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Chemical Stimulation of the Thalamic Reticular Nucleus Inhibits the Neuronal Activity of the Posterior Insular Cortex in Rats

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Abstract

Extracellular neuronal responses were recorded from the posterior insular cortex following electrical and chemical stimulation of the thalamic reticular nucleus (Rt) regions. In the present study, most neurons (29/32) were first characterized for their responses to electrical stimulation of the superior laryngeal (SL) nerve or glossopharyngeal (IXth) nerve. In the first experiment, 15 neurons in the posterior insular cortex were examined for their responses to electrical stimulation of the Rt regions. It was found that effective stimulation sites to evoke action potentials in the posterior insular cortex were the ventromedial portion of the Rt and its adjacent regions. In the second experiment, 17 neurons in the posterior insular cortex were examined for their responses by pressure injection of glutamate (Glu) into the Rt regions. Of the 17 neurons, 13 were inhibited in the spontaneous discharge rate following injection of Glu into the Rt, and the remaining four were unaffected. Histologically, it was demonstrated that Glu injection sites for the case of inhibition were located near or within the Rt. On the other hand, the injection sites for all four non-responsive neurons were located outside of the Rt. These data suggest that excitation of the Rt (GABAergic neurons) causes depression of the neuronal activity in the thalamic relay nucleus and then this may in turn induce depressed neuronal activity in the posterior insular cortex. The results here indicate that neuronal activity in the posterior insular cortex is controlled by the Rt, which has been reported in other sensory systems.

Key words: posterior insular cortex, taste, thalamic reticular nucleus, visceral

Introduction

The anterior and posterior portions of the insular cortex are considered to be a cortical taste area and a visceral sensory area, respectively (Yamamoto *et al.*, 1980b; Cechetto and Saper, 1987, 1990; Ogawa *et al.*, 1990). We have reported that the neurons in the posterior insular cortex (posterior to the region where the chorda tympani projects) respond to gustatory, visceral, and nociceptive stimuli (Hanamori *et al.*, 1997a,b, 1998a,b). However, the functions and neuronal connections in the insular cortex are not still fully understood.

The Rt, which lies in the outer peripheral region of the thalamus, receives inputs from the branched axons of the thalamic relay nucleus, which project into the cortex, and also from branches of the descending axons of the cortical neurons (for a review, see Guillery *et al.*, 1998). Since neurons in the Rt are mostly GABAergic (Houser *et al.*, 1980) and send axons to the thalamus (Ohara and Lieberman, 1985), it is assumed that the Rt has important roles in controlling the neuronal activity in the cortex (Guillery *et al.*, 1998). It has been reported that the Rt receives various sensory inputs: somatosensory (Shosaku *et al.*, 1984); visual (Hale *et al.*, 1982); and auditory (Shosaku and Sumitomo, 1983). Recently, similar neuronal connections between the thalamic relay nucleus, Rt, and the insular cortex have been demonstrated histologically for the visceral (Stehberg *et al.*, 2001) and taste systems (Hayama *et al.*, 1994). Therefore, there is a possibility that neuronal activity in the insular cortex may be controlled by the Rt. However, no electrophysiological studies have been conducted to reply to this issue.

A few electrophysiological studies have shown that electrical stimulation of the insular cortex has an effect (mainly inhibitory) on neuronal activity in the taste thalamic relay nucleus (Yamamoto *et al.*, 1980a; Ogawa and Nomura, 1988). There is a possibility that this depression may be induced via activation of the GABAergic neurons in the Rt.

In the present study, extracellular unit activity of the neurons in the posterior insular cortex was recorded using a glass microelectrode; the activity was characterized for its responses to electrical stimulation of the SL or IXth nerve.

In the first experiment, electrical stimulation of the brain at around Rt was employed to determine the location of the Rt (visceral related region in the Rt). Action potentials of the neurons in the posterior insular cortex evoked by electrical stimulation at the Rt or its adjacent region were further examined whether they are antidromic or orthodromic. In the second experiment, the effects of a Glu (an excitatory transmitter) injection into the Rt on the neuronal activity in the posterior insular cortex were examined.

Materials and methods

Animals and surgery

Experiments were performed on 33 Sprague–Dawley male rats weighing between 220 and 600 g (mean = 328 ± 15 g). Animals were initially anesthetized with an i.p. injection of a mixture of urethane (1 g/kg) and alpha chloralose (0.1 g/kg). The trachea was cannulated for artificial ventilation. The left femoral artery and vein were cannulated for the measurement of blood pressure (BP) and the administration of drugs, respectively.

The right SL nerve was dissected from the surrounding connective tissue and transected for electrical stimulation. In some experiments, the right IXth nerve was dissected and transected for electrical stimulation. The hypoglossal nerves (bilateral) were transected to prevent inadvertent tongue movements. For stimulation and recording from the brain, the head of each animal was fixed in a stereotaxic frame and a 4–5 mm square hole was made on the rightside of the skull around the bregma.

During the recordings, each animal was anesthetized and paralyzed by tubocurarine (2 mg/kg, i.v.) and artificially ventilated using a Harvard respiratory pump. We used paralyzed rats in the present study for the following reasons. First, it is important to keep the neurons isolated and stationary during recordings. Secondly, the effect of respiration on neuronal activity in the posterior insular cortex should be excluded. Whenever paralysis seemed to wear off (~60 min), the level of anesthesia was assessed by pinching the tail. If required, supplemental doses of a mixture of urethane and alpha chloralose were administered. The end-expired CO_2 was constantly monitored and maintained at 4–4.5%. Rectal temperature was maintained at 37–38°C by a thermostatically regulated heating pad.

Stimulation

For electrical stimulation of the peripheral nerve, the central portion of the dissected SL nerve or the IXth nerve was placed on a pair of platinum wire electrodes. The nerves were stimulated with a train of rectangular pulses at 12 V (0.5 ms duration, n = 1–5, at 500 Hz). This stimulus intensity is enough strength for excitation of A-delta fibers in the SL nerve or the IXth nerve (Hanamori *et al.*, 1996).

A tungsten microelectrode insulated except at the tip (resistance, 50–300 kΩ) was used for electrical stimulation of the brain. The electrode was impaled from the dorsal surface of the brain (a reference electrode was connected to the skin of the neck). The stimulus was given with a rectangular pulse of duration 0.01–0.2 ms and amplitude 0.01–0.2 mA.

For chemical stimulation of the Rt, a glass micropipette (tip diameter, ~50 μm) containing Glu (0.5–1 M) was used. The glass micropipette was glued to the tungsten electrode (the tip of the glass micropipette was positioned within 100 μm posterior to the tip of the tungsten electrode) and impaled from the dorsal surface of the brain. Chemical stimulation sites were first identified, where the action potentials or compound action potentials in the posterior insular cortex could be evoked by electrical stimulation at around the Rt regions using the tungsten electrode. The glass micropipette was connected to the PicoPump (WPI, PV830) for pressure ejection of Glu (13–20 PSI; pressure time, 50–100 ms; ejection volume, ~250 nl, calculated from the movement of the meniscus of the Glu solution within the glass micropipette under the lens of a surgical microscope). Glu was dissolved in 0.9 % NaCl.

