Effect of Gap Junction Blocker β-Glycyrrhetinic Acid on Taste Disk Cells in Frog

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Abstract A gap junction blocker, 18β-glycyrrhetinic acid (β-GA), increased the membrane resistance of Ia, Ib and II/III cells of frog taste disk by 50, 160 and 300 MΩ, respectively, by blocking the gap junction channels and hemichannels. The amplitudes of gustatory depolarizing potentials in the disk cells for 4 basic taste stimuli were reduced to 40-60% after intravenous injection of β-GA at 1.0 mg/kg. β-GA of 1.0 mg/kg did not affect the resting potentials and the reversal potentials for tastant-induced depolarizing potentials in any taste disk cells. The percentage of cells responding to each of 4 basic taste stimuli and varying numbers of 4 taste qualities did not differ between control and β-GA-treated taste disk cells. This implies that gustatory depolarizing response profiles for 4 basic taste stimuli were very similar in control and β-GA-treated taste disk cells. It is concluded that β-GA at 1.0 mg/kg

reduced the amplitude of gustatory depolarizing potentials in taste disk cells by strongly blocking depolarizing currents flowing through the gap junction channels and hemichannels, but probably weakly affected the gustatory transduction mechanisms for 4 taste stimuli.

Keywords Frog taste disk cell ・ Depolarizing response profile ・ Gap junction blocker ・ gap junction hemichannel ・ Membrane resistance increase

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Introduction

Gap junction channels basically exist between homogenous cells such as epithelial cells, smooth muscle cells and myocardial cells of the same type (Ganong 2005; Sáez et al. 2003; Evans et al. 2006). On the other hand, gap junction hemichannels exist in most of the cells (Sáez et al. 2003; Evans et al. 2006). A variety of intercellular communications occur through either the gap junction channels or hemichannels where signaling molecules or electrical currents are transmitted to neighboring cells (Sáez et al. 2003; Evans et al. 2006; Rodoriguez-Sinovas et al. 2007).

In the frog taste disk located at the top of the fungiform papillae, six types of cell exist (Richiter et al. 1988; Witt 1993; Osculati and Sbarbati 1995). These are types Ia (mucus) cells, Ib (wing) cells, Ic (glia) cells, II/III (receptor) cells and IV (basal) cells (Osculati and Sbarbati 1995). Dye-coupling studies on frog taste disks have revealed that gap junction channels exist only between Ia cells but no gap junction channels exist among any pairs of taste disk cells (Takeuchi et al. 2001; Suwabe and Kitada 2004).

There are two types of taste receptor cells designated types II and III cells in frog taste disk. Afferent synapses are present in III cells but not in II cells (Witt 1993; Osculati and Sbarbati 1995). Also, in mammalian taste buds there are two types of taste receptor cells designated types II and III cells and only type III cells possess afferent synapses (Murray 1973; Seta and Toyoshima 1995). However, afferent fiber terminals are in close contact with type II cells in amphibian and mammalian

taste organs (Murray 1973; Osculati and Sbarbati 1995). Recently it has been thought that gustatory information formed in mammalian type II cells designated taste transduction cells is transmitted into type III cells, where afferent neural signals are transmitted into the brain (Huang et al. 2007). In frog taste disk system it is possible that the similar relationship is present between II and III cells.

The present studies investigate the effects of a gap junction blocker, 18β-glycyrrhetinic acid (β-GA) on the electrical properties and gustatory depolarizing responses of taste disk cells in the frogs.

Materials and Methods

Preparation

Adult bullfrogs (*Rana catesbeiana*) weighing 320-680 g were used. All the experiments were carried out under a guidance of Nagasaki University Animal Experimentation Committee. The animals were anesthetized by intraperitoneally injecting a urethane solution at a dose of 2-3 g/kg body weight. Care was taken to keep the lingual blood circulation normal as long as possible. The tongue was pulled out from the mouth and pinned on a cork plate. Both the hypoglossal nerves were cut to remove spontaneous twitches of the tongue. Both the glossopharyngeal nerves were severed to avoid the effect

of parasympathetic nerve fibers supplying taste cells (Sato et al. 2005). All the experiments were carried out at room temperature (22-25°C).

Electrical Recording and Chemical Stimulation

The methods of intracellular recordings from taste disk cells in the fungiform papillae were the same as those described previously (Sato et al. 2002, 2004). The fungiform papillae dotted at the apical and middle loci of the tongue were used. The cell bodies of taste disk cells in the central area of the disk are arranged at three layers: Ia cell bodies at the superficial layer, Ib cell bodies at the upper part of intermediate layer and cell bodies of Ic/II/III cells at the lower part of intermediate layer (Richiter et al. 1988; Witt 1993; Osculati and Sbarbati 1995). The criteria for identification of Ia cell, Ib cell and II/III cell in intracellular recordings from taste disks were the same as described previously (Sato et al. 2007). In brief, the cell types were identified by three significantly different resting membrane potentials. Similar identification of taste disk cells in frogs was first attempted by Akaike et al. (1976). The input resistance of taste disk cells was measured by injecting constant hyperpolarizing current pulses into cells.

Taste stimuli used were 1 M NaCl, 1 mM acetic acid, 10 mM quinine-HCl (Q-HCl) and 1 M sucrose. The first two were dissolved in deionized water and the last two were dissolved in 0.1 M

NaCl to remove solvent water-induced hyperpolarization of taste disk cells (Okada et al. 1992; Sato et al. 2005). After taste stimulation the tongue surface was usually rinsed with a frog Ringer solution. The composition of frog Ringer was 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$ and 5 mM HEPES [4-(2-hydroxymethyl)-piperazine-1-ethanesulpfonic acid]. The pH was adjusted to 7.2 by a Tris [tris(hydroxylmethyl)aminomethane] solution. Depolarizing responses of taste cells for acetic acid are composed of ion channel and pump components (Okada et al. 1993. Sato et al. 1995). When the reversal potential of 1 mM acetic acid-induced responses was measured, the tongue was adapted for 3 min to a frog Ringer containing 0.1 mM N,N′-dicyclohexylcarbodiimide to block the pump component and rinsed with the same Ringer after the acid stimulation. The flow rate of stimulating and rinsing solutions was 0.05 ml/s.

Chemical

A popular gap junction blocker 18β-glycyrrhetinic acid (β-GA) (Sigma-Aldrich) was used (Böhmer et al. 2001; Takeda et al. 2005). β-GA shows a low toxicity (Davidson and Baumgarten 1988). The stock solution was prepared with dimethyl sulfoxide (DMSO). Aliquots of the stock solution were added into the frog Ringer solution to obtain desired concentrations. β-GA solutions were injected intravenously (i. v.). The veins used were the precaval vein and postcaval vein.

There are numerous gap junction channels between the myocardial fibers (Ganong 2005). The heart rate of frogs was 58 ± 2/min (N = 12) in control, but decreased to 50 ± 2/min (N = 12) 3 h after intravenous injection of β-GA at 1.0 mg/kg which was used in most of the experiments. All the experiments were conducted for 3 h after injection of β-GA.

Statistics

All data were expressed as means ± standard errors of means (SEMs). The level of significance was set at $P < 0.05$ at a Student's *t*-test. In some experiments Fisher's exact-test was used at the significance level of $P < 0.05$.

Results

Relationship between β-GA Concentration and Membrane Resistance

As revealed in the previous investigation (Sato et al. 2007), the vertical penetration of a microelectrode across the taste disk induced three step-potential changes (arrows in Fig. 1). The first step-potential shows the penetration of the electrode into a Ia cell. The second and third step-potentials

exhibit the penetration into a Ib cell and a II/III cell, respectively (Fig. 1). The vertical hyperpolarizing pulses induced by constant current pulses indicate the amplitudes of membrane resistances in each cell. Fig. 2 illustrates the relationships between the concentration of β-GA injected i. v. and the amplitude of input resistances in Ia, Ib and II/III cells. The input resistance increased with increasing doses of β-GA and reached mostly the maximum at 1.0 mg/kg body weight. No significant increase was seen in the membrane resistance following a further increase in the dose of β-GA ($P > 0.05$, $N = 14\text{-}25$). The amplitudes of increase in membrane resistances at 1.0 mg/kg of β-GA were 50 MΩ in Ia cells, 160 MΩ in Ib cells and 300 MΩ in II/III cells.

Almost all the experiments described below were carried out after intravenous injection of β-GA at 1.0 mg/kg.

