Role of Oral Sensory and Serotonergic Neurons in Dopamine-induced Tongue Movement in Rat

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Synopsis
Background: Oral dyskinesia is a condition in which oral organs exhibit involuntary movements. The aim of this study was to determine the contribution of oral sensation to the control of oral dyskinesia using an animal model that exhibits vacuous tongue movements after systemic administration of dopamine (DA) agonists.

Methods: Adult male Sprague-Dawley rats were used in all experiments. Tongue movement was elicited in a freely moving rat model by systemic administration of a mixture of the D1 receptor agonist SKF38393 and the D2 receptor agonist quinpirole. The number of tongue protrusions was calculated from electrical myographic data obtained from the genioglossal muscle. Extracellular concentrations of DA and serotonin (5-HT) in the nucleus accumbens were determined using in vivo microdialysis. To investigate the effects of oral sensation on vacuous tongue movement, deafferentation of the palatal mucosa was performed, and the above experiments were repeated.

Results: The hyperactivity of tongue protrusion induced by administration of SKF38393 and quinpirole was significantly inhibited by palatal deafferentation. Palatal deafferentation also significantly reduced the increase in extracellular 5-HT concentration in the nucleus accumbens produced by administration of DA agonists.

Conclusions: These results support the important contribution of oral sensation to vacuous tongue protrusion in a rat model of oral dyskinesia.

Key words: oral dyskinesia, oral sensation, nucleus accumbens, dopamine, serotonin

Introduction
Oral dyskinesia is a condition in which oral organs exhibit involuntary movements. It is most commonly observed as tardive dyskinesia in patients taking certain neuroleptic drugs. It is also observed in edentulous patients not receiving these medications, which is sometimes termed as edentulous dyskinesia [1,2]. Unlike oral dyskinesia in tardive dyskinesia, edentulous dyskinesia is caused by ill-fitting dentures, malocclusion, and a lack of sensory contact, although the mechanism remains ill defined. However, it was reported that edentulous patients exhibit increased severity of oral dyskinesia when experiencing tardive dyskinesia [3]. Thus, oral sensation plays an important role in the occurrence or deterioration of oral dyskinesia, but the mechanism and oral factors have not yet been elucidated.
Numerous studies have investigated the mechanisms underlying oral dyskinesia. These studies used models of dyskinesia involving local and/or systemic administration of various neurotransmitter agonists and antagonists to determine possible roles of neurotransmitter receptors. An animal model of oral dyskinesia has emerged from these studies in which apomorphine [4-8], a nonselective dopamine (DA) agonist, is administered systemically to rats. Using this model, many studies have demonstrated that DA receptors in cerebral neural networks play a role in the regulation of involuntary oral movements. In particular, it is now widely accepted that oral stereotypies are because of synergistic effects of D1 and D2 receptors [5, 6, 9].

The nucleus accumbens contains a large number of D1 and D2 receptors [10,11] and receives input from mesolimbic dopaminergic projections from the ventral tegmental area. In turn, mesolimbic dopaminergic projections receive serotonergic input from the dorsal raphe nucleus at the level of DA cell bodies [12, 13], as well as in the ventral tegmental area and nucleus accumbens [14, 15]. In addition, numerous reports have shown that serotonin (5-HT) influences the release of DA in the ventral tegmental area and nucleus accumbens [13, 16-18]. Previous studies also demonstrated that the serotonergic system, particularly 5-HT1A and 5-HT2A/2C receptors, may also be involved in involuntary oral movement [8, 19, 20]. Thus, 5-HT in nucleus accumbens may play an important role in oral dyskinesia caused by neurotransmitter disorders.

Various afferent inputs are present in the oral and facial region and play a role in controlling movement of each oral organ. For instance, mechanical stimulation of the palatal mucosa causes tongue protrusion and jaw movement [21]. Studies showed that sensory stimulation may affect DA metabolism in the striatum [22]. Therefore, we hypothesized that increased input from oral organs, caused by frequent oral movement, also influences the production of involuntary movement and affects central dopaminergic and serotonergic systems. No previous study has investigated the interactions between these contributing factors.

To investigate the contribution of oral sensation to oral dyskinesia, we analyzed the effect of palatal deafferentation (PD) on vacuous tongue protrusions induced by systemic administration of SKF38393, a selective D1 agonist, and quinpirole, a selective D2 agonist. We also measured DA and 5-HT release in the nucleus accumbens using microdialysis and high-performance liquid chromatography with electrochemical detection.