Recording

After surgery the rat was mounted in the stereotaxic frame. Glass microelectrodes (3–5 MΩ) filled with 2% pontamine sky blue in 0.5 M sodium acetate were used for recording from the posterior insular cortex (in some cases, stainless electrodes of 1–2 MΩ were used). Neural activity was amplified (WPI, M-701, USA) and displayed on an oscilloscope (VC-10, band-pass 0.1–3 kHz; Nihon Khoden, Japan). Neurons recorded were first characterized for their responses to electrical stimulation of the SL or IXth nerve.

Instantaneous BP in the femoral artery and heart rate (HR) obtained from pulsation of the blood pressure were amplified using a conventional amplifier and recorded on an eight-channel pen recorder.

Neuronal activity was amplified and displayed on an oscilloscope. Neural discharges were fed into a pulse counter and instantaneous changes in the number of the impulses were continuously recorded on an eight-channel pen recorder. All data of neuronal activity in the posterior insular cortex and BP and HR during recording were also stored in the computer and later analyzed using a data analyzing system (UAS-1; Unique Medical, Japan).

Data analysis

The responses of neurons isolated in the posterior insular cortex were defined as excitatory if the mean number of impulses/s over a 6 s period after chemical stimulation was significantly higher than that over a 30 s period before stimulation and inhibitory if the mean number of impulses was significantly lower (two-tailed Student's t-test, $P < 0.05$). The duration of the depression of the neuronal activity of the posterior insular cortex was estimated as the time between the beginning of the depression (leaving from the baseline) and the end of the depression (reaching to the base-

line). The degree of maximum depression (%) for each sample was defined as a relative value: $(1 - r) \times 100$; r was obtained by dividing the mean number of spikes/s over 6 s in the most depressed phase by the mean number of spikes over 30 s before stimulation.

Histology

At the end of the experiment, the location of the recording site was marked by iontophoretical ejection of the dye from the tip of the recording microelectrode (0.01 mA, 5 min) or was lesioned electrically by passing a cathodal current from the tip of the stainless electrode (0.1 mA, 1.5 min). The location site of chemical stimulation (also stimulated electrically) was lesioned electrically by passing the cathodal current from the tip of the stimulating tungsten electrode (0.1 mA, 1.5 min). The brains were then removed and fixed with 10% formalin. Sections (50 μm) were cut on a freezing microtome and stained with neutral red. In the present study, the anterior edge of the joining of the anterior commissure (APo) was adopted as a standard zero point in the anterior–posterior axis.

Results

Antidromic and orthodromic responses in the posterior insular cortex to electrical stimulation of the Rt and adjacent areas

Fifteen neurons (15 rats, one neuron from each rat) in the posterior insular cortex were found to elicit the action potentials following electrical stimulation of the Rt and internal capsule (*ic*; see Figure 1A,B, lower records). Most of the neurons (12/15) were characterized for their responsiveness to electrical stimulation of the SL nerve (10 neurons) or the IXth nerve (two neurons; see Figure 1A,B, upper records). In many cases, burst responses were produced following electrical stimulation of the SL or IXth nerve. The mean latency of the first spike, which was produced following an electrical stimulation of the SL nerve, was 56.7 ± 10.0 ms (n = 10), and that of the IXth nerve was 28.9 ms (n = 2).

The train pulse stimulation and the collision test were employed to examine whether the action potentials in the posterior insular cortex neuron produced by electrical stimulation of the Rt were antidromic or orthodromic. Responses for eight neurons were found to be antidromic and those for seven were orthodromic. Figure 2 shows a representative record of a neuron showing antidromic action potentials by electrical stimulation of the Rt. This neuron showed an action potential to electrical stimulation of the Rt at a stimulus duration of ≥0.03 ms (stimulus current; 0.05 mA; Figure 2A, asterisk). The action potential (Figure 2B, asterisk; at delays of 3 and 4 ms; stimulus duration, 0.03 ms) disappeared when electrical stimulation was given with a shorter delay of ≤2 ms from the preceding spontaneous spike; this shows a collision between spontaneous spike and an evoked-action potential (Figure 2B, interval; 1 and 2 ms).

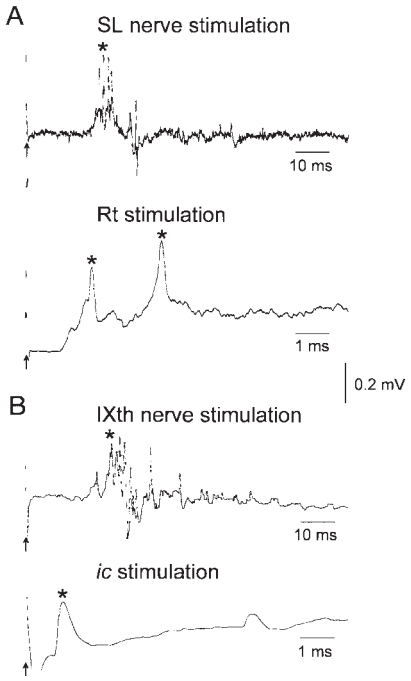

Figure 1 Responses of two neurons (A) and (B) in the posterior insular cortex to electrical stimulation of the peripheral nerves and brain. **(A)** Sample of a neuron responsive to both SL nerve (upper) and Rt (lower) stimulation. The spikes (asterisks) induced by Rt stimulation were identified as an orthodromic response. All records presented in this and following figures show single records. Recording site: 0.9 mm anterior to the APo. Stimulation site: 2.8 mm posterior to the APo. **(B)** Sample of a neuron responsive to both IXth nerve (upper) and *ic* (lower) stimulation. The spike (asterisk) induced by *ic* stimulation was identified as an antidromic response. Recording site: 1.4 mm anterior to the APo. Stimulation site: 2.5 mm posterior to the APo. The intensity for stimulation of the SL or IXth nerve was 12 V (0.5 ms duration). The intensity for stimulation of the Rt and the *ic* was 0.1 mA (duration of 0.06 ms for the Rt and 0.01 ms for the *ic*). Arrows indicate the time of the stimulation. Abbreviations: Rt, thalamic reticular nucleus; *ic*: internal capsule; APo, anterior edge of the joining of the anterior commissure; SL nerve, superior laryngeal nerve; IXth nerve, glossopharyngeal nerve. As shown in (A) and (B), many neurons in the posterior insular cortex show the burst responses to electrical stimulation of the SL or IXth nerve.