Time Course of Membrane Resistance Change in Single Taste Disk Cells

While a microelectrode penetrated a single taste disk cell, the time course of change in the input resistance was measured following intravenous injection of β-GA at 1.0 mg/kg. As shown in an example recorded from a Ib cell (Fig. 3a), the membrane resistance gradually increased during 5-6 min following application of β-GA. This process was due to a gradual diffusion of β-GA released from the capillary vessels underneath the taste disk of a fungiform papilla (Jaeger and Hillman 1976). Fig. 3b, c

and d show step-like changes in the input resistance of Ia, Ib and II/III cells after injection of β-GA at 1.0 mg/kg. Only three step-changes of input resistance occurred in a Ia cell but 5 and 7 step-changes appeared in a Ib and a II/III cell.

Gap junction channels and hemichannels exist in cluster in a small area of the cell membrane (Loewenstein 1981; Bennett et al. 2003; Evans et al. 2006). A cluster of the channels and hemichannels might be closed together by diffusing β-GA molecules. The amplitudes of membrane resistances increased by closing each cluster were 14 ± 2 MΩ (N = 4) in Ia cells, 33 ± 2 MΩ (N = 4) in Ib cells and 41 ± 3 MΩ (N = 4) in II/III cells. The number of clusters of gap junctions, which was counted from the number of steps in step-like changes in the membrane resistance, was 2.4 ± 0.3 (N = 4) in Ia cells, 4.3 ± 0.3 (N = 4) in Ib cells and 6.3 ± 0.5 (4) in II/III cells. The conduction time at which β-GA molecules moved between gap junction clusters was measured from the depth of steps in step-like changes in the membrane resistance. These times were 121 ± 4 s (N = 4) in Ia cells, 69 ± 6 s (N = 4) in Ib cells and 41 ± 5 s (N = 4) in II/III cells.

Resting Potential

Fig. 4 illustrates the resting potentials of Ia, Ib and II/III cells before and after injection of various doses of β-GA. No significant changes in resting potentials occurred in any taste disk cells in the

absence and presence of β-GA ($P > 0.05$, N = 31-83, in any doses of β-GA).

Reversal Potential

After β-GA was injected i. v. at 1.0 mg/kg, the reversal potentials for 1 M NaCl, 1 mM acetic acid, 10 mM Q-HCl and 1 M sucrose were measured in Ia, Ib and II/III cells. Depolarizing potentials of any cells for tastants reached slowly to the peak, maintained the plateau level and fell down slowly to the base line after wash-out (Figs 5a and 7a). Fig. 5b-e exemplify the relationships between the membrane potential levels and the amplitudes of depolarizing and hyperpolarizing potentials for 4 basic stimuli in 4 Ib cells. In Fig. 5a are shown a gradual decrease in depolarizing potentials and the reversal into a hyperpolarizing potential when the membrane potential of a Ib cell was shifted towards the positive direction and stimulated by 1 M NaCl. These data are plotted in Fig. 5b. The reversal potentials in control and β-GA-treated disk cells for the 4 basic stimuli are listed in Table 1. The data in parentheses are control values without β-GA of reversal potentials in Ia, Ib and II/III cells. No differences were found among reversal potentials in all cells for each taste stimulus before and after β-GA was injected i. v. at 1.0 mg/kg ($P > 0.05$, N = 3-7).

Decrease of Depolarizing Response

Fig. 6 shows the amplitudes of depolarizing responses of Ia (a), Ib (b) and II/III cells (c) before and after intravenous injection of β-GA at 1.0 mg/kg. The large response amplitudes for 1 M NaCl and 1 mM acetic acid were reduced to 50-60% following application of β-GA. The small amplitudes for 10 mM Q-HCl and 1 M sucrose were also reduced to 40% and 50% respectively after β-GA.

Membrane Resistance Change

With control and β-GA-injected tongues, changes in the membrane resistance of taste disk cells elicited by 4 basic tastants were compared. Fig. 7a illustrates recordings of changes in the membrane resistance of a II/III cell during taste stimulation after an intravenous injection of β-GA at 1.0 mg/kg. The membrane resistances which were measured from vertically deflected hyperpolarizing pulses greatly decreased during 1 M NaCl stimulation, but slightly decreased during stimulation with 1 mM acetic acid and 1 M sucrose. The resistance was slightly increased during stimulation with 10 mM Q-HCl. Fig. 7b, c and d show membrane resistance changes of Ia (b), Ib (c) and II/III cells (d) for 4 tastants before (control) and after an injection of β-GA at 1.0 mg/kg. In the control and test values the membrane resistance changes are expressed as (membrane resistance during gustatory stimulation)/(membrane resistance at rest) x 100. In any cell types the membrane resistances were

decreased during stimulation with 1 M NaCl, 1 mM acetic acid and 1 M sucrose, but increased during stimulation with 10 mM Q-HCl. The degrees of membrane resistance changes in any types of taste disk cell were not different between control and β-GA-treated cells ($P > 0.05$ in any types of stimuli and cells).

Gustatory Response Profile

Fig. 8 illustrates the percentage of taste disk cells responding to each of 4 basic stimuli in control and β-GA (1.0 mg/kg)-injected tongue. The response percentage for 1 M NaCl and 1 mM acetic acid was 90-100% in any types of cell in the control and β-GA-treated tongue. The response percentage for 10 mM Q-HCl in all cell types was 40-50% in control and β-GA-injected tongue. However, the percentage for 1 M sucrose was 40-50% in Ia and Ib cells, but 70% in II/III cells in either tongue. In each cell type, the response percentage pattern for 4 basic stimuli was similar in control and β-GA-injected tongues (Fisher's exact-test, $P > 0.05$ in each cell type). Fig. 9 shows the percentage of cells responding to varying numbers of 4 taste qualities in Ia (a), Ib (b) and II/III cells (c) in control and β-GA treated tongue. In any cells the response percentage was largest for 3 taste qualities and smallest for one quality. The response percentage pattern for the number of taste qualities was similar in any cells of the control and β-GA- injected tongue (Fisher's exact-test, $P > 0.05$). Fig. 10 shows the

depolarizing response profile of 18 II/III cells for 4 basic taste stimuli in β-GA- injected tongue. This response profile pattern was very similar to that of the control cells excepting the reduced response amplitudes (Sato et al. 2008). The gustatory response profiles of 14 Ia and 22 Ib cells in β-GA injected tongue for 4 basic taste stimuli were also very similar to those of control Ia and Ib cells (Sato et al. 2008) (data not shown).

Discussion

The membrane resistance of a cell is composed of fixed resistance and variable resistance. One of the deficiencies in the microelectrode method is to make a leakage resistance when the electrode is inserted into a cell. The part of fixed resistance is connected in parallel to the leakage resistance, so the input resistance of taste disk cells is smaller than that measured with the patch clamp method (Miyamoto et al. 1991; Okada et al. 1994). However, changes in membrane resistance are exactly recorded by the microelectrode method as shown in Figs 1, 2 and 7. A cluster of gap junction channels and hemichannels exist in a small area of the cell membrane (Loewenstein 1981; Evans et al. 2006). When gap junction blocker molecules are released from blood capillaries under the taste disk of the fungiform papillae, the dotted clusters of gap junction channels and hemichannels in taste disk cells are gradually blocked by a movement of the blocker molecules. The increase in the resistances was 3

times in Ia cells, 6 times in Ib cells and 7 times in II/III cells of each control. The dye-coupling studies show that a few neighboring Ia cells are connected to a Ia cell through the gap junction channels (Takeuchi et al. 2001; Suwabe and Kitada 2004), but no cell types are connected to Ib and II/III cells (Takeuchi 2001; Suwabe and Kitada 2004). Investigation of an increase in membrane resistances with single disk cells provides that the mean number of clusters of gap junction channels is 2 in Ia cells and that of clusters of the hemichannels is 4 in Ib cells and 6 in II/III cells. The time course of membrane resistance changes in single cells (Fig. 3) indicates that the mean conduction time at which the gap junction blocker molecules pass between two gap junction hemichannel (channels in Ia cells) clusters is 121 s in Ia cells, 69 s in Ib cells and 41 s in II/III cells. If the conduction distance between the gap junction clusters is estimated to be 5-15 μm, the conduction velocity of β-GA molecules moving through the complex, barrer-rich extracellular space of in situ taste disk is as slow as 0.2 μm/s. The mean increase in membrane resistance by closing the hemichannel or channel cluster is 14 MΩ in Ia, 33 MΩ in Ib and 41 MΩ in II/III cells. The ratio of the resistance of the gap junction hemichannels (or channels) in a cluster is Ia : Ib : II/III = 1.0 : 2.4 : 2.9.

