Baseline membrane activities of *Onchidium* photoresponsive neurons

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**Abstract**

Baseline stimulus-free activity in *Onchidium* photoresponsive neurons Ip-1 and Ip-2 differs according to the season in which animals are obtained from their natural habitat. Few neurons harvested from animals acquired in winter and early spring show spontaneous membrane activity; in particular, regular patterns of bursting, composed of repetitive firing followed by silent periods, are rarely seen. In contrast, half of the neurons harvested from animals obtained in late spring and summer exhibit membrane activity including bursting. This spontaneous membrane activity may reflect seasonal conditions influencing activities such as reproduction.

**Introduction**

In a previous study, I reported that spontaneous membrane activity of neurons Ip-1 and Ip-2 (Ip-1/2) in the mollusk *Onchidium* show quite different patterns between preparations (Nishi, 2012). However, the cause of these differences is unclear. In the current study, I analyzed the membrane activities according to the following variables: the season in which the animals were acquired from their natural habitat, the length of time the animals were kept in the laboratory after acquisition, as well as the size of animals.

It is suggested that the membrane activities of *Onchidium* neurons, Ip-1/2, vary depending on the season in which the animals were obtained, not on the animal’s size or length of time spent in the laboratory after collection. Bursting tends to occur in the late spring and summer and may be related to the animals’ reproductive cycle.

**Results and Discussion**

The experimental specimens were prepared from *Onchidium verruculatum* from a habitat in Sakurajima, Kagoshima in 2011 and 2012. As *Onchidium* live more than a year, it is possible to obtain mature specimens throughout the year. The aquarium (tank) in which the animals were kept is in a laboratory in which light conditions were not specifically regulated. The temperature of circulating natural sea water of the aquarium was maintained at 15 °C. In these conditions, animals
tend to reside underneath a rock in the tank for extended periods of time without feeding, and do not actively crawl around.

Animals collected on the 9th and 10th of December 2011 are referred to as "winter animals." Likewise, animals collected on the 10th and 11th of March 2012 are referred to as "early spring animals," those collected on the 17th and 18th of May 2012 are referred to as "late spring animals," and those collected on the 17th and 18th of August 2012 are referred to as "summer animals."

In the current study, I analyzed the membrane activities according to the following variables: the season in which the animals were acquired from their natural habitat, the length of time the animals were kept in the laboratory after acquisition, as well as the size of animals. The procedures for recording neuronal membrane activity were as previously described (Shimotsu et al., 2010, Nishi, 2012). Membrane activity was categorized into 3 types according to the previous study (Nishi, 2012). The first type is regular bursting (B), in which the duration of firing and cessation time are relatively constant. The second type is irregular bursting (IB), which is composed of repetitive firing and subsequent cessation times, the durations of which are not constant. The third type is "silent" (S) activity, in which no firing of action potentials is detected. These 3 types of activity are shown Figure 1 schematically. In the previous study, I described 2 types of "silent" activities, where no firing occurs without stimulation. One silent type neuron shows monotonous firing activity, and the other shows small changes in spike frequency during repetitive firing during CO₂ gas stimulus application to the perfusing solution or by depolarizing current injection. In the present study, I classify the latter as IB type, since firings self-terminate after a stimulus of long duration. Conversely, firings continue until the stimulus is removed in the former silent type. A few neurons display stimulus-free spontaneous firing, in which the interval of repetitive firing is fairly constant, there are no obvious cessation periods, and firings occur continuously. In this study, I categorize this type of activity as S type, because firings cease completely with the injection of hyperpolarizing current.

Figure 1.
Schematic diagram showing the membrane activities of 1p-1/2. B: Bursting type of activity, bursting and subsequent cessation time is relatively constant. Duration of bursting and cessation time is on a time scale of minutes. IB: Irregular bursting activity. Duration of bursting and cessation time varies considerably, both bursting and silent period last several minutes in some specimens. S: Silent activity. Membrane potential slowly fluctuates by less than 2-3 mV in the resting potential.
The time periods in which animals were kept in the laboratory were classified into 4 groups (periods I, II, III, and IV). In period I, experimental procedures were conducted less than 20 days after animal collection; period II encompassed 21-40 days, period III 41-60 days, and period IV 61-80 days.

