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# Anti-cholinergics mecamylamine and scopolamine alleviate motion sickness-induced gastrointestinal symptoms through both peripheral and central actions

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## HIGHLIGHTS

- MEC and SCOP as well as HEX and MSCP alleviated MS-related nausea and emesis.
- MEC failed to attenuate MS-related hypothermia as the SCOP did, but aggravated MS-induced balance disorder.
- MEC was more effective for inhibiting Fos expression in the CVN and NTS than SCOP.

## ARTICLE INFO

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## ABSTRACT

Enhanced cholinergic activity contributes to the production of complex autonomic manifestations of motion sickness (MS). However, whether anti-cholinergics exert their anti-MS effects through central or peripheral actions remained unclarified. In the present study, we investigated the effects of mecamylamine (MEC) and scopolamine (SCOP) on rotation-induced gastrointestinal symptoms (conditioned gaping and defecation), locomotion disturbances (hypoactivity and impaired balance performance), hypothermia as well as Fos expression in vestibulo-autonomic regions in rats. We also observed the effects of hexamethonium (HEX) and methyl scopolamine (MSCP) on those MS behavioral responses. The efficacy of all these drugs on rotation-induced emesis and other MS symptoms in cats was also examined. We found that intragastric administration of MEC and SCOP inhibited rotation-induced gaping and defecation in rats, but only MEC showed a dose-dependent manner. MEC aggravated rotation-induced balance disorder and failed to attenuate rotation-induced hypothermia as the SCOP did. MEC was more effective for inhibiting Fos expression in the caudal vestibular nucleus and nucleus of solitary tract than SCOP. Intraperitoneal injection of HEX and MSCP also significantly alleviated rotation-induced gastrointestinal symptoms, and showed benefit to balance performance in rats. In cats, MEC, SCOP and HEX had prophylactic effects against rotation-induced emesis and salivation, and deceased non-retching/vomiting symptoms, but MSCP only attenuated emesis. It suggested that MEC and SCOP might alleviate gastrointestinal symptoms of MS via inhibiting peripheral autonomic nervous system and central vestibulo-autonomic pathways. The nicotinic acetylcholine receptor inhibitors like MEC might be new candidates against gastrointestinal symptoms induced by MS or other vestibular disorders.

## 1. Introduction

Motion sickness (MS), a common problem during travelling and virtual reality immersion in modern society, is induced by sensory conflict that eliciting complex autonomic signs and symptoms (Wang et al., 2017; Zhang et al., 2016). It is hypothesized that release of a cholinomimetic agent might be involved in the production of a mixture of pattern sympathetic and parasympathetic disturbances, such as nausea, vomiting, pallor, sweating, increased salivation as well as cardiovascular responses during MS (Eisenman, 2009; Sheehan et al., 2011; Zhang et al., 2016). Diverse muscarinic acetylcholine receptor (mAChR) and nicotinic acetylcholine receptor (nAChR) subtypes were expressed in the vestibular and autonomic nervous system (Hiel et al., 1996; Li et al., 2007) and is believed to be involved in modifying

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function of both the vestibular and peripheral autonomic nervous system (Spinks and Wasiak, 2011; VanPatten and Al-Abed, 2017). However, the contribution of mAChR and nAChR to the development of MS-related the autonomic responses still remains ambiguous.

Mecamylamine (MEC), a noncompetitive nAchR antagonist, was originally used as an antihypertensive agent via its inhibition effect on nAchR activity at sympathetic ganglion against excitation by Ach (Stone et al., 1956). MEC can also traverses the blood brain barrier easily and exert central effects at much lower doses than those used to treat hypertension (Nickell et al., 2013). Intraperitoneal (i.p.) MEC injection abolished nicotine-conditioned place preference and significantly reduced nicotine-induced Fos protein expression in the rat brain (Pascual et al., 2009; Seppa et al., 2001). Preclinical and clinical studies suggest that MEC may have therapeutic efficacy in a variety of neurological disorders including mood disorders, cognitive impairment (Nickell et al., 2013) and smoking cessation at relatively low doses without significant hypotension or severe side effects in humans (Nickell et al., 2013; Shytle et al., 2002). In our recent study, we found that MEC administrated to the caudal vestibular nucleus (CVN), i.e. the caudal medial vestibular nucleus (MVe) and spinal vestibular nucleus (SpVe), significantly alleviated rotation-induced defecation and hypoactivity in rats (Wang et al., 2015). In vitro studies showed that MEC alone could inhibit Ach-induced gastrointestinal smooth muscle contraction and reduce electrically stimulated twitch responses in isolated phrenic nerve-rat diaphragm preparation (Young et al., 2001). Cerebral ventricles injection of MEC had no significant effect on area postrema-mediated emesis-induced by chemicals such as noradrenaline, dopamine and clonidine in cats, suggesting that MEC might also prevent gastrointestinal events through its peripheral activity. (Beleslin and Strbac, 1987; Japundzic-Zigon et al., 1997; Jovanovic-Micic et al., 1995). However, to our knowledge, the effect of MEC on MS-induced nausea and vomiting has not been investigated yet. Moreover, scopolamine (SCOP), a nonselective competitive mAChR inhibitors, has been widely used as a first line medication against MS for a long period of time (Soto et al., 2013; Spinks and Wasiak, 2011). Nevertheless, whether the anti-MS benefits of MEC and SCOP depend on their central action and/or the peripheral actions still remains controversial and needs verification (Sheehan et al., 2011).

Rodents as the most widely used lab animals can not vomit possibly due to anatomical characteristics such as reduced diaphragmatic muscle and stomach geometry dissimilar to emetic species as well as the lack of critical brainstem emetic circuitry (Horn et al., 2013). Nevertheless, they indeed experience “nausea” or visceral malaise indicated by conditioned gaping, conditioned taste aversion or pica after emetic stimulation, making them a useful animal model for the study of MS (Fox and McKenna, 1988; Morita et al., 1988; Parker et al., 2008). In the present study, we firstly investigated the efficacy of MEC and SCOP via observing their effects on Ferris wheel-like rotation-induced MS responses including gastrointestinal (GI) symptoms: conditioned gaping and defecation (Cordick et al., 1999; Ossenkopp and Frisken, 1982; Ossenkopp et al., 2003), locomotion disturbances: hypoactivity and balance disorder (McCaffrey, 1985; Ossenkopp et al., 1994; Wang et al, 2015, 2017), and rotation-induced hypothermia which has been shown to be associated with nausea in rodents (Nalivaiko et al., 2014; Ngampramuan et al., 2014; Nobel et al., 2010). Secondly, we tried to find out the effects of peripheral nAChR and mAChR blockers, hexamethonium (HEX) and methyl scopolamine (MSCP), on these MS responses in rats. Anatomical and physiological studies have confirmed that activation of CVN and its downstream autonomic area including the nucleus of solitary tract (NTS), the parabrachial nucleus (PBN), the medial and central amygdala (MeA and CeA) and the locus ceruleus (LC) were critical for production of antonomic responses during MS (Balaban et al., 2014; Pompeiano et al., 2004). Thus, using Fos protein as a marker for neuronal activation (Cai et al., 2010; Zhou et al., 2017), we tried to identify the potential central targets of MEC and SCOP via examine the Fos expression in these vestibular-autonomic related areas after rotation in rats. Given that rats don't have the ability of vomit, we finally observed the efficacy of MEC and SCOP as well as HEX and MSCP on rotation-induced emesis and other MS symptoms in the cats.