Figure 3 shows a representative record of a neuron showing orthodromic action potentials. This neuron shows an action potential at a stimulus duration of 0.02 ms, but it did not respond to a stimulus duration of 0.01 ms (stimulus current, 0.05 mA; Figure 3A). However, an action potential was produced by electrical stimulation at a stimulus duration of 0.01 ms with double pulses at intervals of 1 ms (Figure 3B). Furthermore, an evoked-action potential was induced by electrical stimulation at a stimulus duration of 0.01 ms when electrical stimulation was triggered by the preceding spontaneous spike with a delay of 1 ms (Figure 3C, upper). At a stimulus intensity of 0.02 ms, the response

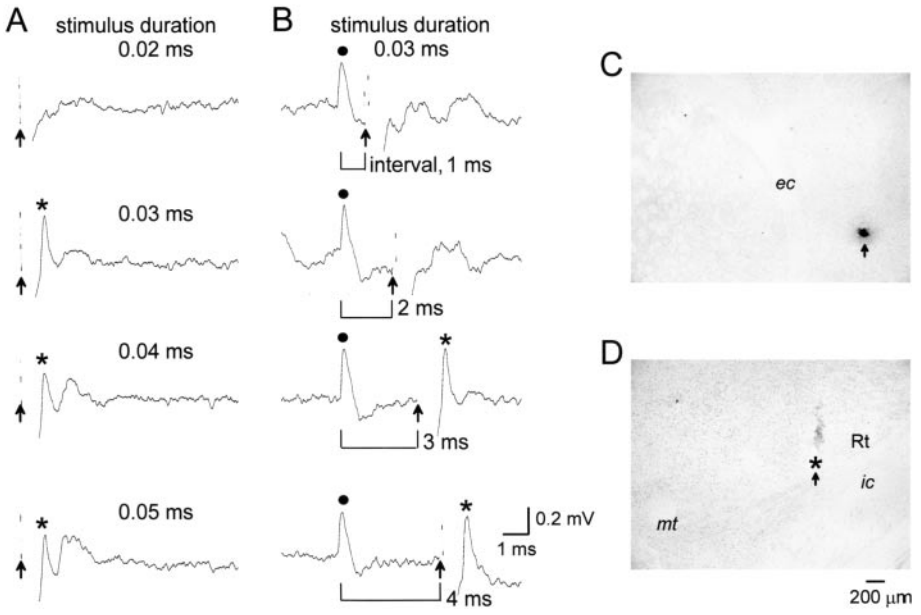

Figure 2 Responses of a neuron in the posterior insular cortex to electrical stimulation of the Rt. This neuron showed an antidoromic response to Rt stimulation. **(A)** Responses of a neuron to Rt stimulation at various intensities (duration from 0.02 to 0.05 ms at 0.05 mA). A spike (asterisk) was evoked by Rt stimulation at intensities above 0.03 ms. **(B)** Stimulation (arrows; stimulus duration, 0.03 ms) was triggered by a preceding spontaneous spike (filled circles) at various delays from 1 to 4 ms. The action potential was not evoked at delays of 1 and 2 ms; collision of spontaneous (filled circles) and antidromic spikes caused the elimination of the antidromic spike. **(C)** Photomicrograph showing the recording site (arrow) (coronal section at 1.5 mm anterior to the APo). **(D)** Photomicrograph showing the location of the stimulation site (arrow; coronal section at 2.8 mm posterior to the APo). Abbreviations: Rt, thalamic reticular nucleus; *ic*, internal capsule; *ec*, external capusule; *mt*, mammillothalamic tract.

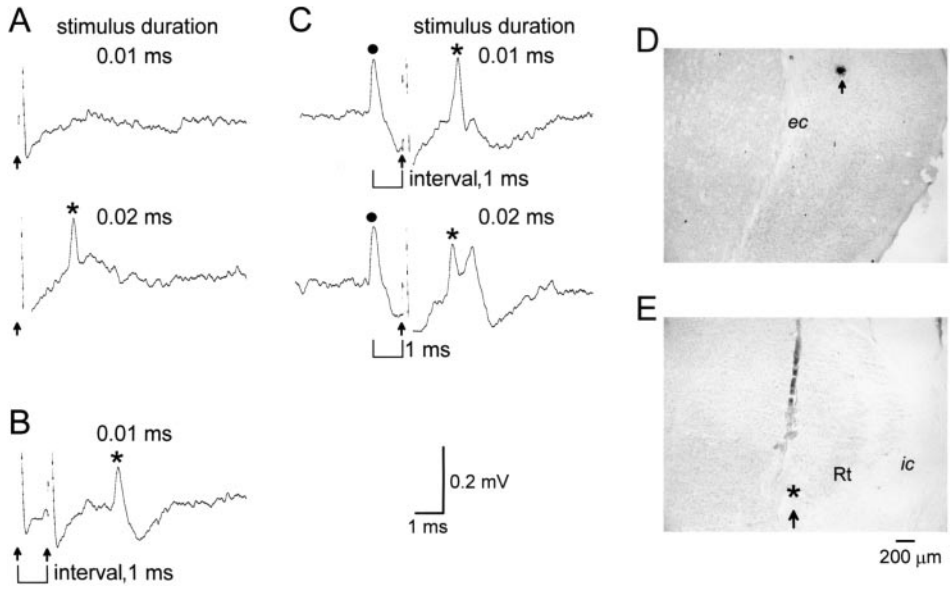

Figure 3 Responses of a neuron in the posterior insular cortex to electrical stimulation of the Rt. This neuron showed an orthodromic response to Rt stimulation (0.05 mA). **(A)** A spike (asterisk) was evoked by Rt stimulation at intensities of 0.02 ms (lower), but no response was evoked at a stimulation of 0.01 ms (upper). **(B)** A spike (asterisk) was evoked by a double-pulse stimulation (interval of 1 ms) of 0.01 ms. **(C)** Upper: the action potential was evoked by a stimulation of 0.01 ms when stimulation was triggered by the preceding spontaneous spike (filled circle; 1 ms delay). Lower: two action potentials were evoked by a stimulation of 0.02 ms when stimulation was triggered by the preceding spontaneous spike (1 ms delay). These results indicate that the action potentials of this neuron evoked by Rt stimulation occurred via synapse(s). **(D)** Photomicrograph showing the recording site (arrow; coronal section at 0.7 mm anterior to the APo). **(E)** Photomicrograph showing the location of the stimulation site (arrow; coronal section at 2.7 mm posterior to the APo). Abbreviations: Rt, thalamic reticular nucleus; *ic*, internal capsule; *ec*, external capusule.

was facilitated by the preceding spontaneous action potential of 1 ms (Figure 3C, lower). The mean latency (peak) for an antidromic response was 1.31 ± 0.11 ms (n = 8), ranging from 0.9 to 1.8 ms and that for an orthodromic response was 4.56 ± 1.20 ms (n = 7), ranging from 1.8 to 10 ms. The mean latency of the antidromic response was significantly shorter than that of the orthodromic response (two-tailed Student's t-test, $P < 0.05$).

The mean location of the recording sites was 0.91 ± 0.14 mm anterior to the APo (n = 15, ranging from 0.1 to 1.7 mm), 5.28 ± 0.09 mm lateral to the midline (n = 15, ranging from 4.8 to 6.0 mm) and 3.29 ± 0.61 mm depth from the dorsal surface (n = 15, ranging from 3.5 to 5.2 mm; see Figure 5, left panel).

The mean location of the stimulation sites was 2.71 ± 0.13 mm posterior to the APo (n = 15, ranging from 1.7 to 3.5 mm), 3.45 ± 0.14 mm lateral from the midline (n = 15, ranging from 2.7 to 4.2 mm) and 6.53 ± 0.15 mm depth from the dorsal surface of the brain (n = 15, ranging from 5.5 to 7.5 mm; see Figure 5, right panel).