The resting potentials in taste disk cells after intravenous injection of various concentrations of β-GA were the same as the controls. The apical receptive and basolateral membranes of taste receptor cells at rest in the frog show a high permeability to Na^+ and K^+ (Okada et al. 1986). Therefore, the same resting potentials in control and β-GA-treated taste disk cells suggest that the permeability of

the apical and basolateral membranes to Na^+ and K^+ is stationary before and after β-GA-induced blockage of ion movement through the gap junction pathways. Stationary situation of the resting potential following treatment with β-GA has been found in hepatocytes (Böhmer et al. 2001) and myocytes (Takeda et al. 2005).

Most of the present experiments were carried out with taste disk cells after intravenous injection of β-GA at 1.0 mg/kg. This value corresponds to 50 μM in blood which was calculated from the injected amount of β-GA (1.0 mg/kg) and 4% plasma volume in frogs (Thorson 1964). We can calculate tastant-induced depolarizing currents passing through the taste disk cells with Figs 2, 6 and 7. In control II/III cells the depolarizing currents are 470 pA for 1 M NaCl, 230 pA for 1 mM acetic acid, 70 pA for 10 mM Q-HCl and 70 pA for 1 M sucrose. The depolarizing currents in control Ib cells and control Ia cells for each of 4 basic taste stimuli were approximately 2 and 3.5 times larger than in the control II/III cells, respectively. The depolarizing currents of 50 μM β-GA-treated cells induced by each of 4 basic taste stimuli were decreased to 7-10% of control in II/III cells, 8-11% in Ib cells and 13-17% in Ia cells. In any types of 50 μM β-GA-treated taste disk cells, the amplitude of decrement in depolarizing currents was larger than the amplitude of increment in input resistances. This indicates that most of the outward components of tastant-induced depolarizing currents in control disk cells flow through the gap junction channels and hemichannels.

Gustatory response profiles of Ia, Ib and II/III cells for 4 basic stimuli of 1 M NaCl, 1 mM

acetic acid, 10 mM Q-HCl and 1 M sucrose are very similar in the control and 50 mM β-GA-treated cells in that the response percentage pattern for each of 4 basic stimuli as well as the number of taste qualities is similar in the control and blocker-treated cells. Although β-GA is an excellent gap junction blocker (Davidson and Baumgarten 1988), the side effects on various cells appear dose-dependently. β-GA at 30-40 μM weakly depresses L-type Ca^{2+} current, Cl^- current, Ca^{2+}-activated K^+ current and delayed rectifier K^+ current in various cells (Böhmer 2001; Takeda et al. 2005; Guan et al. 2007). In the present studies, it was estimated that 85-90% of tastant-induced depolarizing currents in control taste disk cells were suppressed in 50 μM β-GA-treated disk cells. If 50 μM β-GA remarkably depresses specific ion channels for gustatory transduction of a certain stimulus, gustatory response percentage of disk cells responding to 4 basic taste stimuli and various numbers of taste qualities (Figs 8 and 9) may differ between control and β-GA-treated disk cells. This was not the case. Therefore, 50 μM β-GA effects on ion channels of taste disk cells are supposed to be weak. Approximately 90% of II/III cells at the lower part of intermediate layer of taste disk in frogs are composed of type III cells in the apical and middle loci of tongue (Osculati and Sbarbati 1995; Li and Lindemann 2003), so it is suggested that 16 cells of 18 II/III cells examined (Fig. 10) are III cells and the other two are II cells.

The role of gap junction channels and hemichannels is to transmit signaling molecules and electrical currents in a cell into the neighboring cells. These molecules and currents control the functions in the adjacent cells such as membrane permeability, excitability, metabolism and cell

division (Loewenstein 1981; Sáez et al. 2003; Evans et al, 2006). In this study we estimated the transmission and its blockage of tastant-induced depolarizing responses in a taste disk cell into adjacent disk cells.

The tastant-induced depolarizing response in a Ia cell is transmitted into neighboring Ia cells via gap junction channels. The amplitude of transmitted depolarizing response will be reduced. Application of various concentrations of β-GA will further decrease dose-dependently the amplitude of transmitted depolarizing response. The tastant-induced depolarizing responses in a Ib and a III cell will be directly transmitted into an adjacent cell of the same type only when the gap junction hemichannels of two Ib and two III cells are very closely contacted. However, this possibility is low. The gustatory depolarizing responses induced in a II cell may not be transmitted to another II cell because of its dotted distribution. However, depolarizing response in a II cell is possibly transmitted into adjacent III cells via a signaling substance such as ATP, as suggested in mammalian taste bud (Huang et al. 2007).

In mammalian taste bud, type II cells are directly stimulated by some taste quality (bitter, sweet and umami) stimuli. On the other hand, type III cells are directly stimulated by the other taste quality (salty and sour) stimuli and also indirectly excited via ATP released from the gap junction hemichannels of type II cells. This implies that type III cell depolarizing responses are composed of directly induced responses of type III cells themselves and indirectly induced responses from type II cells. Therefore, the depolarizing response profiles elicited by basic taste stimuli are quite different

between types II and III cells.

In frog taste disk, depolarizing response profile of II/III cells (mostly regarded as III cell group in this study) was the same between control and β-GA-treated cells. This is explained by two possibilities. One is that no indirect responses via ATP from II cells are induced in III cells of the frogs. The other possibility is that depolarizing responses in II cells induced by 4 basic taste stimuli are carried to III cells via ATP from II cells, but the own response profiles of II cells induced by 4 taste stimuli may be the same as those of III cells. This results in the same depolarizing response profile in III cells of the control and β-GA injected tongue. Taste response profile of II cells themselves in frogs must be clarified.

The present study suggests that the conduction velocity of signaling molecules such as ATP through barrier-rich extracellular space of in situ taste disk in the frog is 0.2 μm/s. Conduction time at which ATP molecules pass a minimal distance of ~1 μm between II and III cell is estimated to be 5 s. Usually the latency of gustatory neural impulses induced by strong taste stimuli in frogs is 200-500 ms (Sato et al. 1987). The latency of 5 s in III cell responses is extraordinarily longer, so the generation of depolarizing responses in III cells via signaling molecules is questionable.

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Figure Legends

Fig. 1 Three step-potential changes of resting membrane potentials in taste disk cells by a microelectrode after intravenous injection of β-GA. Left arrow shows penetration of microelectrode into a Ia cell, and middle and right arrows show that into a Ib and a II/III cell. Vertically deflected potential pulses denote amplitudes of input resistances of cells. β-GA was injected at 1.0 mg/kg.

Fig. 2 Relationships between doses of β-GA and amplitudes of input resistances in taste disk cells. β-GA was injected i. v. at doses of 0.2-2.0 mg/kg body weight. Vertical bars in this and other figures denote SEM and numerals number of cells tested. Control input resistance was 24 ± 2 MΩ (N = 65) in

Ia cells, 34 ± 3 MΩ (N = 65) in Ib cells and 50 ± MΩ (N = 65) in II/III cells.

Fig. 3 Time course of input resistance changes in taste disk cells following injection of β-GA. **a** Continuous recording of input resistance changes in a Ib cell following intravenous injection of β-GA at 1.0 mg/kg. **b-d** Time courses of input resistances in Ia **(b)**, Ib **(c)** and II/III cell **(d)** following β-GA injection. At time 0 β-GA was injected at 1.0 mg/kg.

Fig. 4 Relationship between doses of β-GA and resting potentials in taste disk cells. Numerals above squares are number of taste disks tested.

Fig. 5 Relationships between membrane potentials and gustatory responses induced by taste stimuli in taste disk cells following β-GA injection. **a** Change in 1 M NaCl-induced depolarizing potential in a Ib cell by altering membrane potential. **b-e** Relationships between amplitude of membrane potentials and amplitude of taste stimulus-induced responses in 4 Ib cells. **(b)** 1 M NaCl. **(c)** 1 mM acetic acid. **(d)** 10 mM Q-HCl. **(e)** 1 M sucrose. Data in **a** are plotted in **b**. β-GA was injected at 1.0 mg/kg.

Fig. 6 Amplitudes of gustatory depolarizing responses of taste disk cells before (control) and after injection of β-GA. **a** Ia cells. **b** Ib crls. **c** I/III cells. Taste stimuli were 1 M NaCl (N), 1 mM acetic acid (A), 10 mM Q-HCl (Q) and 1 M sucrose (S). β-GA was injected at 1.0 mg/kg.

Fig. 7 Changes in membrane resistances of taste disk cells in control and β-GA -injected tongue induced by gustatory stimulation. **a** Recording of membrane resistance changes of a II/III cell by 4 basic taste stimuli. **b** Ia cell. **c** Ib cell. **d** II/III cell. Taste stimuli: 1 M NaCl (N), 1 mM acetic acid (A),

10 mM Q-HCl (Q), 1 M sucrose (S). β-GA was injected at 1.0 mg/kg.