## 2. Materials and methods

### 2.1. Animals and ethics

Adult male Sprague–Dawley rats, weighing 250–300 g, were purchased from Shanghai Laboratory Animal Center. Adult male Domestic Shorthair cats (3–5 kg) were purchased from the Animal Center of the Dalian Medical University. The animals were individually housed under a 12 h light: 12 h dark cycle (temperature: 22 ± 2 °C and lighting: 8:00–20:00) with free access to food (standard chow for the rats and commercial pallet food for the cats) and distilled water. All animals were adapted to the lab environment for at least 2 weeks at room temperature (22–24 °C) before initiation of the experiment. All animal protocols and procedures complied with the Guide for the Care and Use of Laboratory Animals (National Research Council U.S., 2011) and were approved by the Ethics Committee for Animal Experimentation of the Second Military Medical University (Shanghai, PR China). All efforts were made to minimize the number of animals used and their suffering. The rats and the cats received experimental treatments and behavioral testing during 8:00–12:00 p.m. and 8:00–12:00 a.m., respectively.

### 2.2. Rotation device and procedures

The rotation device was modified based on the one created by Crampton and Lucot (1985), and was used in our previous studies (Cai et al, 2007, 2010; Wang et al, 2012, 2017; Zhou et al., 2017). It consisted of two plexiglass boxes suspended on two metal frames which were fixed on the rotation arm of the vertical wheel, like the so called Ferris wheel in the amusement parks. Each plexiglass box was composed of four separate plexiglas chambers (for rat) or one chamber (for cat) with the long axis parallel to rotation axis. The size of each plexiglas chamber for rat is 22.5 cm × 26 cm × 20 cm (Length × Width × Height) and that for cat is 90 cm × 26 cm × 20 cm (Length × Width × Height). During rotation, the animals were placed in separate chambers without restraint to minimize general stress. The frames together with the plexiglass boxes revolved about an rotation axis parallel to the floor. They started to rotate in a clockwise direction at 16°/s$^2$ to reach an angular velocity of 120°/s and then began to decelerate at 48°/s$^2$ to reach 0°/s. After a 1 s pause, the container continued to rotate in a counter-clockwise direction in the same manner as above. The clockwise-pause-counterclockwise cycle lasted approximately 21 s. The magnitude of angular velocity and centrifugal acceleration ranged from 0 to 120°/s to 0–2.22 m/s$^2$, respectively. The animals in the rotation (Rot) groups received 2 h (rats) or 30 min (cats) of rotation stimulation. The rotation time period was determined based on the behavioral observation in our previous studies and the pilot study (Cai et al, 2007, 2010; Wang et al, 2012, 2017; Zhou et al., 2017). The animals in the static control (Sta) groups were kept in the plexiglass chambers near the rotation device without rotation and were only exposed to the noise and the vibrations when the device were being rotated.

### 2.3. Drugs

MEC and SCOP were purchased from Aladdin (Shanghai, China), while HEX and MSCP were purchased from Sigma-Aldrich (St. Louis, USA) and Selleckchem (Houston, USA), respectively. These drugs were dissolved in 0.9% saline (SAL) solution. We used MSCP and HEX to selective block peripheral mAchR and nAchR, respectively, because both drugs have poor penetration of blood brain barrier (Johnson Rowsey et al., 2002; Miyabara et al., 2017; Piotrovskiy et al., 2016). Previous studies have confirmed that i.p. application of HEX at a single

low dose (5 mg/kg) showed no central effects (Piotrovskiy LB et al. Dokl Biochem Biophys. 2016; 468:173–5), while intravenous administration showed apparent penetration into the brain at a high dose (10 mg/kg) (Asghar K and Roth LJ. Biochem Pharmacol. 1971; 20: 2787-95). In the present study, in order to investigate whether peripheral nAchR and mAchR might play a role in production of nausea and vomiting in MS, MSCP and HEX were delivered i.p. in the same sets of experiments for the consistency of administration route. On the other hand, MEC and SCOP were administered intragastrically (i.g.) for evaluating the potential of MEC to be used clinically as an anti-MS drug in the future. All tested drugs and SAL were given 30 min before Rot or Sta treatment. For the rats, the dosages used were selected based on the levels shown to block the mAchR or nAchR effectively in previous studies (Gordon and Grantham, 1999; Johnson Rowsey et al., 2002; Kunisawa et al., 2016; Lenoir et al., 2013; Rowsey and Gordon, 2000). MEC or SCOP (0.1 mg/kg ~ 4 mg/kg) solution in a volume of 1 ml were i.g. administrated rapidly via an oral-gastric gavage. MSCP (1 mg/kg) was i.p. delivered. Given the short action duration, HEX was i.p. administered repeatedly (3 mg/kg every 30 min) for totally 5 doses with the initial dose given at 30 min before Rot or Sta treatment. For the cats, MEC and SCOP (0.1 mg/kg and 0.4 mg/kg) was administrated orally using #4 PCcaps gelatin capsules (CAPSUGEL, Peapack, NJ. USA) into which appropriate amount of each drug or starch (STH) was filled using multiple capsule filler kit according to the instructions of the manufacturer (CAPSUGEL, Peapack, NJ. USA). The cat was fixed and the mouth was pried open gentally by pinching the upper and lower jaw canine teeth. The capsule inserted in the tip of the dosing syringe (CAPSUGEL, Peapack, NJ. USA) was injected rapidly into the cat mouth right onto the root of tongue. Then the upper and lower jaw was rapidly closed and the capsule was swallowed consequently. HEX and MSCP were i.p. injected at a single dose of 1.2 mg/kg and 0.4 mg/kg, respectively. The drug dosages used in the cats were converted from the effective doses in the rats based on body-surface-area.

### 2.4. Experiment design and grouping

#### 2.4.1. Experiment 1

Two sets of experiment were performed using 180 animals for conditioned gaping test and balance beam test, and another 180 animals for defecation and spontaneous locomotion observations. In each set of experiment, animals were randomly divided into 9 Rot groups or 9 corresponding Sta groups (n = 10 in each group), and received SAL or different doses of MEC or SCOP. Similarly, additional two batches of rats were used for examine the effects of HEX and MSCP on MS-related behavior responses. Each batch of animals was randomly divided into 6 groups: HEX/Rot, MSCP/Rot, SAL/Rot, HEX/Sta, MSCP/Sta and SAL/Sta groups (n = 8 in each group).