Effective electrical stimulation sites in the Rt

Figure 4A shows the compound action potentials and field potentials of a neuron in the posterior insular cortex (0.8 mm anterior to the APo; recording site shown in Figure 4C) evoked by electrical stimulation at various depths in the brain (records 1–10; locations shown in Figure 4B; 2.6 mm posterior to the APo; stimulus current and duration, 0.05 mA and 0.08 ms, respectively). In this neuron, the affected stimulation sites for the evoked response were localized in a small area, namely, the ventral part of the Rt (record; 4 in Figure 4A; location, 4 in Figure 4B). Figure 5 shows plots of all (15) recorded insular sites (left panel) and stimulated Rt sites (right panel) on the coronal sections of the brain. Eight of 15 neurons showing an antidromic response, seven were found to be stimulated within the internal capsule (*ic*) and only one was found within the Rt (Figure 5, right panel; filled circles). The remaining seven neurons showing orthodromic responses were found to be stimulated within the Rt (Figure 5, right panel; open circles).

Chemical stimulation of the Rt and adjacent areas

The effects of chemical stimulation to the Rt on the neuronal activity in the posterior insular cortex were investigated in 17 rats (one neuron from each rat). The mean spontaneous activity of the neurons in the posterior insular cortex for the chemical stimulation study was 5.2 ± 1.4 spikes/s (n = 17, ranging from 0.1 to 16.4 spikes/s). Seventeen neurons in the posterior insular cortex were responsive to electrical stimulation of the SL nerve as well as to electrical stimulation of the Rt (Figure 6B) or its outside areas (Figure 8B). The mean latency of the first spike elicited following electrical stimulation of the SL nerve was 55.0 ± 7.5 ms (n = 17, ranging from 15.6 to 148.9 ms). The mean latency of the spike (or compound action potentials) evoked by the electrical stimu-

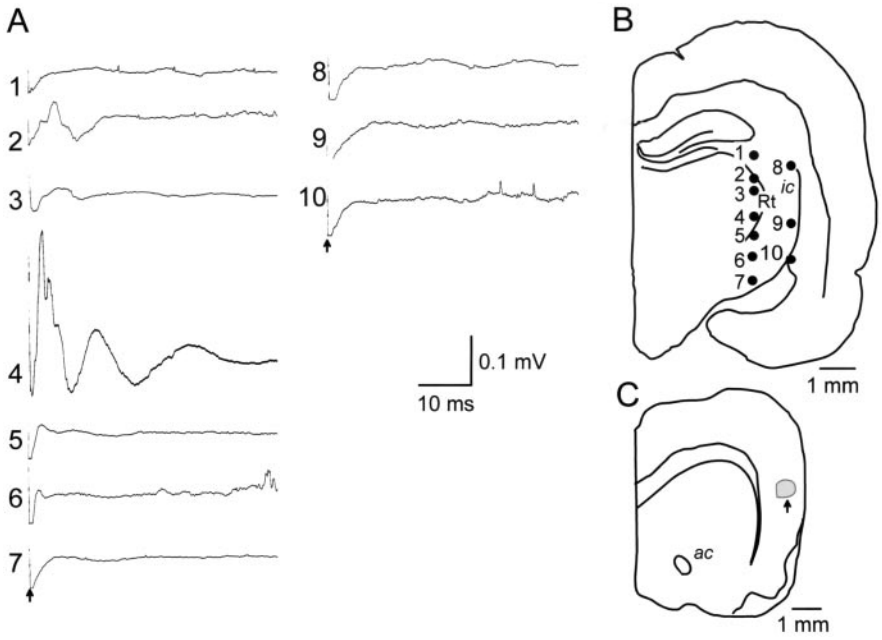

Figure 4 Compound action potentials and field potentials of a neuron in the posterior insular cortex **(A)** to electrical stimulation at various depths (1–10; locations are indicated in B). A remarkable response was obtained only by electrical stimulation at location 4 (ventral part of the Rt; see in B). **(B)** Locations of the stimulation sites. **(C)** Location of the recording site (arrow; marked by electric lesion using a stainless recording electrode). The locations of the recording and stimulation sites were 0.8 mm anterior and 2.6 mm posterior to the APo, respectively. The stimulus intensity was 0.08 mA of 0.05 ms duration. Abbreviations: Rt, thalamic reticular nucleus; *ic*, internal capsule; *ac*, anterior commissure.

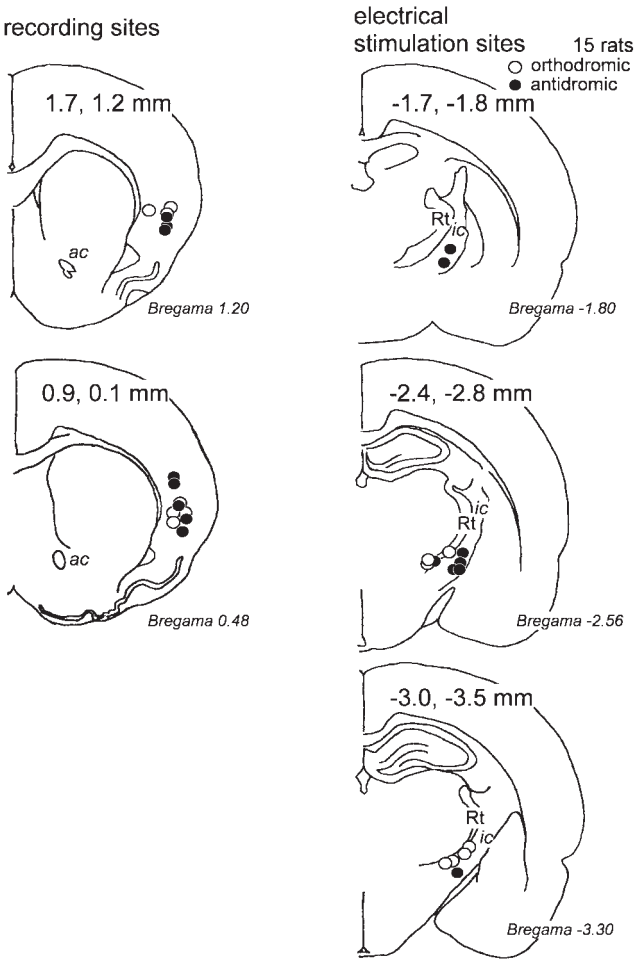

Figure 5 Location of recording (left) and electrical stimulation sites (right) for 15 rats. The recording and stimulation sites identified histologically are plotted on the coronal sections of the brain atlas of Paxinos and Watson (1986) from anterior (top) to posterior (bottom) levels (distances from the bregma are indicated in the bottom right area of each section). Numerals (upper central in each section) indicate the locations of the recording and stimulation sites in the range of the distance anterior or posterior to the APo. Filled or open circles indicate cases that showed antidromic or orthodromic responses, respectively. Abbreviations: Rt, thalamic reticular nucleus; *ic*, internal capsule; *ac*, anterior commissure.

lation of the Rt or its outside areas was 6.9 ± 2.4 ms (n = 17, ranging from 1.4 to 41.5 ms).

The mean location of the recording sites was 0.26 ± 0.11 mm anterior to the APo (n = 17, ranging from 0.8 to –0.3 mm), 5.87 ± 0.09 mm lateral to the midline (n = 17, ranging from 5.4 to 6.7 mm) and 4.27 ± 0.15 mm depth from the dorsal surface (n = 17, ranging from 3.4 to 5.2 mm; see Figure 9, left panel).