**Materials and Methods**

**Animals**
Adult male Sprague-Dawley rats weighing 250–320 g (Nihon Animal, Japan) were used in all experiments. Animals were housed in a temperature- and humidity controlled room maintained at 23 °C with a relative humidity of 55% under a 12-hour day-and-night cycle, with free access to food and water. All experiments were conducted according to the institutional guidelines for the care and use of experimental animals of the Osaka University Graduate School of Dentistry (Authorization number: 02-015).

**Drugs**
The following drugs were used: the selective D1 receptor agonist SKF38393 [(±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride; Sigma-Aldrich, USA], the selective D2 receptor agonist quinpirole [trans-(-)-4aR-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-pyrazolo(3,4-g) quinoline hydrochloride; LY-171,555; Sigma-Aldrich], and the selective 5-HT1A receptor agonist 8-OH-DPAT [(±)-8-hydroxy-2-dipropylaminotetralin hydrobromide; Tocris, USA]. Each substance was dissolved in 1 mL of sterile saline immediately before injection. A mixture of SKF38393 and quinpirole was administered intravenously (i.v.), and 8-OH-DPAT was administered subcutaneously (s.c.).

**Preparation**
Each rat was anesthetized intraperitoneally with sodium pentobarbital (50 mg/kg), ensuring that body movement evoked by pinching the tail or hind limb was abolished. Animals were then placed on their backs and the left femoral vein was cannulated for i.v. administration of the drugs. A silicon tube (Fuji Systems, Tokyo, Japan) filled with saline containing heparin was inserted into the left femoral vein and then passed under the skin to the parietal region and soldered to a mini-connector (Unique...
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Medical, Tokyo, Japan), which was fixed to the surface of the cranial bone with dental resin (GC, Tokyo, Japan).

To analyze the concentration of DA and 5-HT in the nucleus accumbens by microdialysis, rats were placed on a stereotaxic apparatus and a guide cannula (outer diameter: 0.50 mm; inner diameter: 0.30 mm; Eicom, Kyoto, Japan) was stereotaxically implanted using a dummy probe (Eicom). A dummy probe was implanted into the left nucleus accumbens (planar coordinates: AP + 1.6 mm from bregma, ML = 0.9 mm, V = 7.7 mm from dura). The guide cannula was fixed to the skull surface with 5 mm titanium screws, in addition with instant surgical glue (Sankyo, Osaka, Japan) and acrylic resin. Following these procedures, rats were injected with the antibiotic fosfomycin (0.1 g/kg, i.v.) to prevent infection, and the animals were allowed to recover for at least 5 days before experimental procedures were initiated.

To analyze the effect of palatal sensation loss, PD was carried out by surgical removal of all palatal mucosa anterior to the molars on both sides by electrocautery 3 days before experimental procedures were initiated. These rats were referred to as the PD group. In these rats, the periosteum held for palatal bone protection, and they recovered food intake after 1 or 2 days with no significant weight loss on the experimental day.

Behavior analysis

We continuously recorded the occurrence of tongue protrusions of the freely moving rat from 1 hour prior to administration of the SKF38393 (5 mg/kg) and quinpirole (10 mg) mixture until 3 hours after administration. Tongue protrusions are mainly produced by genioglossus muscle activity and were operationally defined as a visible extension movement of the tongue outside the mouth. EMG signaling from the genioglossus muscle was recorded on a polygraph system (LEG-1000, Nihon Kohden, Tokyo, Japan) through a preamplifier (MEG-1100, Nihon Kohden), and the EMG signal was transformed into an integrated wave on the polygraph system. The integrated wave was counted in 20-minute periods over the duration of the experiment to longitudinally determine the number of tongue protrusions.

Microdialysis analysis

Extracellular DA and 5-HT concentrations were analyzed using a microdialysis system. On each day of the experiment, a microdialysis probe with an active dialyzing length of 2.0 mm was inserted into the guide cannula, and each rat was placed in an individual clear plastic cage (30 x 30 x 40 cm) with a stainless-steel grid floor in a quiet, well-lit room. Ringer’s solution (147 mM Na+, 4 mM K+, 2.3 mM Ca2+, and 155.6 mM Cl-) was continuously perfused through the microdialysis probe by a microinfusion pump (Eicom) at a flow rate of 2.0 μL/min. The probe was connected by Teflon tubing (Eicom) to an EAS-20 online injector (Eicom), and the perfusate (40 μL) was automatically injected every 20 minutes through the apparatus into a HTEC-500 HPLC-ECD system (Eicom) equipped with an ODS reverse-phase column (Eicom-pac MA-5 ODS, Eicom). The mobile phase consisted of 79% (v/v) 0.1 M phosphate buffer (pH 6), 21% (v/v) methanol, 550 mg/L sodium octane sulfonate (Nacalai Tesque, Kyoto, Japan), and 50 mg/L of EDTA 2Na. After perfusion was complete, DA and 5-HT levels became constant (occurring approximately 120 minutes after probe insertion). The mean basal DA and 5-HT release values were determined by averaging values of three consecutive perfusate collections.