Fig. 8 Percentage of taste disk cells responding to each of four basic stimuli before (control) and after β-GA injection. **a** Ia cells. **b** Ib cells. **c** II/III cells. Taste stimuli: 1 M NaCl (N). 1 mM acetic acid (A), 10 mM Q-HCl (Q), 1 M sucrose (S). β-GA was injected at 1.0 mg/kg. Control data in Ia and Ib cells were from Sato et al. (2008). Number of cells tested was 11 Ia, 11 Ib and 31 II/III cells in control tongues, and 14 Ia, 22 Ib and 18 II/III cells in β-GA-injected tongues.

Fig. 9 Percentage of taste disk cells responding to various numbers of 4 basic taste qualities before (control) and after β-GA injection. **a** Ia cells. **b** Ib cells. **c** II/III cells. Taste stimuli: 1 M NaCl (N), 1 mM acetic acid (A), 10 mM Q-HCl (Q), 1 M sucrose (S). β-GA was injected at 1.0 mg/kg. All percentage patterns in Ia, Ib and II/III cells were obtained using same data described in Fig. 8.

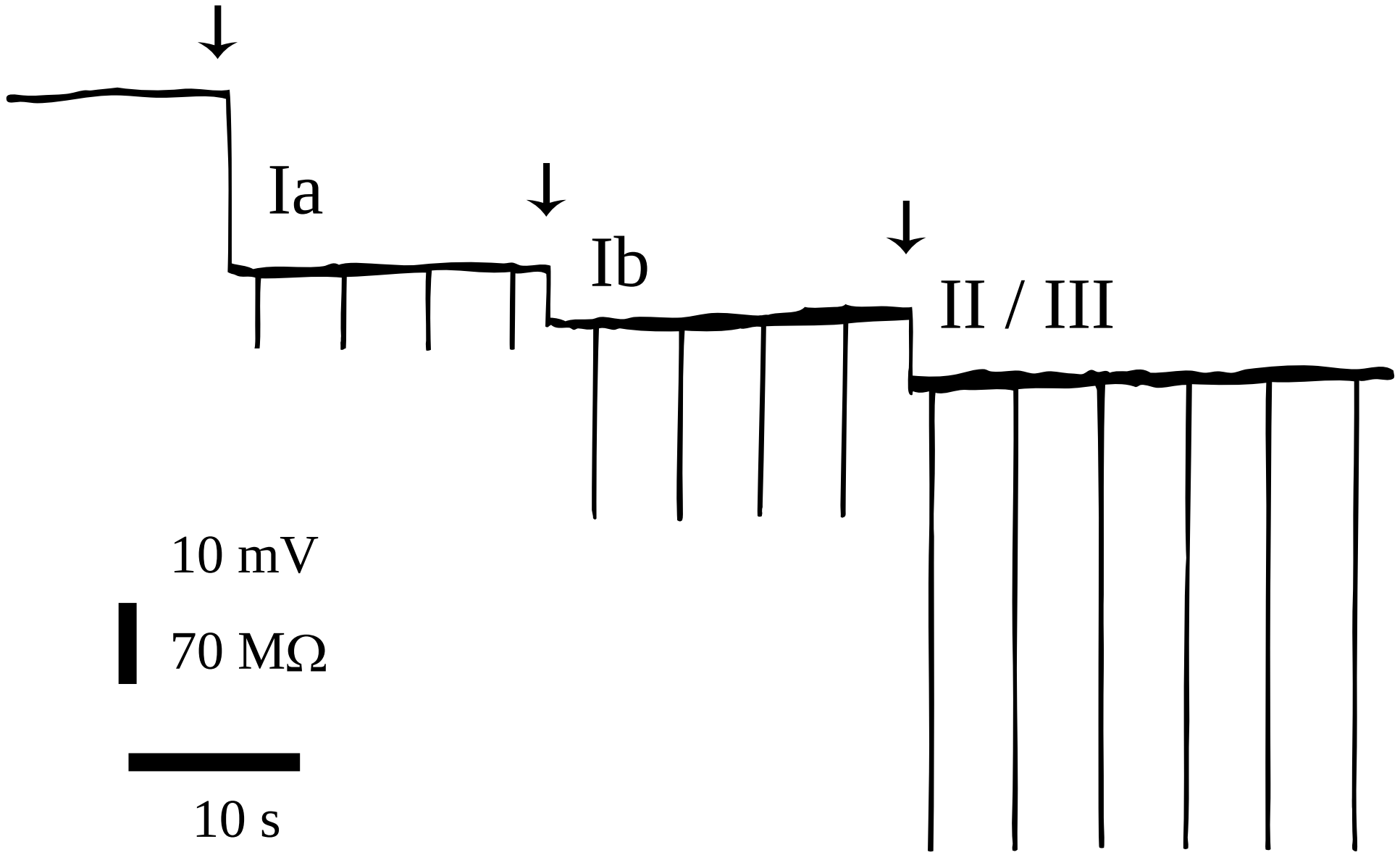
Fig. 10 Gustatory response profile of β-GA-treated II/III cells for 4 basic taste qualities. Taste stimuli: 1 M NaCl, 1 mM acetic acid, 10 mM Q-HCl and 1 M sucrose. β-GA was injected at 1.0 mg/kg.

Table 1 Reversal potential of taste disk cells for 4 basic taste stimuli

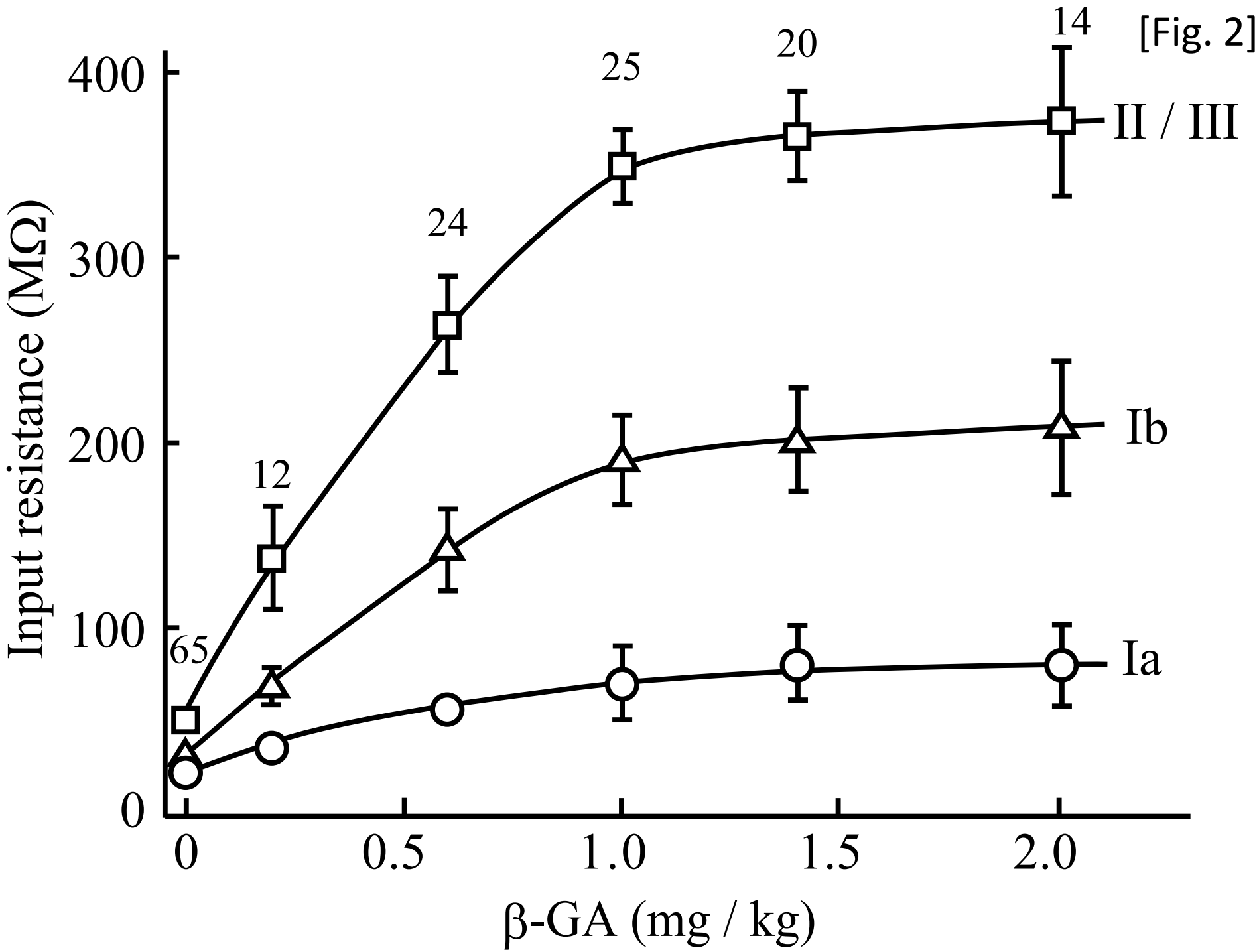
Cell	1 M NaCl mV (*N*)		1 mM acetic acid mV (*N*)		10 mM Q-HCl mV (*N*)		1 M sucrose mV (*N*)	
Ia	21 ± 4	(3)	81 ± 7	(3)	∞	(3)	20 ± 4	(5)
	(21 ± 3)	(3)	(82 ± 4)	(4)	(∞)	(3)*	(11 ± 8)	(5)*
Ib	24 ± 4	(4)	83 ± 7	(3)	∞	(3)	18 ± 5	(3)
	(23 ± 2)	(3)	(86 ± 3)	(5)	(∞)	(3)*	(13 ± 6)	(3)*
II/III	29 ± 3	(3)	89 ± 6	(3)	∞	(5)	15 ± 2	(4)
	(23 ± 4)	(3)	(88 ± 5)	(7)	(∞)	(2)*	(12 ± 5)	(3)*