The mean location of the stimulation sites was 2.57 ± 0.10 mm posterior to the APo (n = 17, ranging from 1.7 to 3.4 mm), 3.09 ± 0.12 mm lateral from the midline (n = 17, ranging from 2.1 to 4.0 mm) and 6.24 ± 0.11 mm depth from the dorsal surface of the brain (n = 17, ranging from 5.5 to 7.0 mm; see Figure 9, right panel).

Of the 17 neurons, 13 showed depressed spontaneous activity by chemical stimulation of the Rt (Figures 6A and 7A–C; INS, upper; spikes/s, lower; original record). Chemical stimulation also induced changes in BP, as shown in Figures 6A and 7C (MAP). However, changes in BP were not associated with the depression of the neuronal activity in the posterior insular cortex (see Figure 7A,B; MAP). Chemical stimulation of the Rt sometimes induced changes in the HR (Figure 7C; HR). Similarly, changes in the HR did not associate with the depression of the neuronal activity in the posterior insular cortex (see Figure 7A,B; HR).

As shown in Figure 7, the depressed activity of a neuron in the posterior insular cortex could be clearly observed in three instances after chemical stimulation of the Rt at different times (Figure 7A–C). Figure 7E shows that the responses evoked by the SL nerve or Rt stimulation were also inhibited during the depressed periods following chemical stimulation (A; Rt, SL nerve stimulation, *d* and *e*). The evoked responses of this neuron by electrical stimulation of the SL nerve did not disappear after the chemical stimulation site had been electrically lesioned (Figure 7F, SL nerve stimulation; after).

The remaining 4 of the 17 neurons did not show any change in neuronal activity by chemical stimulation of the outside Rt (a representative record is shown in Figure 8A). It was histologically found that, in the case of the neurons showing an inhibitory response to chemical stimulation, the location of the stimulation sites was limited within or near the Rt (Figure 9, right panel, open circles; see also Figures 6D and 7H). On the contrary, for all four neurons that were not sensitive to chemical stimulation, the stimulation sites were found to be located out of the Rt (Figure 9, right panel, filled circles; see also Figure 8D).

Of the 13 neurons that responded to chemical stimulation, three showed increased activity above the baseline spontaneous activity after recovery from depression (data not shown).

Discussion

Responsiveness and localization of the posterior insular cortex neurons

The neurons in the anterior portion of the insular cortex are known to be sensitive to gustatory stimuli (Yamamoto *et al.*, 1980b, 1984; Ogawa *et al.*, 1990) and those in the posterior portion of the insular cortex, to visceral sensory stimuli (Cechetto and Saper, 1987; Yasui *et al.*, 1991). Although the boundary between the taste and visceral areas is not clear, several previous studies suggest that the mean location for the taste area is ~1.3–1.7 mm anterior to the APo (cf. Hanamori *et al.*, 1998b). The mean location of the recording sites in the present study (for both the electrical stimulation and chemical stimulation studies) was 0.91 (n = 15) and 0.26 (n = 17) mm anterior to the APo, respectively. Thus, it is considered that the neurons recorded in the present study

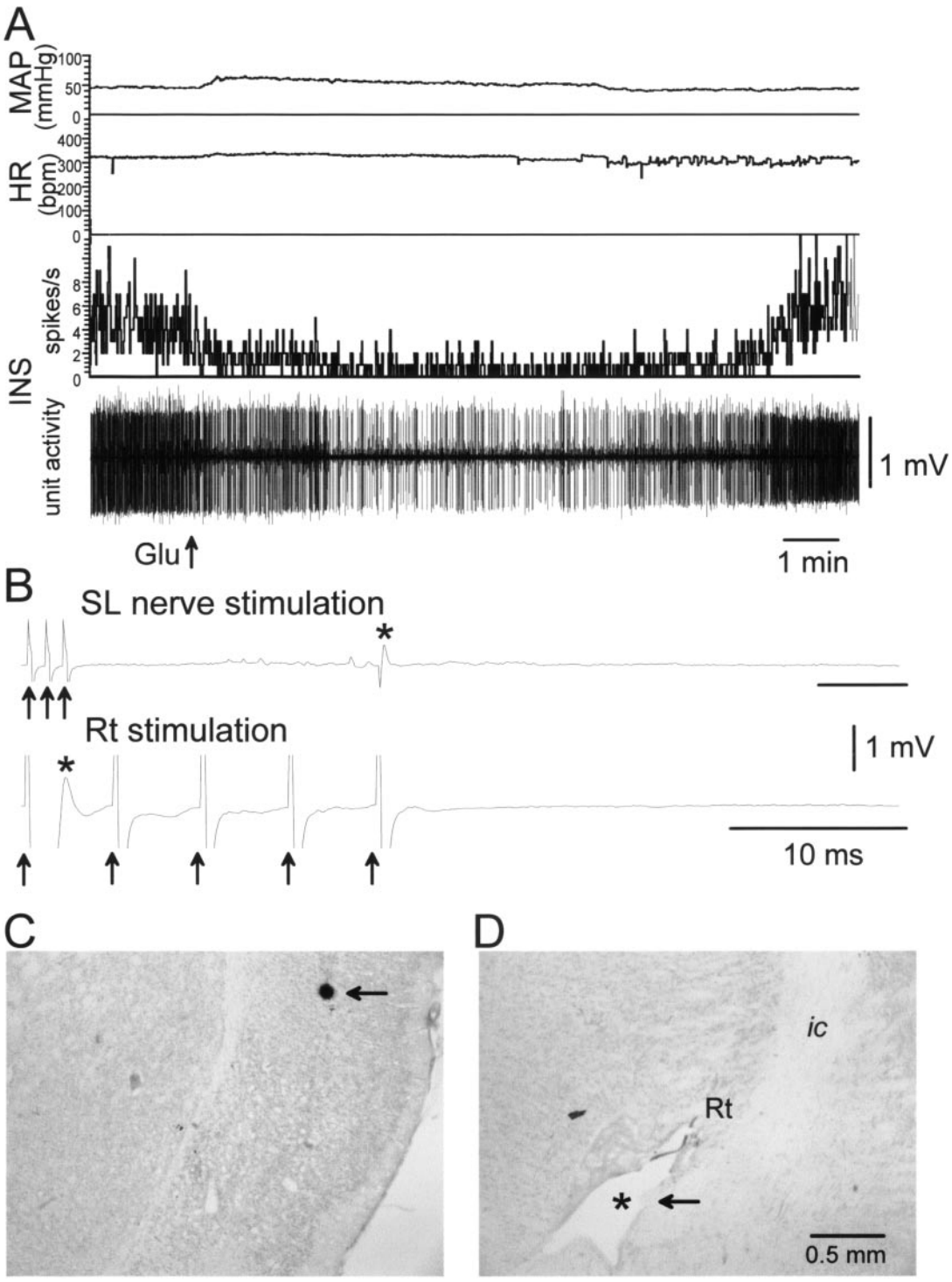

Figure 6 Responses of a neuron in the posterior insular cortex to chemical stimulation of the Rt. **(A)** The spontaneous activity of a posterior insular cortex neuron (INS) decreased following injection of Glu into the Rt. Arrow shows the time of Glu injection. **(B)** This neuron is responsive to electrical stimulation of the SL nerve (upper, train with three pulses at 2 ms intervals) and Rt stimulation (lower, train with five pulses at 5 ms intervals; 0.08 ms, 0.1 mA). The response of a neuron to stimulation of the Rt seemed to be orthodromic, since only the first stimulus evoked the action potential. Asterisk indicates the evoked action potential. Arrows indicate the time of electrical stimulation. **(C)** Photomicrograph showing the location of the recording site (arrow; 0.3 mm posterior to the APo). **(D)** Photomicrograph showing the location of chemical stimulation site (arrow; marked by electric lesion using a tungsten stimulating electrode; 2.4 mm posterior to the APo). Abbreviations: MAP, mean arterial blood pressure (mmHg); HR, heart rate (beats/min); INS, neuronal activity of a neuron; Glu, glutamate; Rt, thalamic reticular nucleus; *ic*, internal capsule; SL nerve, superior laryngeal nerve.

