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# Odor Detection Ability and Thallium-201 Transport in the Olfactory Nerve of Traumatic Olfactory-Impaired Mice

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## Abstract

Although olfactory nerve damage is a contributing factor in the diagnosis of posttraumatic olfactory loss, at present, there are no methods to directly assess injury to these nerves. We have shown that following olfactory nerve injury in mice, thallium-201 ($^{201}$Tl) transport from the nasal cavity to the olfactory bulb decreases. To determine if olfactory function after nerve injury could be assessed with nasal administration of $^{201}$Tl, we measured the correlation between odor detection ability (ODA) and the rate of transport of $^{201}$Tl in olfactory nerves. Both ODA and $^{201}$Tl transport were measured after bilateral olfactory nerve transection for a 4-week period. Cycloheximide solution was used for ODA against tap water. $^{201}$Tl transport was measured as the ratio of radioactivity in the nasal cavity and olfactory bulb with gamma spectrometry. There was a significant correlation between ODA and the rate of $^{201}$Tl transport in the olfactory nerve. These findings suggest that olfactory function after nerve injury can be objectively evaluated with the nasal administration of $^{201}$Tl.

**Key words:** odor detection, posttraumatic olfactory impairment, thallium-201

## Introduction

Injuries to the olfactory bulb and tract (88% of patients), subfrontal region (60%), and temporal lobe (32%) have been shown using magnetic resonance imaging (MRI) in patients with posttraumatic olfactory dysfunction; however, this did not correlate well with olfactory test scores (Yousem et al. 1996), and it is difficult to visualize damage to olfactory nerve fibers with MRI (Fujii et al. 2002). It would be useful to use objective measurements to assess damage to olfactory nerve fibers and also to predict the course of traumatic olfactory impairment.

Functional MRI (fMRI) signals by response to odor stimuli in the piriform cortex, amygdala, and orbitofrontal cortex have been shown in humans (Zatorre et al. 1992; Zald and Pardo 1997; Sobel et al. 1998; Gottfried et al. 2002); however, the possibility of diagnosing a lesion in olfactory nerve fibers with fMRI is unconfirmed. Furthermore, MRI and fMRI are not available for individuals who have metal in their bodies. Other neuroimaging techniques therefore need to be developed to diagnose patients with traumatic olfactory impairment.

We have previously established a simple and reliable method to examine olfactory function in rodents by the cycloheximide solution avoidance behavior (Miwa 1989; Moriizumi et al. 1994; Fukushima et al. 2002; Miwa et al. 2004). This is based on the ability to detect the odor of cycloheximide solution.

Thallium-201 ($^{201}$Tl) is transported in the olfactory nerve of mice when administered intranasally (Kanayama et al. 2005). The transport of $^{201}$Tl in the olfactory nerve is decreased following transection of the olfactory nerve fibers (Kinoshita et al. 2008). $^{201}$Tl has been widely used by systemic administration in isotope imaging for clinical diagnosis (Iida and Eberl 1998; Higuchi et al. 2005).

The purpose of this study was to determine whether olfactory impairment due to head injury can be diagnosed based on the rate at which $^{201}$Tl is transported in olfactory nerve fibers after nasal administration of $^{201}$Tl. Our results show a positive correlation between odor detection ability (ODA) and the $^{201}$Tl transport rate in the olfactory nerve of mice.

## Materials and methods

### Materials

Fifteen Male ICR mice, 8 weeks of age (Japan SLC, Shizuoka, Japan), were divided into 5 groups by survival day after surgery (2 days, 7 days, 14 days, and 28 days) and sham operation. Mice were housed in a 22 °C air-conditioned room with a 12:12 h light:dark cycle and freely provided food (Charles River Laboratories Japan, Inc., Yokohama, Japan) and water. The Kanazawa University Animal Experiment Committee approved all animal experimental procedures in advance (No. 26142).