Histological analysis

At the end of each experiment, animals were euthanatized with sodium pentobarbital (200 mg/kg, i.v.) and perfused transcardially with a 10% formaldehyde solution, after which brains were carefully removed and fixed in 10% formaldehyde for at least 2 days. Brains were then sectioned (50 μm thick) and stained with cresyl violet to verify probe placement microscopically (Fig. 1). Data were discarded if the animal was not euthanatized by sodium pentobarbital administration (200 mg/kg, i.v.) or if any portion of the dialysis probe was not placed in the intended region.

Figure 1 Location of microdialysis probe.

In nucleus accumbens stained with crystal violet (arrow), the location of the microdialysis probe inserted through the guide cannula was implanted stereotaxically 1.6 mm anterior from the bregma.
Statistical analysis
All values are expressed as the mean ± standard error of the mean. Data for the number of tongue protrusions, as well as extracellular DA and 5-HT concentrations for each 20-minute period, were analyzed using a two-factor factorial analysis of variance (ANOVA). In all tests, the criterion for statistical significance was $p < 0.01$.

Results
Tongue protrusion frequency
All rats administered the dopamine (DA) agonist mixture (DAM group) of SKF38393 and quinpirole showed an increase in the frequency of tongue protrusions. This effect was observed immediately after systemic administration until the end of the experiment 3 hours later. The number of tongue protrusions in the DAM group was significantly higher than in rats administered saline (open circles, $n = 7$) at all time points ($**p < 0.01$). After pretreatment with 8-OH-DPAT (DPAT group, closed triangles, $n = 7$), an increased number of tongue protrusions was observed 60 minutes after systemic administration of the DA agonist mixture, peaking 160 minutes after administration. The number of tongue protrusions in the palatal deafferentation (PD) group (closed squares, $n = 5$) gradually increased after systemic administration of the DA agonist mixture, whereas the number of tongue protrusions in the DPAT and PD groups was significantly lower than in the DAM group throughout the observation period ($**p < 0.01$). There was no significant difference between the DPAT and PD groups.

Extracellular DA concentration in nucleus accumbens
Extracellular DA concentration in the nucleus accumbens gradually decreased following administration of the DA agonist mixture in DAM, DPAT, and PD groups (Fig. 3). These changes were significantly different from the extracellular DA concentration after saline administration; however, there was no significant difference in the decrease of extracellular DA concentration between the DAM and PD group. Further, the extent of this decrease in the DPAT group was significantly less than that of the DAM and PD group.
Extracellular 5-HT concentration in nucleus accumbens

In the DAM group, the extracellular 5-HT concentration in the nucleus accumbens gradually increased following administration of the DA agonist mixture, peaking 60 minutes after administration (Fig. 4). Similar to the DAM group, the extracellular 5-HT concentration in the nucleus accumbens of the PD group increased following administration of the DA agonist mixture, peaking 60 minutes after administration (Fig. 4).
nucleus accumbens of the PD group increased immediately after DA agonist mixture administration, but then decreased 40 min after administration. This observed change in 5-HT concentration was significantly lower than that found in the DAM group. Further, the increase in 5-HT concentration after administration of the DA agonist mixture was completely inhibited in the DPAT group, which was significantly different compared with the DAM and PD group.

Discussion
Oral dyskinesia is a condition comprised of various vacuous oral movements. It is most often caused by systemic administration of neuroleptic drugs, but is also frequently observed in edentulous patients who wear ill-fitting dentures. Because of the link with neuroleptic drugs, numerous studies have attempted to clarify neural control mechanisms of oral dyskinesia. However, limited attention has been paid to the contribution of oral sensation despite this contribution being considered as a major cause of the syndrome in edentulous patients [1-3]. To our knowledge, this study is the first to demonstrate the contribution of oral sensory input to oral dyskinesia in a rat model.