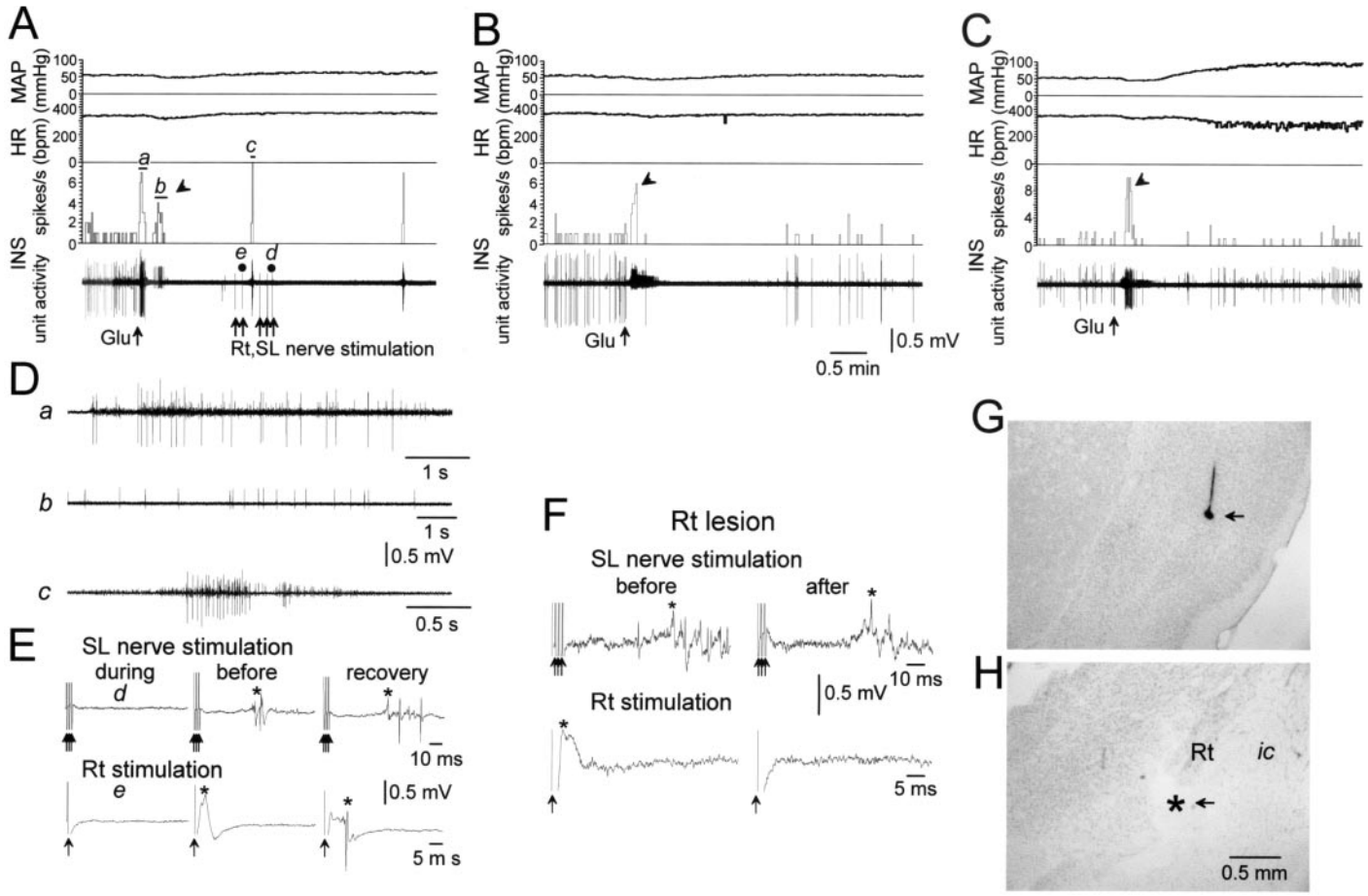

Figure 7 Responses of a neuron in the posterior insular cortex to chemical stimulation of the Rt. The spontaneous activity disappeared following injection of Glu into the Rt; three injection times; **(A–C)**. Arrows show the time of Glu injection. **(D)** *a*, *b*, and *c* are expanded records of the unit activity in A (INS, marked by the same character). **(E)** Responses to electrical stimulation of the SL nerve and Rt before (middle records; 0.08ms, 0.1 mA), during (left; *d* and *e*, expanded records of unit activity in A) and after (right, recovery) chemical stimulation. **(F)** Evoked responses to electrical stimulation of the SL nerve and Rt before (left) and after (right) electric lesion of the Rt (chemical stimulation site). **(G)** Photomicrograph showing the location of the recording site (arrow; 0.4 mm anterior to the APo). **(H)** Photomicrograph showing the location of the stimulation site (arrow; marked by electric lesion using a tungsten stimulating electrode; 2.8 mm posterior to the APo). Abbreviations: MAP, mean arterial blood pressure (mmHg); HR, heart rate (beats/min); INS, neuronal activity of a neuron; Glu, glutamate; Rt, thalamic reticular nucleus; *ic*, internal capsule; SL nerve, superior laryngeal nerve.

were from the posterior insular cortex. This might correspond to the area identified as the rostral half of the posterior insular cortex by Yasui *et al.* (1991). It has been reported that the neurons in this area are responsive to various sensory stimuli: visceral, taste and nociceptive (Cechetto and Saper, 1987; Hanamori *et al.*, 1997b, 1998a,b; Ito, 1998; Zhang and Oppenheimer, 2000). Recently, it has been reported that the neurons in the anterior portion of the insular cortex (taste area) are also responsive to nociceptive stimuli (Ogawa and Wang, 2002). In the present study, all neurons examined were sensitive to SL or IXth nerve stimulation as has been reported by Hanamori *et al.* (1997a).

Histological studies have shown that the retrograde-labeled cell bodies in the insular cortex are in layer VI. These studies were conducted with an injection of tracer into the ventroposteromedial parvicellular thalamic neucleus (VPMpc; Hayama *et al.*, 1994) or the ventroposterolateral parvicelluar thalamic nucleus (VPLpc; Stehberg *et al.*, 2001). In the present study, all neuron were located in layer V or VI, although we did not delineate the cortical layers in detail (Figures 5 and 9, left panels).