Animals were weighed prior to experimental procedures to determine size and divided into 4 groups: small (S) for less than 10 g, medium-small (MS) for 10-15 g, medium (M) for 15-20 g, and large (L) for above 20 g. Animals less than about 7 g or greater 25 g are inappropriate for experiments and thus not collected. These small or large animals are infrequently detected; thus, the animals used in these experiments are thought to reflect a natural distribution of animals in their natural habitat, although this was not analyzed rigorously. The mean weights (± SD) of *Onchidium* were as follows: 15.6 ± 3.4 g (n = 19, in winter animals), 16.9 ± 3.7 g (n = 34, in early spring animals), 14.3 ± 4.1 g (n = 41, in late spring animals), 14.2 ± 3.3 (n = 30, in summer animals). There was no statistical difference in the weights of animals according to season (P > 0.95). However, very large animals (>30 g) were seen exclusively in the winter, and very small animals (<1 g) were only detected in the late spring. At that time, I found about 10 small animals together in one “nest” underneath rocks in several places. These extremely small animals are thought to have recently metamorphosed into adults and it is thought that many animals spawn in this season.

In Figure 2, all graphs show the numbers of animals as vertical scale. They are classified by size and membrane activity on the 2 axes, according the keeping period I, II, III and IV. The numbers of animals indicate the animals of which membrane potentials were analyzed, i.e., there were a few animals that were weighed but not experimented on; these are not included in the graphs.

In this study, it was clarified that the “silent” activities without bursting did not simply reflect a deteriorated condition of the animals. Although animals are thought to be freshest just few days after collecting, the silent activities were observed throughout the year. It is difficult to determine which factors are related to the membrane activities as shown in Figure 2. However, when B and IB types of activities are examined together, it is clear that the number of animals with these activities increase with time in the summer and late spring animals, in contrast to the early and winter animals, as shown in Figure 3.

There seems to be a tendency for a difference in the appearance of genital organs (especially the hermaphroditic gland, ovotestis) between bursting and silent animals. The color of ovotestis is brown with black spots when the neurons show bursting, whereas it is bright yellow when neurons show silent activities. This may suggest that the color of ovotestis indicates the state of the reproductive organ development, and neurons secrete a reproductive related hormone. In that case, Ip-1/2 is the neurosecretory cell. It was observed that neurons swell with transparent liquid sometimes, more often in early spring rather than in summer or winter.
Figure 2.
Membrane activities among collecting season, keeping period, and weight. Each season is divided into 3 (4) groups from the date of collecting the experimental animals. In the panels, I indicates that animals are kept less than 20 days in the laboratory after collection, II means 21 to 40 days, III means kept 41 to 60 days, IV means 61 to 80 days. Vertical axis indicates the number of animals. Animals are classified by weight (L, M, MS, or S) and membrane activities (B, IB, or S).
Properties of membrane potential activities in *Onchidium* photoresponsive neurons

Figure 3.
The ratio of bursting activities. The vertical scale shows the ratio of animals that show B or IB type activities in each season. The ratios are shown along the period, I to IV, in abscissa axis.

Ip-1/2 are known to be intrinsically photoresponsive neurons; therefore, light stimulus may control hormone production or secretion, which is found in quail brain (Nakao et al., 2008). Compared to vertebrates, there are few studies concerning the endocrine system of mollusks. Studies using mollusks today are done mainly with the related animal *Aplysia*, because *Aplysia* is one of the invertebrates whose genome is clarified as well as drosophila. In *Aplysia*, bag cells are neurosecretory cells and secrete several kinds of peptide hormones. One of these peptides is egg-releasing hormone, it stimulates egg release from the ovotestis, and bursting occurs in bag cells in prior to egg-releasing behavior (Kachoei et al., 2006). *Aplysia* live 1 year; thus, this phenomenon happens only once a year, and *Aplysia* die after releasing egg. In contrast to *Aplysia*, *Onchidium* live a few years and their spawning period is rather wider than *Aplysia*, from early spring to fall (early November). The variation of membrane activities may reflect the wide period of spawning.

Long-term bursting, which is composed of repetitive firing and silent periods on a time scale of minutes, requires intracellular calcium (Ca) stores, namely from the endoplasmic reticulum (ER) (Chay, 1996). It has been suggested that the silent activities subsequent to bursting correspond to the period of absorption of Ca ions into the Ca store in both theoretical and experimental studies (Goforth et al., 2002).

The results of this study indicate that membrane activities of *Onchidium* neurons are quite different among preparations, and activities differ depending on seasons. To the best of my knowledge, this is the first study to examine and identify seasonal changes of membrane activities in identified mollusk neurons. My findings indicate that membrane activities of *Onchidium* neurons are quite different among preparations and differ according to season; it is thus suggested that the properties and function of Ca stores also change seasonally. Future studies should investigate the relationship between the seasonal changes in membrane activity and intracellular Ca stores in Ip-1/2 neurons.
References


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