### Odor detection ability

Cycloheximide has a peculiar odor and unpleasant taste. After being deprived of water for 48 h, mice were trained to avoid the cycloheximide solution. The cycloheximide solution avoidance behavior was examined by testing the ability to distinguish a 0.01% cycloheximide solution from tap water. The positions of the cycloheximide solution bottle and tap water bottle were randomized according to a uniform random number (the cycloheximide bottle on the right side of the cage if an odd number; the water bottle on the right if an even number). Each mouse was examined twice before nerve transection for conditioning to the odor of cycloheximide. Ten trials were repeated each time.

### Bilateral olfactory nerve transection

The olfactory nerve fibers in mice were transected according to a method previously described (Kinoshita et al. 2008). We exposed both the right olfactory bulb (OBR) and the left olfactory bulb, cutting the frontal bones of mice under anesthesia (ether inhalation; intraperitoneal administration of pentobarbital sodium, 0.03 mg/g). The olfactory nerve fibers were carefully transected bilaterally with a Teflon knife avoiding damage to olfactory bulbs (bilateral olfactory nerve transection, BNTX). The skin incision was closed using a nylon suture. Sham-operated mice had their olfactory bulbs exposed, but the olfactory nerves were not transected.

### ODA test and $^{201}$Tl experiments

Mice were assessed for both ODA and the measurement of $^{201}$Tl transport at days 2 ($N = 3$), 7 ($N = 3$), 14 ($N = 3$), and 28 ($N = 3$) following nerve transection (BNTX olfactory-impaired mice). The ODA was 60% or less in all BNTX mice at 2 days after nerve transection. Control mice ($N = 3$) were observed 28 days after the sham operation. $^{201}$TlCl saline solution (78 MBq/ml) was obtained from Nihon Medi-Physics (Kobe, Japan), and 10 µl was carefully instilled into the right nostril of each mouse while avoiding sneeze with a microinjection pipette under anesthesia (ether inhalation). The mice were sacrificed under ether anesthesia 3 h later. Tissue samples were obtained from the right nasal turbinate and the OBR.

The radioactivity of the samples was measured with gamma spectrometry using the Auto Well Gamma System (model ARC-380; Aloka, Tokyo, Japan) after weight measurement. The uptake percentage (percent dose) of the isotope in each sample was calculated as a percentage of the radioactivity of each sample per radioactivity of 1% of 10-µl $^{201}$TlCl solution. The $^{201}$Tl uptake percentage per weight (percent dose per gram) was calculated as a percentage of the uptake percentage of each sample per wet weight.

The transport of $^{201}$Tl by olfactory nerve fibers was calculated as a percentage of the uptake percentage per weight (percent dose per gram) of the isotope in the OBR divided by the isotope uptake percentage per weight (percent dose per gram) in the right nasal turbinate.

### Autoradiography

The mice were given $^{201}$TlCl solution (10 µl) for autoradiography into their nasal cavity. Three hours later, they were sacrificed under ether anesthesia and the head was dissected. After removal of the skin and muscle, the head was embedded in Tissue-Tek OCT Compound (Sakura Finetechnical, Tokyo, Japan) and frozen in liquid nitrogen. The frozen heads were sectioned at 50 µm. Coronal sections were obtained and dried. The sections were adhered to an isotope imaging plate (FUJIFILM, Tokyo, Japan) and then assessed with a Bio-imaging Analyzer (FUJIFILM). Three mice from each group (control, 2-day BNTX, and 28-day BNTX) were assessed.

### Statistical analysis

The Kruskal–Wallis test was applied to compare ODA in multiple groups. The Spearman correlation was applied to compare ODA and the transport of $^{201}$Tl in olfactory nerve fibers. A statistical comparison of median values was performed using the Mann–Whitney $U$ test. All $P$ values were 2 tailed (Prism 5, GraphPad, San Diego, CA). A $P$ value $<0.05$ was considered significant.