#### 2.4.2. Experiment 2

Forty eight rats were randomly assigned to 6 groups: MEC (1 mg/kg, i.g.)/Rot, SCOP (1 mg/kg, i.g.)/Rot and SAL/Rot, as well as corresponding 3 Sta groups (n = 8 in each group). The core body temperature was continuously monitored from 120 min before drug administration to 30 min after Rot or Sta treatment. Animals in each group were sacrificed immediately thereafter for further Fos immunostaining in the brain.

#### 2.4.3. Experiment 3

Twelve male MS susceptible cats which showed retching, vomiting and/or salivation during an initial 2 h Rot were used. A repeated-measure design was performed to evaluate the anti-MS effects of MEC and SCOP. The animals received the following treatments in sequence: 1st STH/Rot, 0.4 mg/kg MEC/Rot, 0.1 mg/kg MEC/Rot, 0.4 mg/kg SCOP/Rot, 0.1 mg/kg SCOP/Rot and 2nd STH/Rot. Each treatment session was separated by a washout interval of 20 days in order to avoid the MS habituation effect. The second STH/Rot treatment was performed to verify the MS susceptibility after the prior 6 sessions of repeated Rot treatments.

Additional 13 male MS susceptible cats were used and randomly divided into two groups which received HEX/Rot (n = 6) or MSCP/Rot (n = 7) treatment 20 days after the SAL/Rot treatment (self control).

### 2.5. Telemetric sensor implantation

The implantation surgery was carried out according to the manufacturer's instructions (DSI, St. Paul, USA). A radiotelemetric sensor (TA10TA-F40) was implanted intra-abdominally after midline laparotomy. The abdominal muscles and skins were then sutured under sodium pentobarbital (40 mg/kg, i.p.) anesthesia. After the surgery, all animals received antibiotics penicillin (400000 U/kg, i.p.) and analgesics ibuprofen (30 mg/kg, in the drinking water) once a day for 3 days and were allowed a 7-day recovery before further experiment. During the recording period, the telemetric receiver was fixed tightly on to the metal frame just under the rotation chamber where the animals were positioned. The core body temperature was continuously collected immediately after the radiotelemetric sensor was activated with a magnet. Data were sampled every 10 min and the mean temperature during the Rot or Sta treatment session was calculated and analyzed statistically.

### 2.6. MS-related behavior testing in rats

#### 2.6.1. Conditioned gaping test

The devices and detailed procedures were described in previous studies (Parker et al., 2008; Zhou et al., 2017). In brief, a 10 ml test tube containing a cotton dental roll saturated with vanilla flavor extract (McCORMICK; 35% alcohol) was attached to a hole at one side of the plexiglass chamber of the rotation device. The Rot paired simultaneously with the odor served as a conditioned stimulus (paired condition) and the gastric discomfort served as an unconditioned stimulus. The Sta-treated rats were only exposed to an odor context without rotation. All animals received three sessions of conditioning trial with 24 h interval. Twenty-four hours following the last conditioning trial, they received a 1 h test trial with the odor present but without rotation. The orofacial and somatic responses were recorded using a video camera (SONY, HDR-PJ670, Japan) mounted to the plexiglass chamber. Two raters who were blind to the experimental design and grouping counted the numbers of the gaping behavior (rapid, large-amplitude opening of the mandible with lower incisors exposed) by viewing the videotapes at a later time. The inter-rater reliability was examined for each group of all conditioned gaping experiments in the rats ($r_{(1, 30)} > 0.95$ based on the Cronbach's α model).

#### 2.6.2. Defecation and spontaneous locomotion test

The rats were taken out of the plexiglass containers for spontaneous locomotion testing immediately after Rot or Sta treatment. The number of fecal granules deposited by each animal in the plexiglass container was counted. Spontaneous locomotion was recorded by an animal behavior test system (RD1112-IFO-R-4, Mobiledatum, Shanghai, China) in a soundproof room. The apparatus consisted of a dark 40 × 40 × 45 cm rectangular chamber with the floor marked with a 16 × 16 grid. Each animal was gently placed in the center of the chamber and left undisturbed for 5 min. Locomotion tracking of the animals were recorded by an infrared digital video camera. The total distance travelled (cm) were measured with commercially available software (EthoVision XT 8.5, Noldus, Netherlands) (Wang et al., 2015).

#### 2.6.3. Balance beam test

Before testing, each animal was trained daily for 5–9 consecutive days in order to achieve a stable performance on an elevated (90 cm) narrow wooden beam (2.5 × 100 cm). During the training process, each rat was put into the black box for 2 min to let it be familiar with

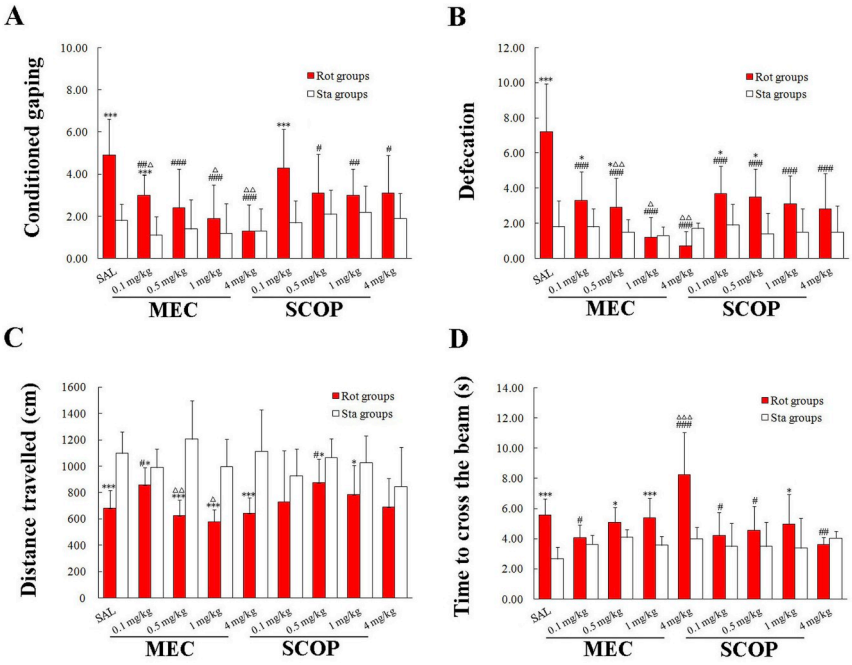


**Fig. 1. Effects of mecamylamine (MEC) and scopolamine (SCOP) at different doses on rotation-induced MS responses in rats assessed by the GI symptoms: conditioned gaping (A) and defecation reaction (B), and the locomotion measurements: total distance travelled (C) in the spontaneous locomotion test and time to cross the beam (D) in the balance beam performance.** SAL, saline; Rot, rotation; Sta, static. Data are represented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 compared with the corresponding Sta controls; #P < 0.05, ##P < 0.01, ###P < 0.001 compared with the SAL/Rot group; △P < 0.05, △△ P < 0.01, △△△ P < 0.001 compared with the corresponding SCOP/Rot groups with the same dose.