Data (means ± SEMs) in parentheses are obtained from taste disk cells in control preparations. Data above controls were obtained from β-GA-injected preparations (1.0 mg/kg). Values marked by * were from control data in previous study (Sato et al. 2008). ∞ denotes that reversal potential was not measurable.

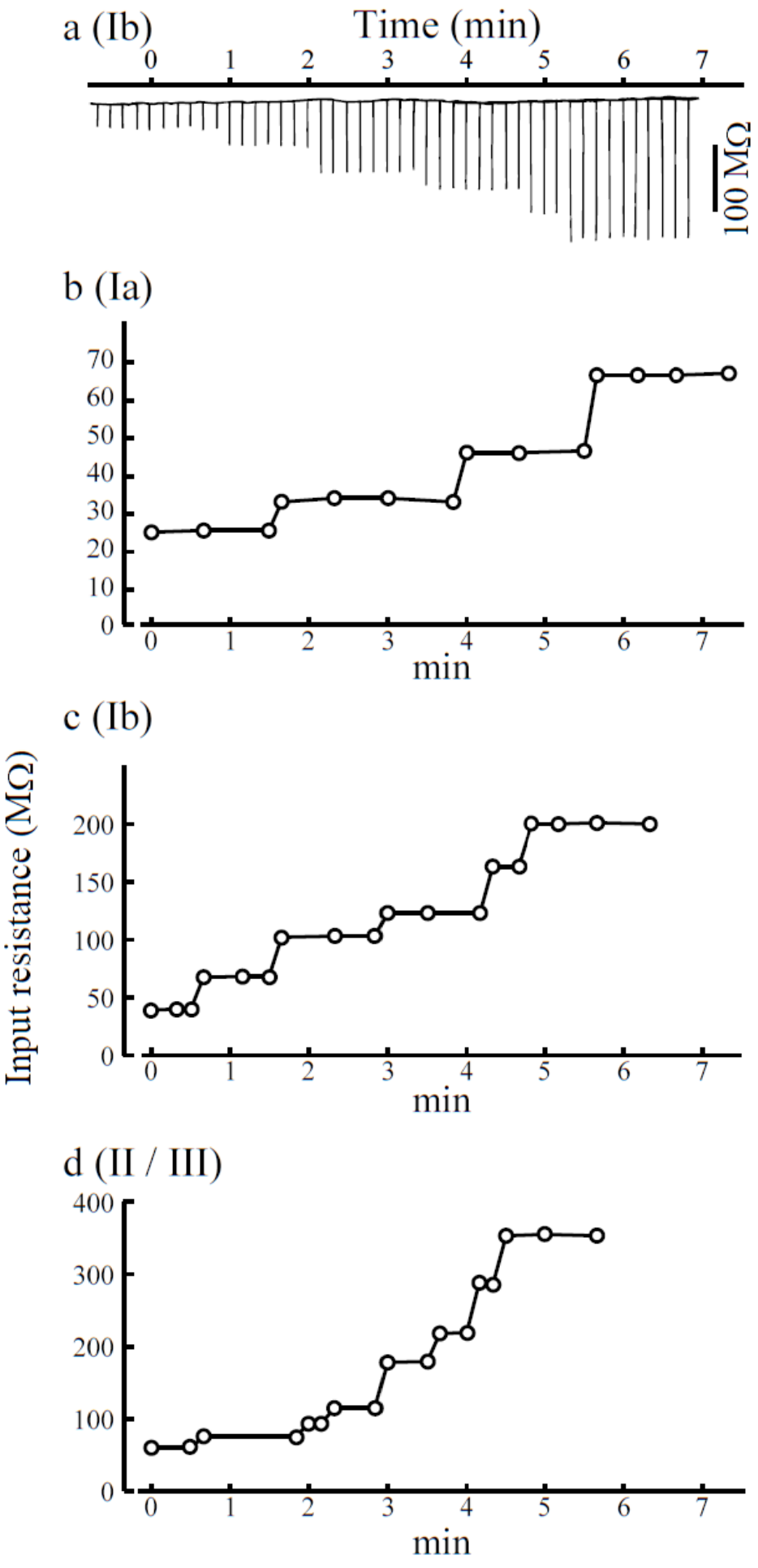
[Fig. 1]



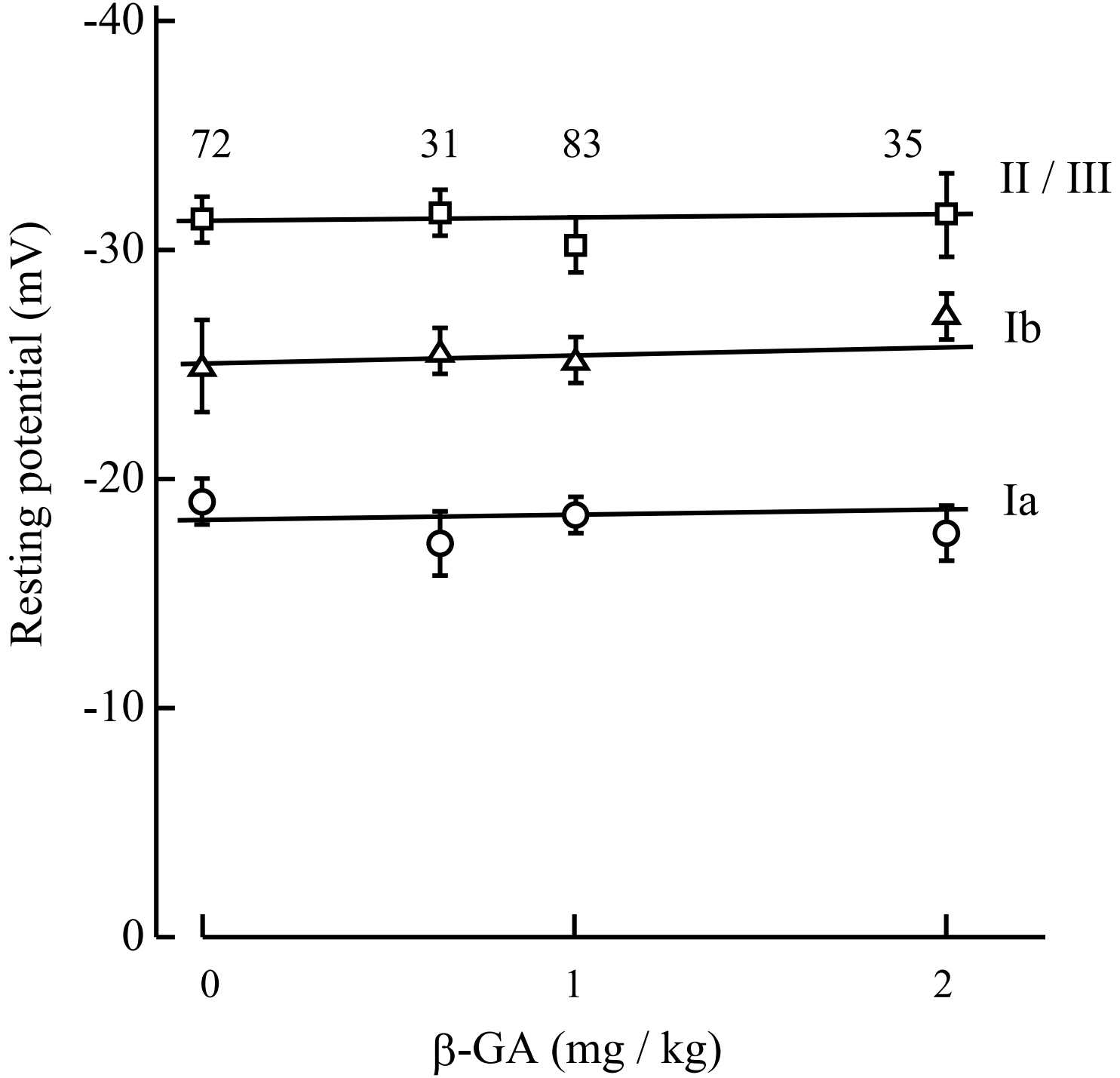
[Fig. 2]