## Results

### Odor detection ability

To determine the change of olfactory ability after the transection of olfactory nerve, ODA was assessed based on the cycloheximide solution avoidance behavior. ODA improved with time after recovery from nerve transection (Figure 1; $N = 3$ for each group; Kruskal–Wallis test, $^*P < 0.03$).

### Correlation between ODA and the transport rate of $^{201}$Tl in the olfactory nerve

To determine the transport rate of $^{201}$Tl between the nasal cavity and the olfactory bulb, we divided the uptake percentage per weight (percent dose per gram) in the OBR by the uptake percentage per weight (percent dose per gram) in

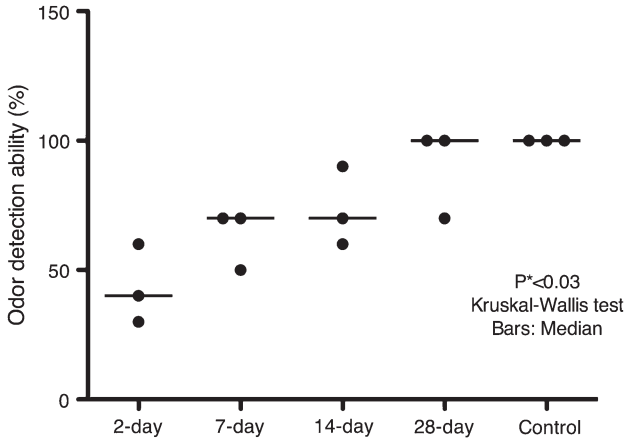


**Figure 1** ODA after transection of olfactory nerve fibers in 2-day, 7-day, 14-day, and 28-day BNTX mice and controls. ODA improved with time after transection ($N$ = 3 for each group; Kruskal–Wallis test, $*P < 0.03$). Bars indicate medians.

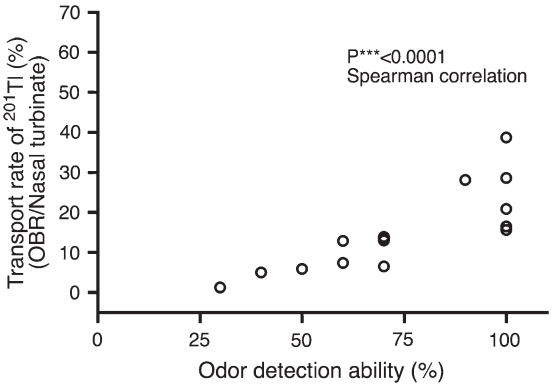


**Figure 2** Correlation between ODA and the transport rate of $^{201}$Tl in the olfactory nerve in 2-day, 7-day, 14-day, and 28-day BNTX mice and controls. The transport rate of $^{201}$Tl in olfactory nerve fibers was significantly correlated with ODA ($N$ = 15; Spearman correlation, $***P < 0.0001$).

the right nasal turbinate. The transport rate of $^{201}$Tl in olfactory nerve fibers was significantly correlated with ODA in 2-day, 7-day, 14-day, and 28-day bilateral olfactory nerve transection (BNTX) mice and the control (Figure 2; $N = 15$, Spearman correlation, $***P < 0.0001$). The transport rate of $^{201}$Tl between the nasal cavity and the olfactory bulb following nasal administration may reflect the level of damage to olfactory nerve fibers in mice.

### $^{201}$Tl transport in olfactory nerve in the early and late posttransection groups

The transport rate of $^{201}$Tl in olfactory nerve fibers was significantly higher in 14-day and 28-day BNTX mice (late post-nerve transection group) than in the 2-day and 7-day BNTX mice (early post-nerve transection group) (Figure 3; $N = 6$ for each group; Mann–Whitney $U$ test, $**P < 0.005$; early group: minimum, 1.312; median, 6.269; maximum, 13.08; 25th percentile, 3.183; 75th percentile, 10.27; late group: minimum 12.89; median, 14.8; maximum, 28.63; 25th percentile, 13.28; 75th percentile, 28.38). The transport rate of $^{201}$Tl in the olfactory nerve improved with time after nerve transection.