the dark environment. Then we put the animals on the beam with the distance to the box from near to far and let the animal walk to the dark box. During testing, 3 trials were performed immediately after Rot or Sta control treatment and the average time to traverse the beam from one end to enter the black plastic box (15 cm × 15 cm × 8 cm) at the other end for each animal were used for statistical analysis (Zhou et al., 2017). The animals were allowed a 60 s rest between trials to reduce stress and fatigue.

### 2.7. MS evaluation in cats

The MS-related behaviors during the Rot or Sta period were recorded using a video camera (SONY, HDR-PJ670, Japan). Emesis was assessed by examining the number of retching and vomiting (retching/vomiting), and the sum of retching/vomiting duration for each bout by viewing the videotapes at a later time. Salivation was judged by continuous visible drooling and/or foaming at the mouth. The number of salivation episodes and salivation duration were also recorded. MS severity was evaluated using a Suri symptom rating scale described in previous studies (Lucot, 2017; Suri et al., 1979). Each non-retch/vomit symptom was rated from 1 to 8 points according to the severity while 16 points was awarded for the presence of retching/vomiting. Both the non-retching/vomiting symptom scores and the total symptom scores were calculated and included in the further statistical analysis.

### 2.8. Immunohistochemistry

Animals were anesthetized with an overdose of sodium pentobarbital (100 mg/kg, i.p.) and perfused transcardially with 100 mL chilled saline and 500 mL of 0.1 mol/L phosphate buffer (PB, pH 7.4) containing 4% paraformaldehyde in sequence. The brains were removed, postfixed with 4% paraformaldehyde at 4 °C for 1 h, and were immersed in the 0.1 mol/L PB containing 30% sucrose overnight at 4 °C. The brain blocks containing the CVN (Bregma −11.6 ~ -12.3 mm), the NTS (Bregma −12.7 ~ -14.3 mm), the PBN (Bregma −8.7 ~ -10.0 mm), the MeA and CeA (Bregma −1.8 ~ -3.6 mm) and the LC (Bregma −9.10 ~ -10.0 mm) were cut into 20 μm-thick sections throughout. One out of every 3 consecutive sections was selected and washed in 0.01 M phosphate-buffered solution (PBS) (pH 7.4). They were then incubated in a rabbit anti-Fos IgG (Santa Cruz Biotechnology Inc.; 1:1000) for 24 h at 4 °C. After washing in PBS, the sections were incubated in biotinylated goat anti-rabbit IgG (Jackson; 1:200) for 4 h. Fos labeling (Fos-LI) was visualized using ABC method according to the manufacturer's instruction (Vector Laboratories, Burlingame, CA). Under a light microscope, the number of Fos-LI neurons was counted by a rater who was unaware of the experimental conditions and the photographs were taken with a digital camera.

### 2.9. Statistical analysis

All statistical analyses were performed with the SPSS v13.0 program. Two-way ANOVA was performed using General Linear Protocol to analyze the main effect of Rot or drugs and their interactions in behavioral tests and body temperature recordings of the experiment 1 and 2. One-way ANOVA analysis was used to assess the numbers of Fos-LI neurons in experiment 2. In experiment 3, ANOVA analysis for repeated-measures or paired *t*-test were conducted to analyze the non-retching/vomitng symptom scores and total symptom scores, while the nonparametric Friedman test and Wilcoxon test were used for analyzing other indices due to heterogeneity of variance. Bonferroni post hoc analysis was conducted when a significant main effect was obtained. Pearson $\chi^2$ analysis was performed to test the occurrence of retching/vomiting and salivation in cats. All data are expressed as the mean ± S.D. Statistical significance was judged at P < 0.05.

## 3. Results

### 3.1. MEC and SCOP significantly inhibited Rot-induced GI symptoms in rats

Fig. 1 showed that Rotation stimulation significantly increased the number of gaping [two-way ANOVA Rot effect: F (1, 179) = 50.65, P < 0.0001; post hoc: P < 0.001] and defecation responses [Rot effect: F (1, 179) = 61.76, P < 0.0001; post hoc: P < 0.001] compared with the Sta controls in rats (Fig. 1A and B). There were also significant drug effects [gaping: F (8, 179) = 3.59, P < 0.001; defecation: F (8, 179) = 16.07, P < 0.0001] and a Rot × drug interaction [gaping: F (8, 179) = 3.89, P < 0.001; defecation: F (8, 179) = 5.64, P < 0.0001] on these two measurements. SCOP treatment significantly alleviated conditioned gaping at the doses of 0.5, 1 and 4 mg/kg (post hoc: P < 0.05 or 0.01 vs. SAL/Rot) and reduced defecation responses at all

doses ($P < 0.001$ vs. SAL/Rot). Meanwhile, MEC significantly suppressed Rot-induced conditioned gaping ($P < 0.01$ or 0.001 vs. SAL/Rot) and defecation responses ($P < 0.001$ vs. SAL/Rot) at all doses in a dose dependant manner. However, the Rot animals treated with MEC or SCOP at low doses still had higher numbers of gaping (0.1 mg/kg, $P < 0.001$) and defecation responses (0.1 and 0.5 mg/kg, $P < 0.05$) compared with the corresponding Sta controls. Rot animals treated with the MEC also showed lower numbers of gaping (doses: 0.1, 1 and 4 mg/kg; $P < 0.05$ or 0.01) and less defecation responses (doses: 0.5, 1 and 4 mg/kg; $P < 0.05$ or 0.01) compared with the corresponding SCOP-treated ones. No significant differences were observed in either conditioned gaping or defecation responses among Sta groups.

In the spontaneous locomotion test, rotation stimulation significantly reduced total distance travelled in the SAL-treated animals compared with the corresponding Sta controls [two-way ANOVA Rot effect: $F(1, 179) = 74.25$, $P < 0.0001$; post hoc: $P < 0.001$, Fig. 1C]. Although MEC at 0.1 mg/kg and SCOP at 0.5 mg/kg slightly alleviated Rot-induced hypoactivity compared with the SAL/Rot group [Rot × drug effect: $F(8, 179) = 4.91$, $P < 0.001$; post hoc: $P < 0.05$], the MEC- or SCOP-treated Rot animals still showed a remarkable decrease in total distance travelled compared with corresponding Sta controls ($P < 0.05$ or 0.001). The Rot rats treated with MEC at 0.5 and 1 mg/kg also showed lower spontaneous locomotion activity compared with the corresponding SCOP-treated ones ($P < 0.01$ and 0.05).