Input resistance (MΩ)
400
300
200
100
0
0
0.5
1.0
1.5
2.0
β-GA (mg / kg)
65
12
24
25
20
14
II / III
Ib
Ia

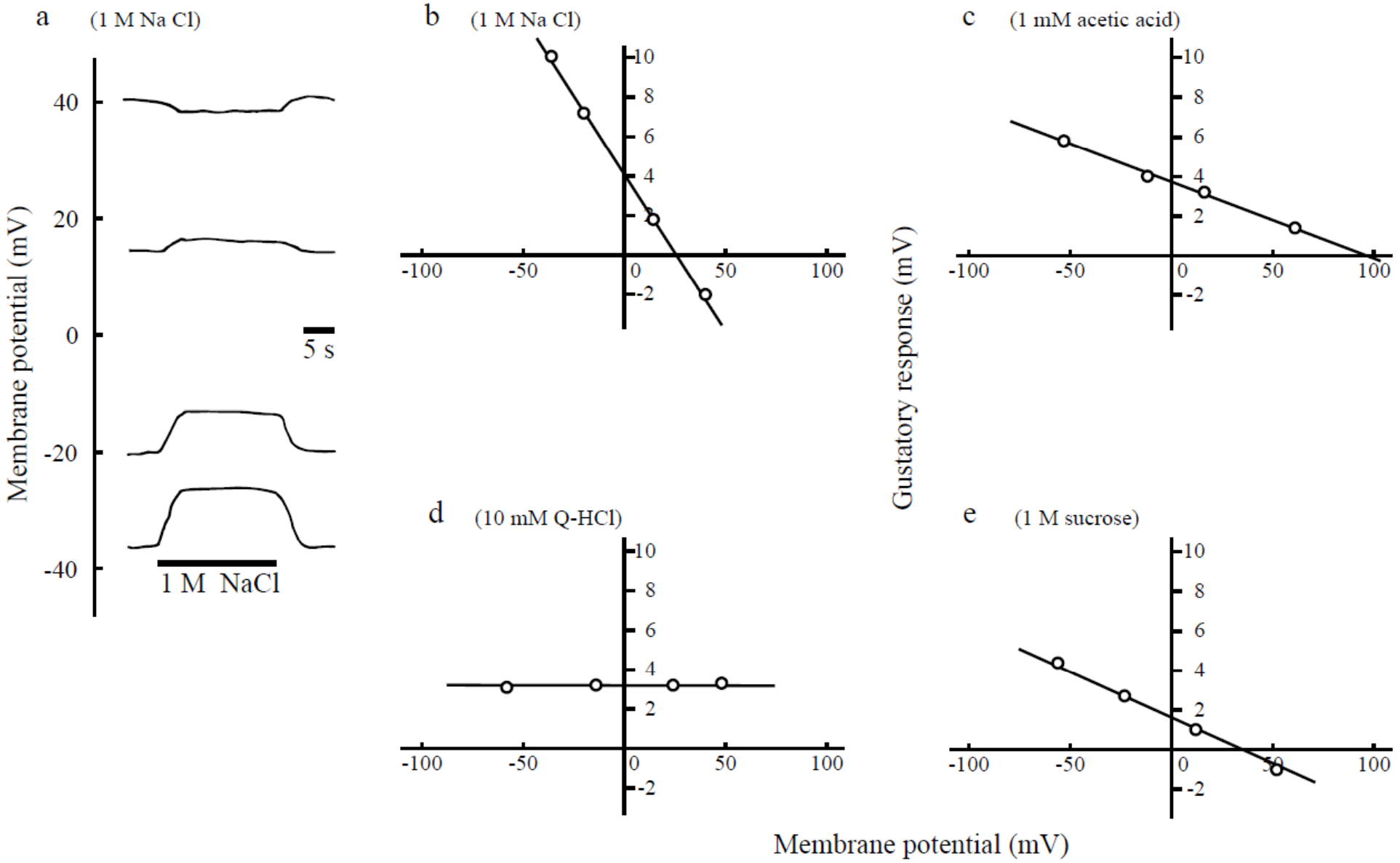
[Fig. 3]


a (Ib)
Time (min)
0 1 2 3 4 5 6 7
100 MΩ
b (Ia)
70 60 50 40 30 20 10 0
0 1 2 3 4 5 6 7
min
c (Ib)
Input resistance (MΩ)
200 150 100 50 0
0 1 2 3 4 5 6 7
min
d (II / III)
400 300 200 100 0
0 1 2 3 4 5 6 7
min

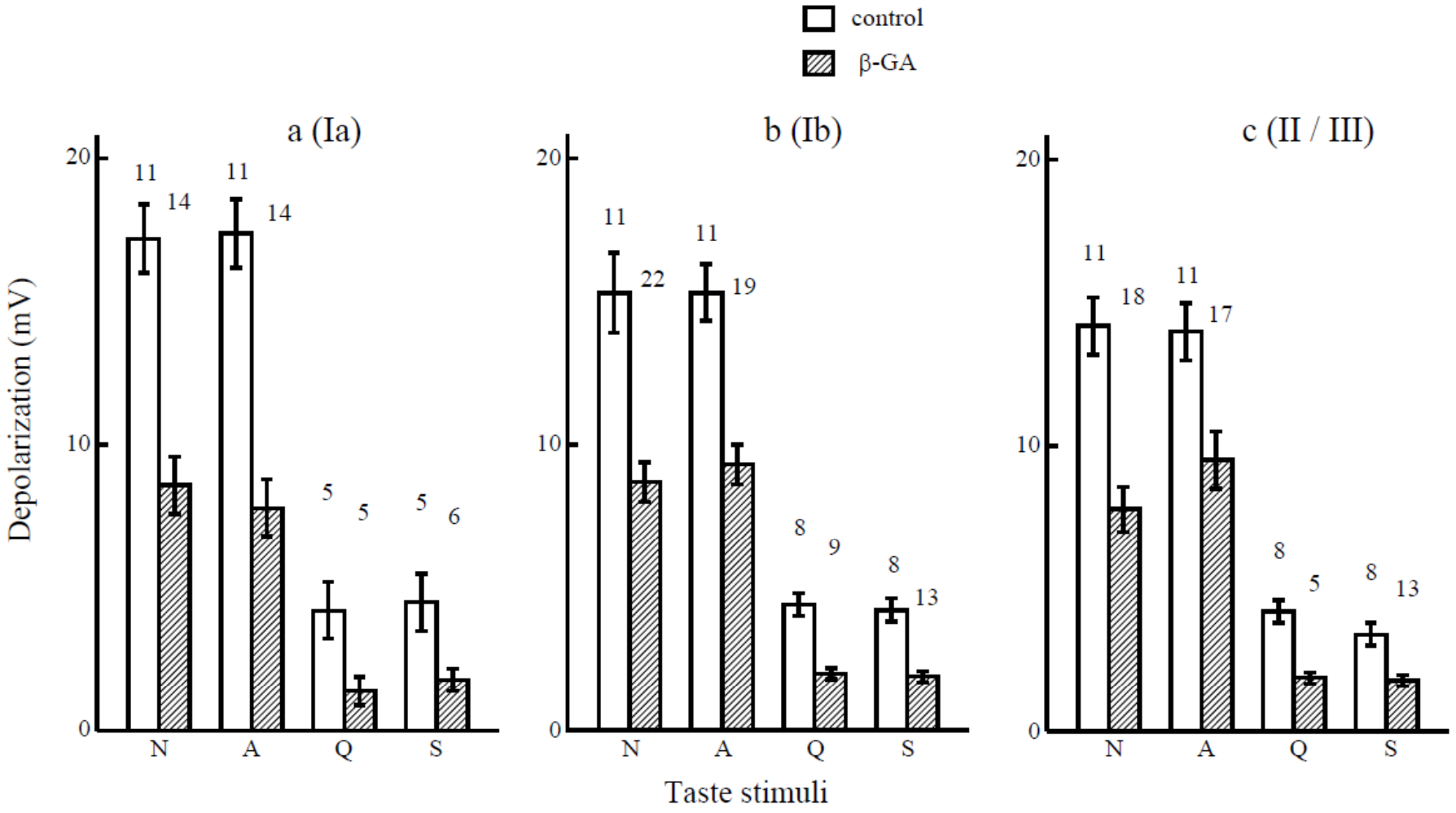
[Fig. 4]