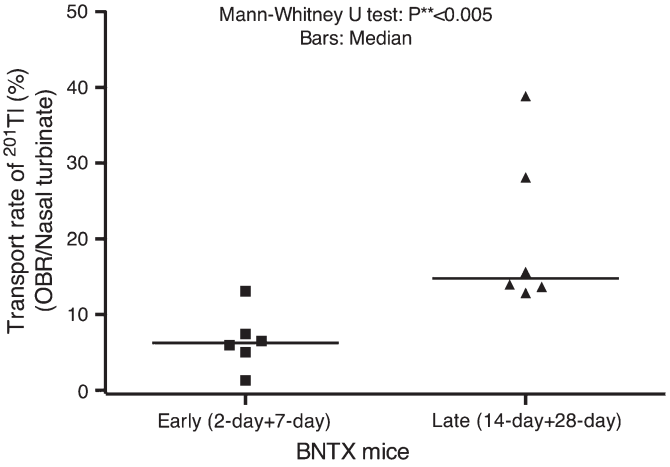


**Figure 3** The significant difference between the early posttransection group (2-day and 7-day BNTX mice) and late posttransection group (14-day and 28-day BNTX mice) in the transport rate of $^{201}$Tl to the OBR from the right nasal cavity. The transport of $^{201}$Tl in olfactory nerve fibers ($^{201}$Tl uptake percentage per weight in OBR/$^{201}$Tl uptake percentage per weight in the right nasal turbinate) was significantly higher in the late group than in the early group ($N$ = 6 for each group; Mann–Whitney $U$ test, $**P < 0.005$). Bars indicate medians.

### $^{201}$Tl uptake images with autoradiography

To show the relationship between $^{201}$Tl uptake images in the olfactory bulb and the extent to which olfactory nerve fibers were damaged, we compared $^{201}$Tl uptake images of mouse heads among control, 2-day BNTX mice, and 28-day BNTX mice using autoradiography. $^{201}$Tl uptake in the OBR 2 days after nerve transection and $^{201}$Tl administered into the right nasal cavity were significantly lower than the increased uptake in 28-day BNTX mice (Figure 4). The regeneration of olfactory nerve fibers could be assessed using this $^{201}$Tl-based imaging method.

## Discussion

We demonstrated a significant correlation between ODA (the cycloheximide solution avoidance behavior) and the $^{201}$Tl transport rate in the olfactory nerves of mice with olfactory nerve transection and controls. The regeneration and recovery of olfactory nerve fibers may be related to the transport rate of thallium from the nasal cavity to the olfactory bulb. Furthermore, our results show that the transport rate of $^{201}$Tl in the olfactory nerve improved with time after nerve transection, and the regeneration of olfactory nerve fibers could be determined using $^{201}$Tl-based imaging method. Our $^{201}$Tl-based imaging method may be useful for the