In the balance beam test, rotation stimulation significantly increased the time to cross the beam in the SAL/Rot animals compared with the SAL/Sta controls [Rot effect: $F(1, 179) = 67.86$, $P < 0.0001$; post hoc: $P < 0.001$, Fig. 1D]. The Rot animals pretreated with MEC at 0.5, 1 and 4 mg/kg [Rot × drug effect: $F(8, 179) = 6.51$, $P < 0.0001$; post hoc: $P < 0.05$ or 0.001] or SCOP at 1 mg/kg ($P < 0.05$) still showed balance disturbances compared with the corresponding Sta controls. The balance disorder was slightly improved by MEC at 0.1 mg/kg or SCOP at 0.1 mg/kg and 0.5 mg/kg ($P < 0.05$) and was completely inhibited by the SCOP at 4 mg/kg ($P < 0.01$) when compared with the SAL/Rot animals. However, the time to cross the balance beam was significantly longer in Rot animals treated with 4 mg/kg MEC compared with those treated with SAL or 4 mg/kg SCOP ($P < 0.001$).

### 3.2. HEX and MSCP also alleviated Rot-induced GI symptoms in rats

Experiment 1 showed that both MEC and SCOP alleviated MS-induced gastrointestinal responses in rats. In this experiment, we investigated the effects of peripheral AchR inhibitors HEX and MSCP on these measurements. As before, the conditioned gaping [Rot effect: $F(1, 47) = 30.54$, $P < 0.0001$] and defecation reactions [Rot effect: $F(1, 47) = 100.116$, $P < 0.0001$] were remarkably induced in the SAL/Rot animals compared with the SAL/Sta controls (post hoc: $P < 0.001$, Fig. 2A and B). HEX and MSCP injection (i.p.) completely inhibited Rot-induced conditioned gaping [Rot × drug effect: $F(2, 47) = 20.98$, $P < 0.0001$; post hoc $P < 0.001$ and 0.01 vs. the SAL/Rot group], but only partly alleviated Rot-induced defecation responses [Rot × drug effect: $F(2, 47) = 18.002$, $P < 0.0001$; post hoc: $P < 0.01$ vs. SAL/Rot group; $P < 0.05$ and 0.01 vs. corresponding drug-treated Sta controls]. MSCP had no significant effect on Rot-induced hypoactivity [Rot effect: $F(1, 47) = 86.18$, $P < 0.0001$; Rot × drug effect: $F(2, 47) = 3.87$, $P < 0.05$; post hoc: $P < 0.01$ vs. Sta controls] which was slightly aggravated by HEX pretreatment ($P < 0.01$ vs. SAL/Rot group Fig. 2C). In contrast, HEX significantly inhibited Rot-induced balance disorder [Rot effect: $F(1, 47) = 84.18$, $P < 0.0001$; Rot × drug effect: $F(2, 47) = 20.68$, $P < 0.0001$; post hoc: $P < 0.001$ vs. the SAL/Rot group) which was also alleviated by MSCP ($P < 0.01$ vs. SAL/Rot and MSCP/Sta groups, Fig. 2D). No significant differences between MEC/Rot and HEX/Rot groups were observed in all these behavior measurements.

### 3.3. SCOP but not MEC attenuated Rot-induced hypothermia in rats

Animals in all of the three Rot groups showed a abrupt decline in core body temperature during the initial 30 min of rotation (Fig. 3A). The averaged body temperature during the whole rotation period in Rot animals was significantly lower than the corresponding Sta controls [Rot effect: $F(1, 47) = 189.553$, $P < 0.0001$; post hoc: $P < 0.05$ or 0.01, Fig. 3B]. SCOP (1 mg/kg) slightly increased the core body temperature during rotation (Rot × drug effect: $F(2, 47) = 9.60$, $P < 0.0001$; post hoc: $P < 0.05$ vs. the SAL/Rot group), while MEC (1 mg/kg) had no significant effect. No difference was observed among Rot groups before or after the rotation period. There was also no difference among the Sta groups at all three stages, suggesting that the drugs alone had no effect on the body temperature.

### 3.4. MEC and SCOP significantly altered Fos expression pattern after rotation in rats

Rotation stimulation significantly increased the numbers of Fos-LI neurons in the CVN [one-way ANOVA: $F(3, 31) = 537.15$, $P < 0.0001$; Fig. 4A, 5A1 and A4], the NTS [$F(3, 31) = 179.27$, $P < 0.0001$; Figs. 4B and 5B1 and B4], the PBN [$F(3, 31) = 216.31$, $P < 0.0001$; Figs. 4C and 5C1 and C4], the MeA [$F(3, 31) = 149.97$, $P < 0.0001$; Figs. 4D and 5D1 and D4], the CeA [$F(3, 31) = 119.67$, $P < 0.0001$; Figs. 4E and 5D1 and D4] and the LC [$F(3, 31) = 245.77$, $P < 0.0001$; Figs. 4F and 5E1 and E4] compared with the SAL-Sta controls (post hoc: $P < 0.01$). Both MEC and SCOP significantly decreased the numbers of Fos-LI neurons in the CVN (Figs. 4A and 5A2 and A3) and the NTS (Figs. 4B and 5B2 and B3) after rotation ($P < 0.05$, 0.01 or 0.001 vs. SAL/Rot). MEC-treated animals also showed lower numbers of Fos-LI neurons in the CVN (Figs. 4A and 5A2 and A3) and the NTS (Figs. 4B and 5B2 and B3) than the SCOP-treated ones ($P < 0.05$). In contrast, Fos expression in the MeA and the CeA was significantly higher in the MEC/Rot group than those in the SCOP/Rot group ($P < 0.05$, Fig. 4D, E, 5D2 and D3). No significant difference was observed among Rot groups in Fos expression of the PBN or the LC. MEC/Rot and SCOP/Rot animals still had increased Fos-LI neuron numbers in all these brain regions compared with the SAL/Sta ones ($P < 0.01$).

