal number to the PO in this study. The subdivisions of the chicken IOC that are suggested by Furber [10] in term of the olivocerebellar projection, however, are suitable for this study.

The avian cerebellum consisted of 10 lobules. The number of PCs was 353,834 on average. The numbers were highest in the lobule IX (21.8%) and lowest in the lobule I (2.6%) (Table 2).

In this study, IOC neurons were about 21,600, and PCs were about 354,000 in the total average numbers. The ratio of the former to the latter, therefore, was about 1:16. In the previous studies, the chick IOC neurons in both sides are 14,350 [13] or about 14,000 [2] in number. The number of PCs is 262,000 [2]. The ratio of both, therefore, is about 1:18. That is similar to our result.

The total numbers of IOC neurons have been estimated in the human, vampire bat, cat and rat. These are about 15 million in the human [14, 16, 17], 1,440,000 to 1,809,000 in the cat [15, 16], and 254,000 to 360,000 in the rat [1, 11, 16]. The chicken cerebellum has as many PCs as the rat, unlike with the number of IOC neurons.

From the above mentioned data, the estimated numerical ratio of IOC neurons to PCs is about 1:15–17 in the human, 1:10–13 in the cat, and 1:4–7 in the rat. According to Sugihara et al. [19] who observed the complete trajectories of the single olivocerebellar fiber, the single fiber branches off seven climbing fibers on average in the rat. Their result nearly corresponds to the above described data on the rat. Therefore, the numerical ratio of IOC neurons to PCs in the chicken falls within the range of mammals. It is suggested that the chicken olivocerebellar system works with the same principle as mammalian one.

### References