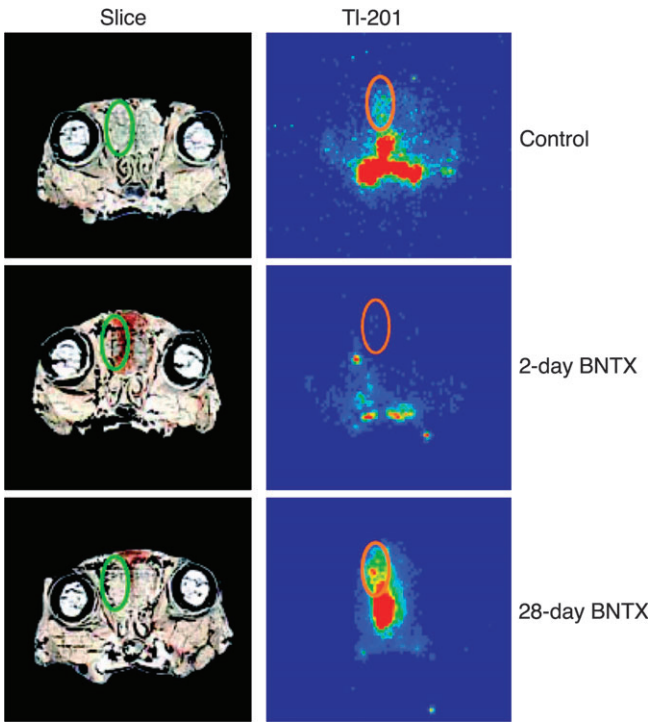


**Figure 4** Representative isotope images of coronal sections of the head 3 h after the unilateral nasal administration of $^{201}$TlCl solution (control, 2-day, and 28-day BNTX mice). Circles outline the olfactory bulb.

analysis of the connections between nasal cavity and olfactory bulb in olfactory-impaired animal models.

Meredith and O'Connell reported horseradish peroxidase (HRP) uptake from the nasal cavity to the brain in mammals (Meredith and O'Connell 1988). HRP reaction was assessed by histochemistry in the previous report. $^{201}$Tl uptake in samples can be counted by gamma spectrometry; therefore, thallium may be more simple and quantitative than HRP. Furthermore, if $^{201}$Tl uptake in the olfactory bulb and nasal cavity could be visualized with a gamma camera in live animals, follow-up would be available in vivo without sacrifice after nerve transection.

Current olfactory function tests (the University of Pennsylvania Smell Identification Test [Doty et al. 1984], the Connecticut Chemosensory Clinical Research Center Test [Cain et al. 1988], and Sniffin sticks [Hummel et al. 1997; Kobal et al. 2000]) depend on the patient's voluntary response to odor. So far, we have no method to visualize the connection between olfactory epithelium and the olfactory bulb in the clinic.

$^{201}$Tl is routinely used intravenously in nuclear medicine. If we could adapt a nasal $^{201}$Tl administration technique to a patient with head injuries resulting in anosmia, the decrease of odor-evoked signals in fMRI due to disconnection of olfactory nerve fibers could be validated when damage to olfactory nerve fibers is assessed with fMRI. We are planning to test the new olfactory nerve imaging technique with thallium on humans after approval from the ethics committee of Kanazawa University Hospital.

It was shown that the inhalation of manganese-54 ($^{54}$Mn) results in extensive uptake of $^{54}$Mn by the olfactory bulb in rats (Brenneman et al. 2000). The detection of damage to the olfactory system with fMRI in live animals administered manganese into the nasal cavity has been reported (Drobyshevsky et al. 2006); however, high doses of manganese inhalation are known to be associated with neurotoxicity in both animals and humans (Aschner et al. 2007). Therefore, $^{201}$Tl scintigraphy with nasal administration of $^{201}$Tl may be more safety than imaging with $^{54}$Mn administration. The effects of the nasal $^{201}$Tl administration technique on olfactory function are under investigation in animals.

Recovery of chemosensory deficits occurs up to 12–18 months after a traumatic event (Reiter et al. 2004). In humans, the recovery rate in patients with traumatic olfactory impairment is less than 30% (Fujii et al. 2002). Patients with intact olfactory nerve fibers may be well selected by means of a new isotope imaging technique for the long-term treatment of olfactory dysfunction. $^{201}$Tl scintigraphy with nasal administration of $^{201}$Tl may be also useful for the analysis of treatment efficacy with new medicines for patients with post-traumatic olfactory impairment.

In conclusion, damaged olfactory nerve fibers could be assessed in an animal model of olfactory impairment through the nasal administration of $^{201}$Tl. The uptake of $^{201}$Tl in the olfactory bulb correlated with the ODA of the mice.

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