-40
-30
-20
-10
0
Resting potential (mV)
72
31
83
35
II / III
Ib
Ia
0
1
2
β-GA (mg / kg)

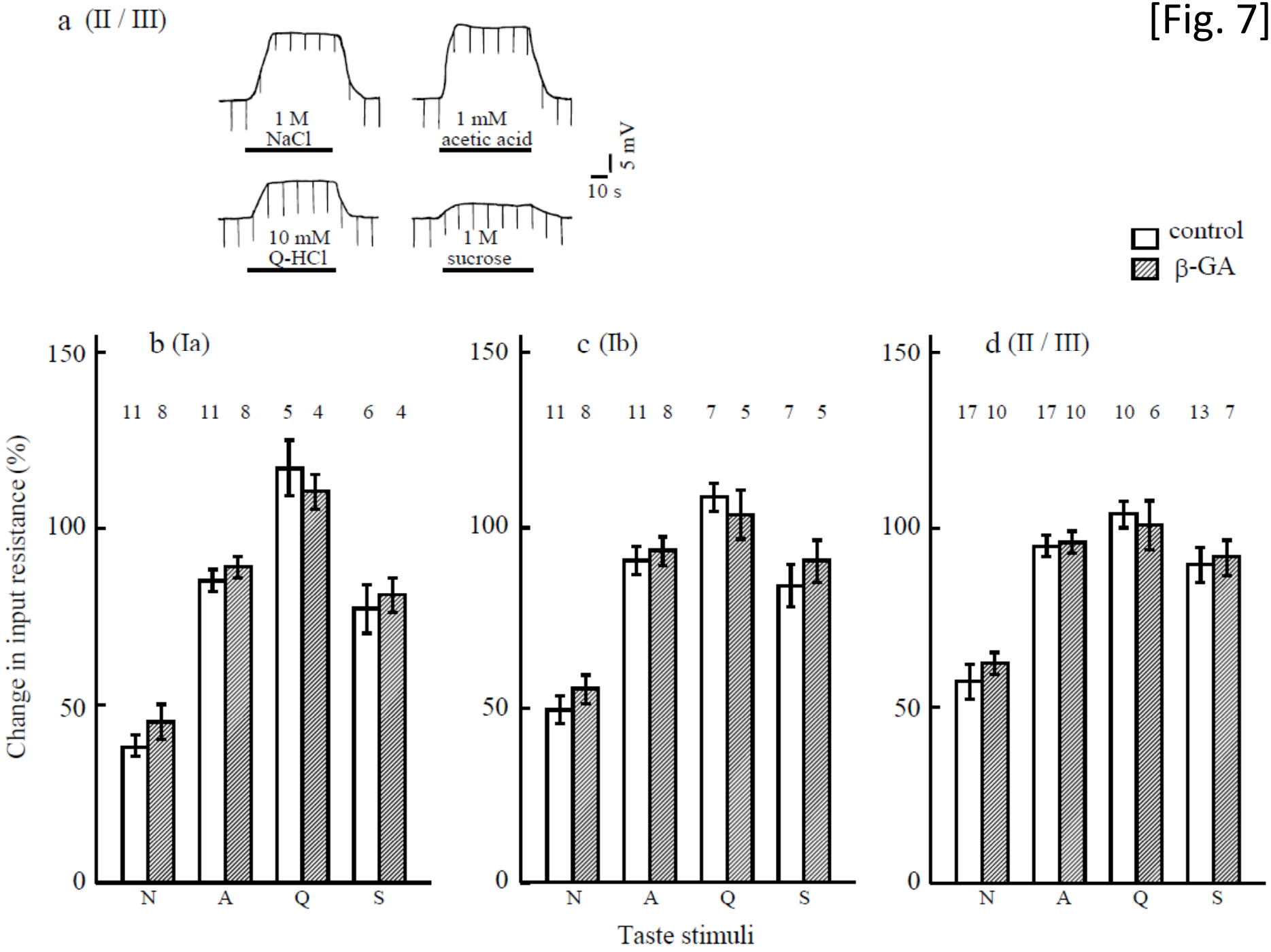
[Fig. 5]


a
(1 M Na Cl)
40
20
0
-20
-40
Membrane potential (mV)
5 s
1 M NaCl
b
(1 M Na Cl)
c
(1 mM acetic acid)
d
(10 mM Q-HCl)
e
(1 M sucrose)
10
8
6
4
2
-2
-100
-50
0
50
100
Gustatory response (mV)
Membrane potential (mV)

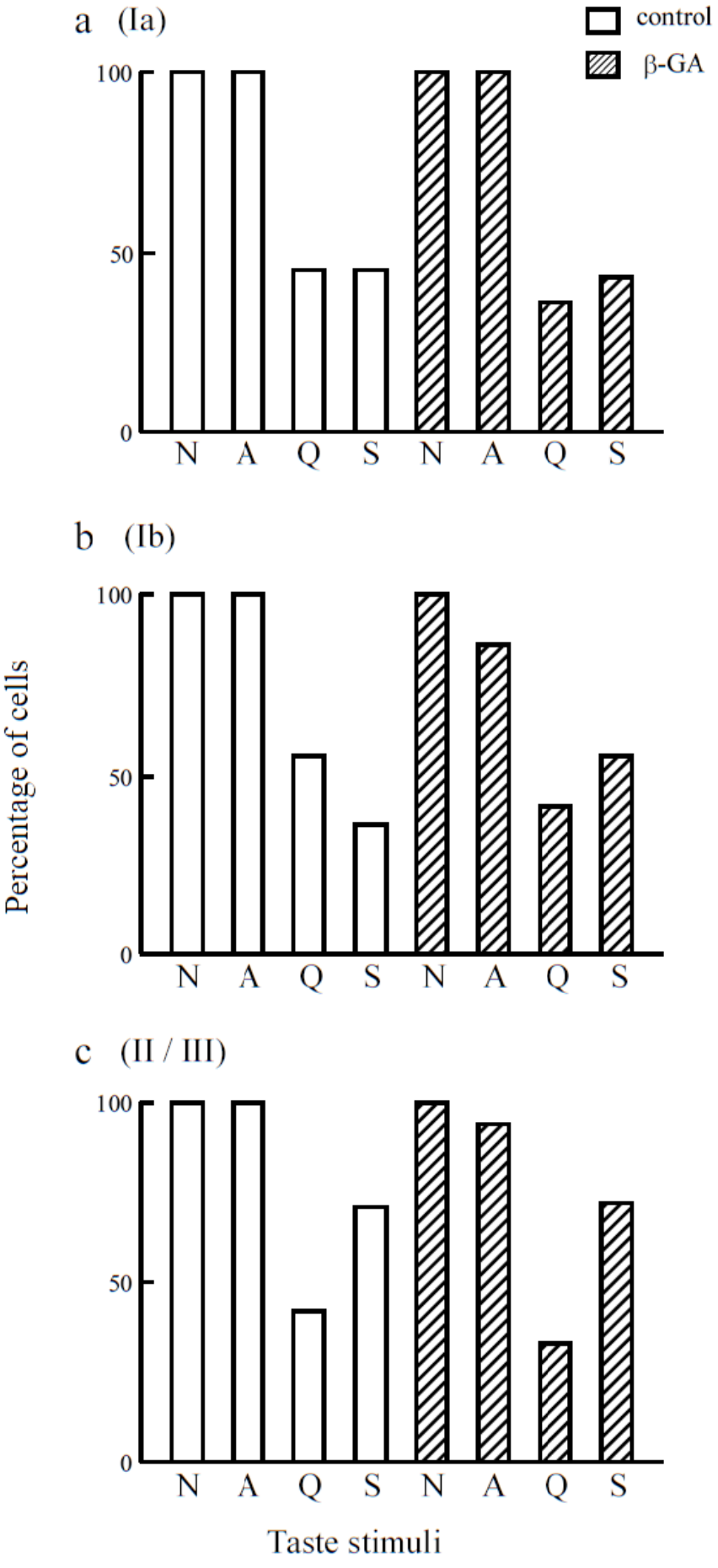
[Fig. 6]


control
β-GA
a (Ia)
b (Ib)
c (II / III)
Depolarization (mV)
20
10
0
11
14
11
14
5
5
5
6
11
22
11
19
8
9
8
13
11
18
11
17
8
5
8
13
N
A
Q
S
Taste stimuli