### 3.5. MEC and SCOP as well as HEX and MSCP attenuated MS-induced emesis in cats

Fig. 6 showed that rotation significantly induced retching/vomiting and salivation responses in 80% and 90% animals during the 1st STH/Rot session. Both MEC and SCOP produced dose-dependent decreases in retching/vomiting episode numbers (Friedman $\chi^2 = 11.80$, $P < 0.05$; Fig. 6A) and duration (Friedman $\chi^2 = 11.42$, $P < 0.05$; Fig. 6B). MEC at 0.4 mg/kg significantly reduced the incidence of retching/vomiting (Pearson $\chi^2 = 5.05$, $P < 0.05$) and its episode numbers and duration (post hoc: $P < 0.05$ or 0.01), while SCOP at 0.4 mg/kg only decreased retching/vomiting episode numbers and duration ($P < 0.05$) when compared with those of the 1st STH/Rot. Animals also showed a shorter retching/vomiting duration when they were treated with 0.4 mg/kg MEC than those treated with 0.1 mg/kg MEC or 0.4 mg/kg SCOP ($P < 0.05$). Furthermore, salivation episode numbers (Friedman $\chi^2 = 13.87$, $P < 0.05$; post hoc: $P < 0.05$) and duration (Friedman $\chi^2 = 33.01$, $P < 0.001$; post hoc: $P < 0.01$) were significantly decreased during the 2nd STH/Rot session when compared with 1st one, suggesting a possible habituation effect on salivation responses after six sessions of 30 min rotation. Although neither MEC nor SCOP at any doses significantly affected numbers of salivation episodes (Fig. 6C), these two drugs remarkably deceased salivation duration at the dose of 0.4 mg/kg compared with that of the 0.1 mg/kg treatments ($P < 0.05$) and the 1st ($P < 0.01$) and 2nd STH/Rot controls ($P < 0.001$, Fig. 6D). Moreover, MEC at 0.4 mg/kg also significantly decreased the

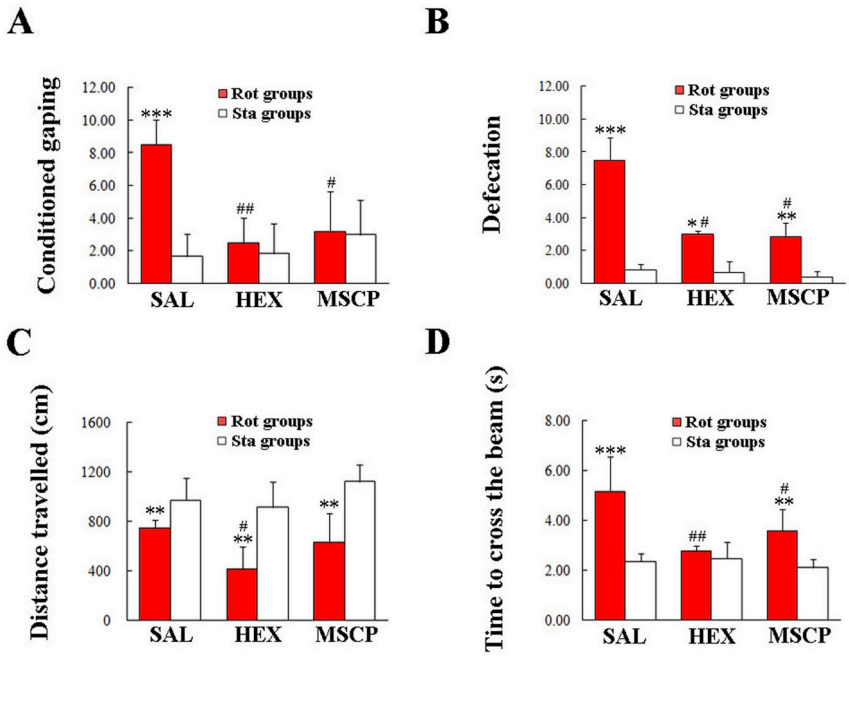


**Fig. 2. Effects of hexamethonium chloride (HEX) and methyl scopolamine (MSCP) pretreatment on rotation-induced MS responses in rats.** MS responses were evaluated by the GI symptoms: conditioned gaping (A) and defecation reaction (B), and the locomotion measurements: total distance travelled (C) in the spontaneous locomotion test and time to cross the beam (D) in the balance beam performance. SAL, saline; Rot, rotation; Sta, static. Data are represented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 compared with the corresponding Sta controls; #P < 0.01, ##P < 0.001 compared with the SAL/Rot group.

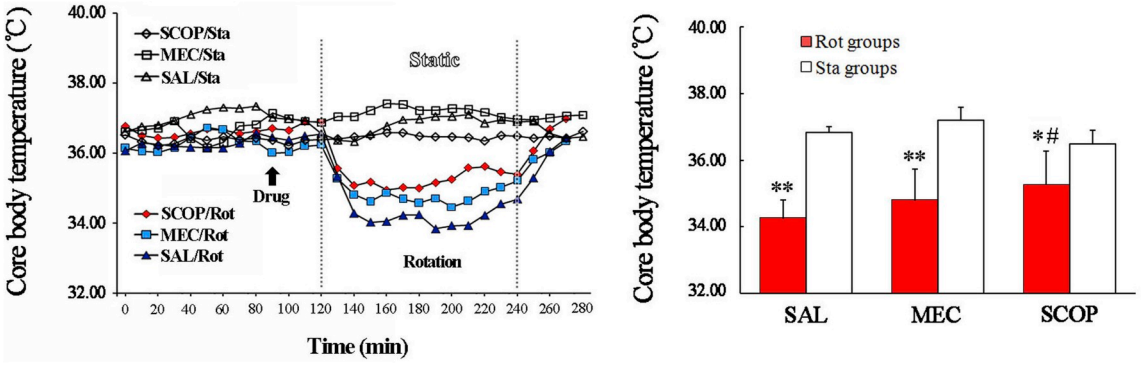


**Fig. 3. Effects of mecamylamine (MEC) and scopolamine (SCOP) pretreatment on rotation-induced hypothermia in rats.** SAL, saline; Rot, rotation; Sta, static. (A) mean group data of each time point for continuous recording of the core body temperature in Rot and Sta groups. The arrow indicates the time point of drug administration i.e. 30 min prior to rotation or static treatment. (B) Histogram showing the mean body temperature during the Rot or Sta period. Data are represented as mean ± SEM. *P < 0.05, **P < 0.01 compared with the corresponding Sta controls; #P < 0.05 compared with the SAL/Rot group.

non-retching/vomiting symptom score [F (2.50, 22.48) = 4.29, P < 0.05] and the total symptom score [F (3.35, 30.13) = 3.56, P < 0.05] compared with the 1st and 2nd STH/Rot controls (post hoc: P < 0.05 or 0.01, Fig. 6E and F).