Systemic administration of dopaminergic agonists evokes frequent involuntary tongue protrusions and vacuous chewing movements. Many oral dyskinesia models have been reported since the introduction of an animal model of oral dyskinesia evoked by systemic administration of the nonselective DA agonist apomorphine. These models often used combinations of selective and nonselective DA receptor agonists, antagonists, and various neuroleptic drugs. Models of oral dyskinesia have also been generated by administration of other neurotransmitter agonists and antagonists into the cerebral area. In this study, we used an established male rat model of oral dyskinesia that displays vacuous oral movements elicited by the simultaneous stimulation of D1 and D2 receptors [5,6,9]. We counted the number of tongue protrusions with mechanical contact to palate as oral dyskinesia behavioral measures to study the influence of palatal sensory input for oral movements. Because it should be noted that hormonal differences in male and female animals may influence behavior, metabolism, and deafferentation, we used only male rats. The drugs and doses administered systematically have previously been found to be effective in the study of involuntary oral movement [5].

It is considered a locomotor stimulant effect because of the circuit via D2 receptors from the nucleus accumbens in basal ganglia and direct activation of postsynaptic D2 receptors on dopaminergic neuron terminals [23-25]. Systemic administration of a mixture of SKF38393 and quinpirole in this study induces tongue protrusion and reduces extracellular DA concentration in the nucleus accumbens. Previous reports have shown that the administration of apomorphine reduces extracellular DA in the nucleus accumbens [24], and that this reduction is mainly attributed to negative feedback via presynaptic D2 autoreceptors on dopaminergic nerve terminals and somatodendrites, both of which participate in the inhibition of DA release. Further, past reports have demonstrated that D2 receptor activation increases the excitability of serotonergic neurons in the dorsal raphe nucleus, and increases local extracellular 5-HT concentration [24]. In addition, a previous study found that an increase in 5-HT concentration in dorsal raphe following systemic administration of apomorphine was antagonized by administration of a selective D1 receptor antagonist [26]. These findings all support the fact that dopaminergic and serotonergic neurons interact and regulate each other. Previous work has shown that the dorsal raphe nucleus contains dopaminergic neurons, and provides both dopaminergic and serotonergic innervation to the nucleus accumbens [24]. However, the role of DA receptors on the regulation of 5-HT in nucleus accumbens and dorsal raphe nucleus is still unclear.

5-HT neurons project directly into the nucleus accumbens from the dorsal raphe nucleus [14, 15]. 8-OH-DPAT is a selective agonist of 5-HT1A that strongly binds to receptors in the dorsal raphe nucleus of rat, and suppresses the firing rate of 5-HT neurons. Our results do not examine the effect of 8-OH-DPAT in specific cerebral regions that control oral movements, but we found that 8-OH-DPAT pretreatment by systemic administration inhibits 5-HT increase, suppresses DA decrease, and suppresses increase of tongue protrusion. A past study showed that systemic administration of 8-OH-DPAT decreases vacuous chewing movements induced by haloperidol [19], which is consistent with our findings. Therefore, we consider that involuntary oral movements induced by a DA agonist is con-
trolled by an interaction between dopaminergic and serotonergic neurons.

Our study also showed that tongue protrusions elicited by DA administration were significantly inhibited by PD. This result may indicate that tongue protrusions in vacuous oral movements are affected by afferent inputs from the palate, which is known to cause reflexive oral movements, including tongue protrusion by contact between the palate and tongue. A previous study reported that the frequency of rhythmic jaw movement can be increased by mechanical stimulation of the palate [21]. Thus, our palatal surgery for deafferentation may exert an influence on our findings, and using a different animal model, such as a neuropathic pain model, may have provided clearer results. Furthermore, it was reported that extracellular 5-HT concentration in brain regions innervated by dorsal and median raphe nuclei increases under stress conditions. In our results, rats that underwent PD also showed a decrease in 5-HT in the nucleus accumbens. Without any adverse effect of our surgery, tongue protrusions evoked by DA agonists and oral sensory input were related, suggesting that serotonergic neurons are interacting with dopaminergic neurons. However, it is unclear whether the observed decrease in 5-HT was induced by decreased afferent input because of the movement of each organ, or whether the decrease in 5-HT itself leads to suppression of these behaviors. Therefore, continued studies are needed to confirm the involvement of palatal sensory in DA-induced oral movement, the modulation of 5-HT by oral sensation, and the influence of oral sensation on neurotransmitters in the central nervous system.

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