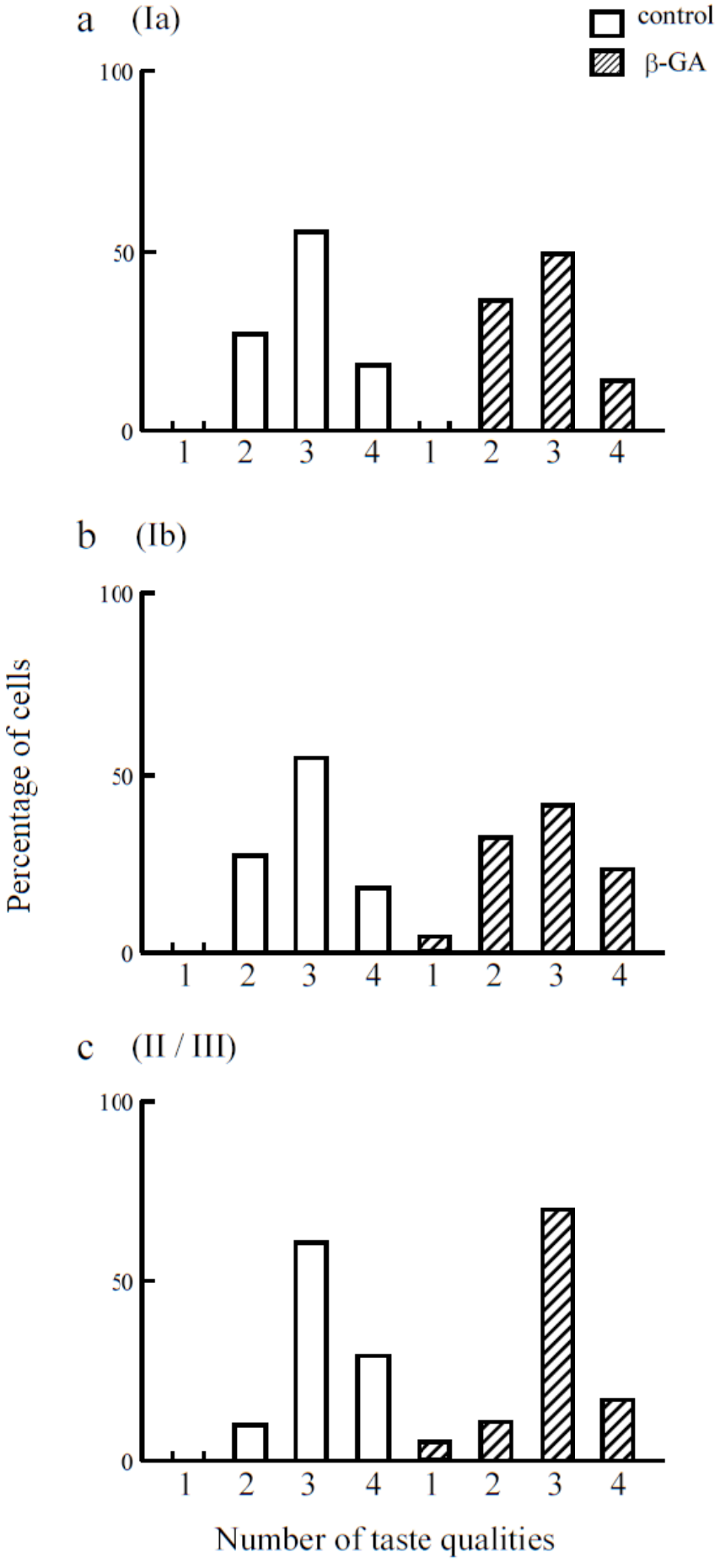
[Fig. 7]


a (II / III)
1 M NaCl
1 mM acetic acid
10 mM Q-HCl
1 M sucrose
5 mV
10 s
control
β-GA
b (Ia)
c (Ib)
d (II / III)
Change in input resistance (%)
150
100
50
0
11 8 11 8 5 4 6 4
11 8 11 8 7 5 7 5
17 10 17 10 10 6 13 7
N A Q S
Taste stimuli

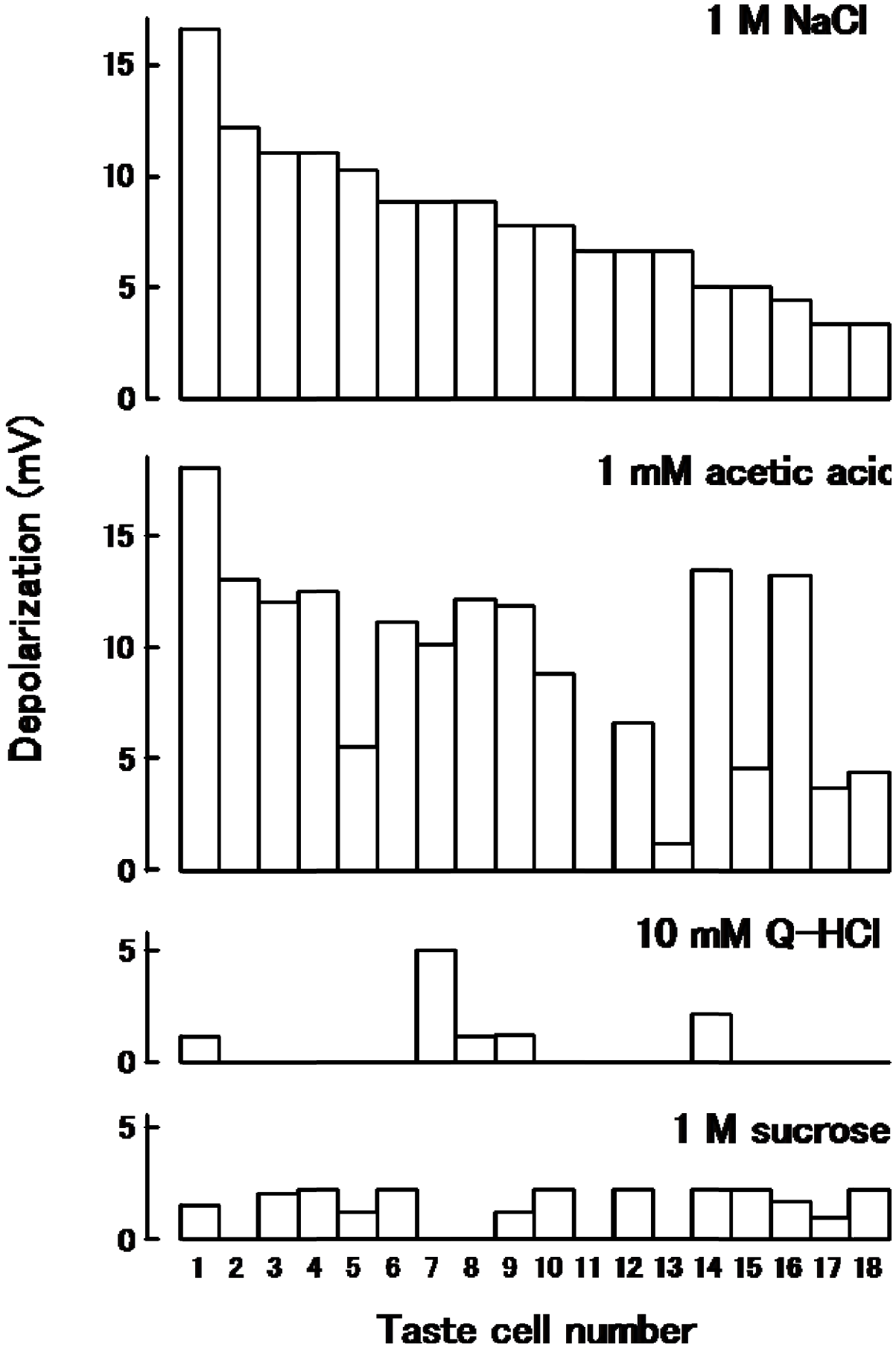
[Fig. 8]


a (Ia)
control
β-GA
100
50
0
N A Q S N A Q S
b (Ib)
Percentage of cells
100
50
0
N A Q S N A Q S
c (II / III)
100
50
0
N A Q S N A Q S
Taste stimuli

[Fig. 9]


a (Ia)
control
β-GA
100
50
0
1 2 3 4 1 2 3 4
b (Ib)
100
50
0
1 2 3 4 1 2 3 4
Percentage of cells
c (II / III)
100
50
0
1 2 3 4 1 2 3 4
Number of taste qualities

[Fig. 10]


1 M NaCl
1 mM acetic acid
10 mM Q-HCl
1 M sucrose
Depolarization (mV)
0
5
10
15
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Taste cell number