HEX administration significantly decreased the number of retching/vomiting episodes (Wilconxon Z = −1.89, P < 0.05 vs. SAL/Rot session, Fig. 7A) and salivation episodes (Wilconxon Z = −2.12, P < 0.05 vs. SAL/Rot session, Fig. 7 C) and concomitantly shortened their duration (Wilconxon Z = −2.02, P < 0.05, Fig. 7B and D) during rotation. HEX also decreased the incidence of salivation (Pearson $\chi^2$ = 5.33, P < 0.05). In the meantime, MSCP significantly decreased retching/vomiting episode numbers and duration (Wilconxon Z = −1.94, P < 0.05; Fig. 7A and B), but failed to affect salivation compared with those of the SAL/Rot treatment (Fig. 7C and D). Additionally, HEX also decreased the non-retching/vomiting symptom scores (t = 7.05, P < 0.001 vs. SAL/Rot session, Fig. 7E) as well as the total symptom scores (t = 5.14, P < 0.01, Fig. 7F), while MSCP only reduced total symptom scores (t = 3.200, P < 0.05) during rotation.

## 4. Discussion

Nausea and vomiting are the cardinal and severe manifestations of MS (Lackner, 2014). As laboratory rodents cannot vomit, conditioned gaping reactions elicited by a emetic stimulation-paired context, taste and/or odor has been used as a rat model of anticipatory nausea (DeVuono et al., 2018; Limebeer et al., 2008; Parker, 2014; Parker et al., 2016; Rock et al., 2016; Sticht et al., 2016). Numerous studies have demonstrated successful utilization of conditioned gaping as a rat model of MS-related nausea (Bolognini et al., 2013; Cordick et al., 1999; Limebeer et al., 2008; Ossenkopp et al., 2003; Zhou et al., 2017). In the present study, we successfully produced rotation-induced conditioned gaping and other commonly reported MS-related symptoms including defecation incontinence and hypoactivity in rats (Limebeer et al., 2008; McCaffrey, 1985; Ossenkopp and Frisken, 1982; Ossenkopp et al., 1994; Parker and Limebeer, 2006; Wang et al., 2015; Zhou et al., 2017). During the balance beam test, we observed that animals had the intention to walk but exhibited significant hesitation and imbalance-like behaviors, including head shaking, unstable stepping and tail swing. This kind of imbalance-like behaviors during balance beam test was also observed in a previous study which clearly demonstrated that mice showed hesitation and instability during balance beam performance leading to increased time to traverse the beam after vestibular challenge (Tung et al., 2014). In this study, the authors also reported that vestibular challenge lead to falling from the beam in some mice

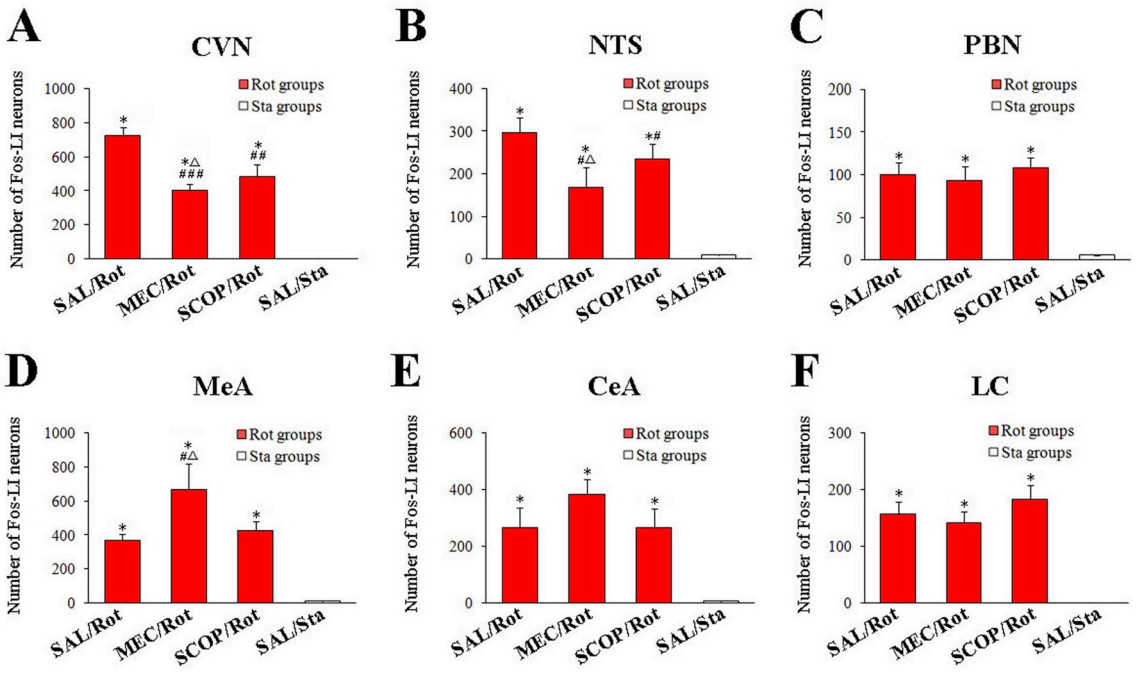


**Fig. 4. Effects of mecamylamine (MEC) and scopolamine (SCOP) pretreatment on the numbers of Fos-labeled (Fos-LI) neurons in the caudal vestibular nucleus (CVN, A), the nucleus of solitary tract (NTS, B), the parabrachial nucleus (PBN, C), the medial amygdala (MeA, D), the central amygdala (CeA, E) and the locus ceruleus (LC, F) after rotation in rats.** SAL, saline; Rot, rotation; Sta, static. Data are represented as mean ± SEM. *P < 0.01 compared with the SAL/Sta group; #P < 0.05, ##P < 0.01, ###P < 0.001 compared with the SAL/Rot group; △P < 0.05 compared with the SCOP/Rot group.