Electrical stimulation of the Rt

It has been reported that the Rt can be divided into several sectors (Guillery *et al.*, 1998). In addition, it is known that the regions in the Rt receiving somatosensory, visual, and auditory inputs are different from each other (Shosaku *et al.*, 1984). Stehberg *et al.* (2001) have demonstrated histologically, after a fluorescent tracer injection into the VPLpc, that retrograde-labeled cells are present in the ventral part of the Rt at 2.1–3.1 mm posterior to the bregma. A similar distribution of the labeled cells in the Rt (2.4–2.8 mm posterior to the bregma) has been reported by Hayama *et al.* (1994) after a fluorescent tracer or WGA-HRP injection into

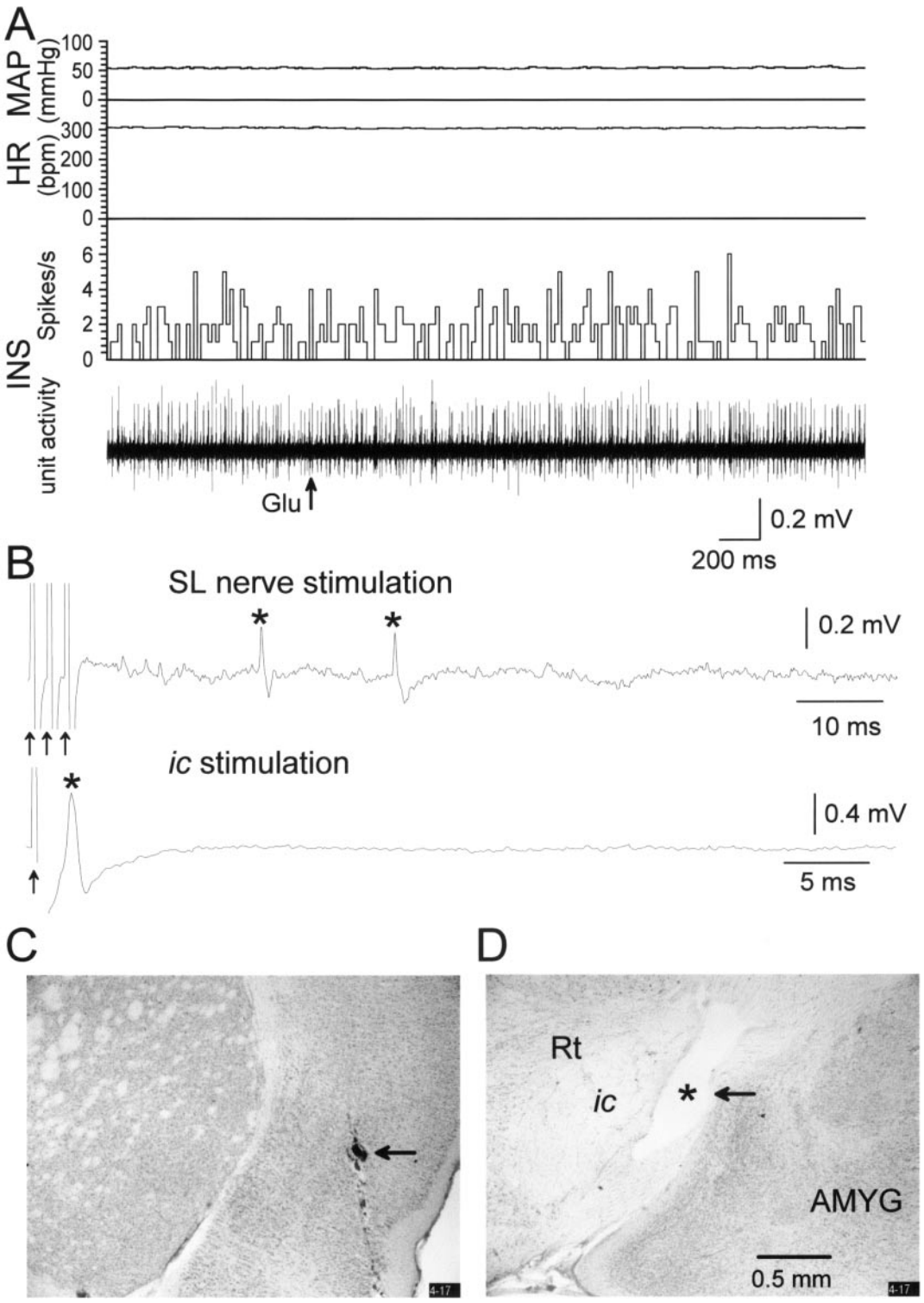

Figure 8 Responses of a neuron in the posterior insular cortex to chemical stimulation to the *ic*. **(A)** Spontaneous activity was unaffected following injection of Glu into the *ic* (INS). Arrow shows the time of Glu injection. **(B)** Responses to electrical stimulation of the SL nerve (train with three pulses at 2 ms intervals) and *ic* (0.08 ms, 0.1 mA). Arrows indicate the time of electrical stimulation. Asterisks show the evoked action potentials. The evoked action potential to *ic* stimulation was larger than that to the SL nerve stimulation. It seems to be recorded as summated action potentials from two cells in the *ic* stimulation. **(C)** Photomicrograph showing the location of the recording site (arrow; 0 mm anterior to the APo). **(D)** Photomicrograph showing the location of chemical stimulation site (arrow; marked by electric lesion using a tungsten stimulating electrode; 2.6 mm posterior to the APo). Abbreviations: MAP, mean arterial blood pressure (mmHg); HR, heart rate (beats/min); INS, neuronal activity of a neuron; Glu, glutamate; Rt, thalamic reticular nucleus; *ic*, internal capsule; SL nerve, superior laryngeal nerve; AMYG, amygdala.

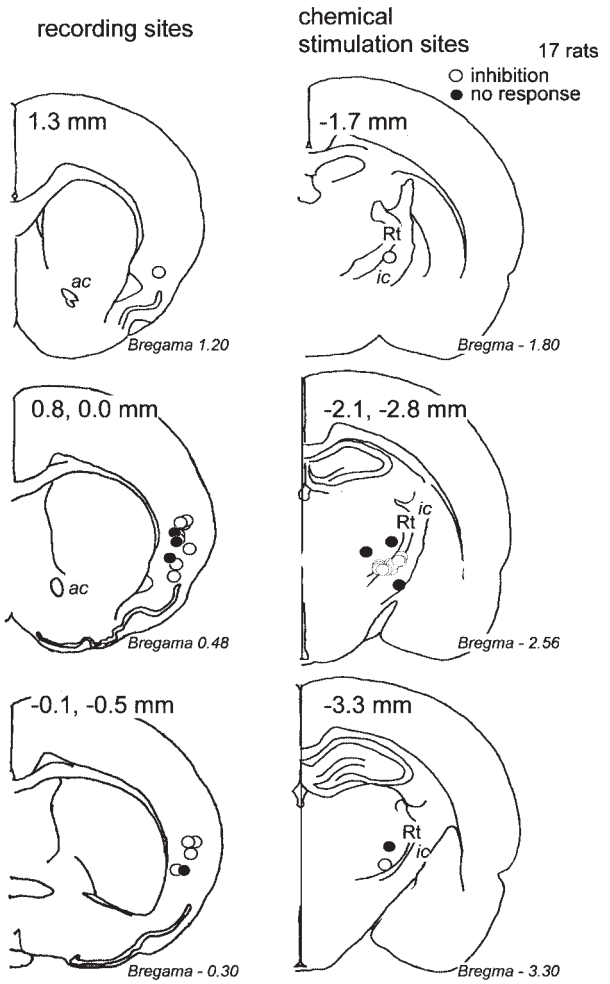

Figure 9 Location of recording (left) and chemical stimulation sites (right) for 17 rats. The recording and stimulation sites identified histologically are plotted on the coronal sections of the brain atlas of Paxinos and Watson (1986) from anterior (top) to posterior (bottom) levels (distances from the bregma are indicated in the bottom right area of each section). Numerals (upper central in each section) indicate the locations of the recording and stimulation sites in the range of the distance anterior or posterior to the APo, respectively. Open circles indicate a case in which spontaneous activity was depressed by chemical stimulation and filled circles indicate a case in which spontaneous activity was unaffected by chemical stimulation. For all neurons that were inhibited, the locations of stimulation sites were found to be within or near the Rt. Abbreviations: Rt, thalamic reticular nucleus; *ic*, internal capsule; *ac*, anterior commissure.

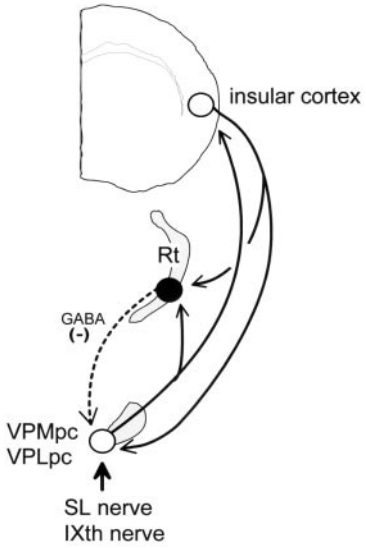

Figure 10 Summary diagram of connections between the thalamic relay nucleus (VPMpc, VPLpc), Rt and insular cortex. Abbreviations: Rt, thalamic reticular nucleus; VPMpc, ventroposteromedial parvicellular thalamic nucleus; VPLpc, ventroposterolateral parvicellular thalamic nucleus; SL nerve, superior laryngeal nerve; IXth nerve, glossopharyngeal nerve. Filled and open circles indicate inhibitory and excitatory neurons, respectively.

the VPMpc. Therefore, both the taste and visceral areas of the Rt seem to be present in similar parts of the Rt (the ventromedial part). In the present study, the location of the stimulation site that could induce the evoked responses for the neurons in the posterior insular cortex was found to be a similar part of the Rt, as reported in the above histological studies [ranging from 1.7–3.5 mm posterior to the APo (n = 32); the mean value was 2.71 mm for the electrical stimulation study (n = 15) and 2.57 mm (n = 17) for the chemical stimulation study, see Figures 5 and 9, right panels]. The difference of the standard zero point in the anterior–posterior axis between the bregma and the APo was small (our previous data showed that the bregma was only 0.06 mm anterior to the APo; n = 32).

Most of the posterior insular cortex neurons examined in the present study were responsive to electrical stimulation of the Rt or a region adjacent to the Rt. However, 7 (/15) neurons for the electrical stimulation study and 4 (/17) neurons for the chemical stimulation study were responsive to electrical stimulation outside of the Rt (Figures 5 and 9; right panels). Two reasons may be considered for this. First, the axons from neurons in the posterior insular cortex or the thalamic relay nucleus pass in the areas surrounding the Rt. Therefore, the responses in the posterior insular cortex neurons could be induced by electrical stimulation of these fibers. Secondly, the fibers passing in the Rt could be stimulated by the current spreading from the stimulating tungsten electrode which was placed outside of the Rt. However, the effect of the electrical stimulation used in the present study might be localized, as suggested by the localized effect of the electrical stimulation shown in Figure 4.

The neurons (GABAergic) in the Rt could be also activated by electrical stimulation of the Rt and surrounding areas. This may induce the depressed activity of the posterior insular cortex neurons via the GABAergic inhibition in the thalamic relay neurons. However, this indirect effect on the neuronal activity in the posterior insular cortex was not examined in detail in the present study.

Most neurons (8/9) showing antidromic response in the electrical stimulation study were obtained from electrical stimulation outside of the Rt (Figure 5, right panel). On the

other hand, all seven neurons showing an orthodromic response had received electrical stimulation of the Rt (Figure 5, right panel). This difference may not be statistically meaningful because of the small number of sampling data. However, this deflection might be due to the anatomical differences of the fibers in the Rt passing from cortex neurons and from thalamic relay neurons.

Chemical stimulation of the Rt

Only the neurons whose injection sites were histologically ascertained to be within or near the Rt showed depressed spontaneous activity following chemical stimulation (13/17, Figure 9, right panel). The mean degree of the depression (see Materials and methods) was 84.2 ± 6.1% (n = 13, ranging from 35 to 100%). The mean duration of the depression (see Materials and methods) was 4.0 ± 1.2 min (n = 13, ranging from 0.2 to 12.6 min). The variation of the degree of the depression and duration might mainly depend on the volume of the injection of Glu (40–300 nl).

From the results of the histological studies for the visceral and taste system, the connections of the Rt, INS and thalamic relay nucleus can be drawn diagrammatically as in Figure 10. In the present study, it was concluded that the spontaneous activity of the neurons in the posterior insular cortex was depressed by the decreased neuronal activity in the thalamic relay nucleus, which was induced by activation of the GABAergic neurons in the Rt following an injection of Glu into the Rt (Figure 10). It has been reported that the spontaneous activity of the neurons in the thalamic relay nucleus showed a long lasting inhibition and was followed by rebound facilitation by electrical stimulation of the insular cortex (Ogawa and Nomura, 1988). A similar biphasic effect (first depression and then facilitation) on the taste relay neurons in the thalamic relay nucleus by cortical stimulation has been reported by Yamamoto *et al.* (1980a). These corticofugal effects on the activity of the thalamic relay nucleus may possibly be explained by the role of the Rt.

In Figure 7A, a brief burst is seen before the start of the depression (*a*, see also Figure 7D; *a*) followed by a burst with a smaller spike and a different shape of the action potential (Figure 7A, *b*, arrowhead; see also Figure 7D, *b*). These phenomena are also observed in Figure 7B,C (arrowheads), but, in Figure 7B,C, no brief burst (Figure 7A, *a*) is observed before the start of the depression. In Figure 7A, a brief burst occurs during a silence period (*c*, see also Figure 7D; *c*). Burst discharges in the early phase of the depression (Figure 7A, *b*) were seen in 4 of the 13 neurons that had an inhibitory response. In the present study, three neurons showed an increased spontaneous discharge following depression. A biphasic effect has been shown in electrical stimulation of the gustatory cortex as described above (Yamamoto *et al.*, 1980a; Ogawa and Nomura, 1988). We cannot explain yet how rebound facilitation results from the neuronal networks shown in Figure 10. Neither can we explain the the burst discharges observed at the beginning of and during the depression phase in the neuronal activity of the posterior insular cortex. In order to understand fully the neuronal activity in the insular cortex, we have to know the modulatory systems in the cortex itself (also, the modulatory systems in the Rt or thalamus itself). Although further detailed studies are needed to explain the responses in the posterior insular cortex neurons following chemical stimulation of the Rt, the present study showed that neuronal activity in the posterior insular cortex is controlled by the Rt, as has been reported for other sensory systems.

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