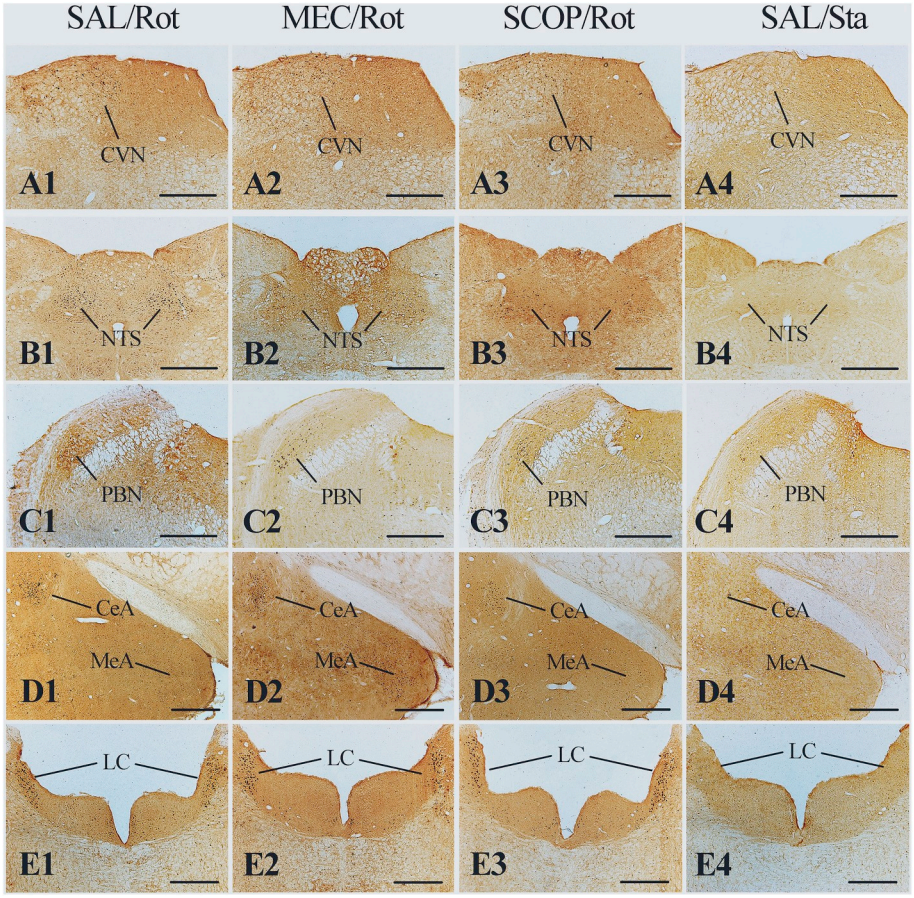


**Fig. 5. Representative photomicrographs showing Fos-labeled (Fos-LI) neurons in the caudal vestibular nucleus (CVN, A1-A4), the nucleus of solitary tract (NTS, B1—B4), the parabrachial nucleus (PBN, C1—C4), the medial amygdala (MeA, D1-D4), the central amygdala (CeA, D1-D4) and the locus ceruleus (LC, E1-E4) in rats of different groups.** MEC, mecamylamine; SCOP, scopolamine; SAL, saline; Rot, rotation; Sta, static. Bar = 500 μm.

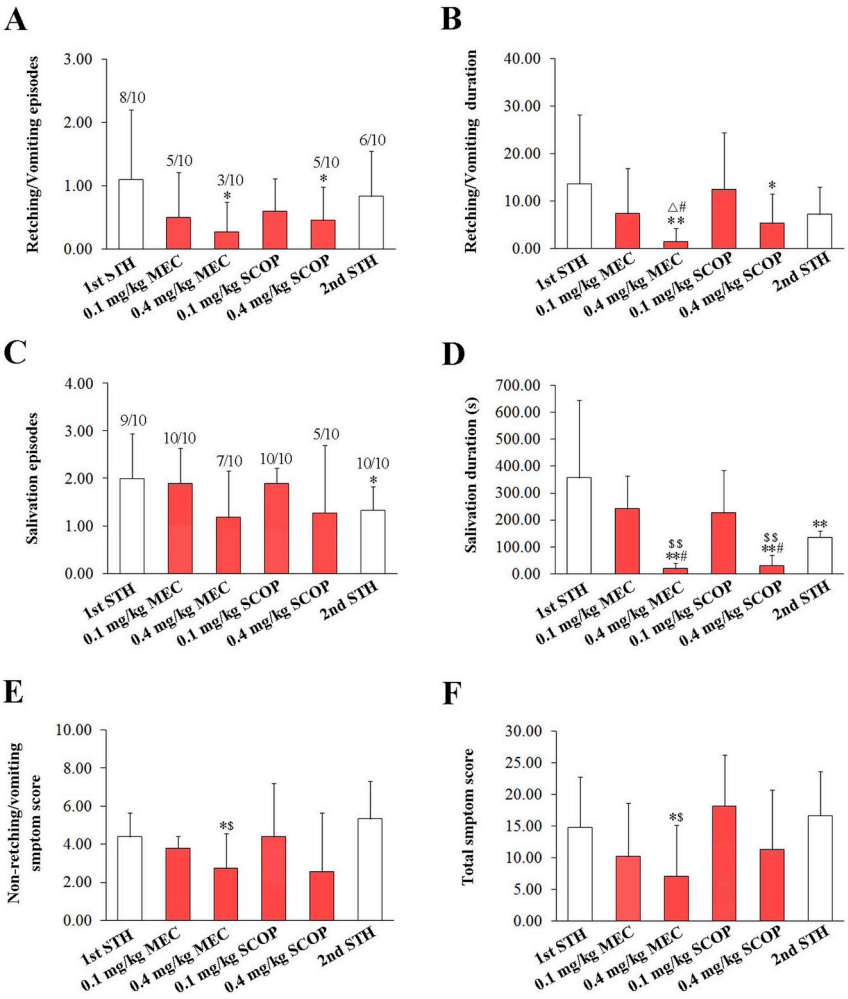


**Fig. 6. Effects of mecamylamine (MEC) and scopolamine (SCOP) at different doses on rotation-induced MS symptoms in cats.** Emesis was assessed by measuring the number of retching/vomiting and salivation episodes (A and C) and the sum of duration for each episode (B and D) in each cat. The occurrence of retching/vomiting and salivation is shown above the column in A and C, respectively. MS severity was evaluated by the non-retching/vomiting score (E) and total symptom score (F) determined by the Suri rating scale. STH, starch; Rot, rotation; Sta, static. Data are represented as mean ± SEM. *P < 0.05, **P < 0.01 compared with the data of the 1st STH/Rot treatment; #P < 0.05 compared with the 0.1 mg/kg MEC/Rot or SCOP/Rot treatment; △ P < 0.05 compared with the data of the corresponding SCOP/Rot treatments with the same dose. $ P < 0.01, $$ P < 0.001 compared with the data of the 2nd STH/Rot.

during testing, strongly suggesting the balance disturbance after vestibular challenge. It suggested that rodents might also manifest balance disorders like humans during or after passive motion challenge (Aykent et al., 2014; Koslucher et al., 2016; Stoffregen et al., 2013), and the imbalance-like behaviors but not the reduced motivation of locomotion could be the main reason for the increased time to traverse the beam.

The present study showed that oral administration of nAchR inhibitor MEC, like SCOP, dose-dependently alleviated MS-induced nausea-related conditioned gaping and defecation response in rats and also attenuated emesis and salivation in cats. Previous studies showed that intracerebroventricular nicotine injection or peripreral administration of nAchR agonists such as varenicline and cytisine can produce MS like symptoms including salivation, pallor as well as nausea and vomiting which could not be prevented by anti-muscarinic drugs, adrenergic receptor antagonists, dopamine antagonists, antihistamines or 5-hydroxytryptamine antagonists (Beleslin and Krstic, 1987; Beleslin et al., 1983; Cahill et al., 2016; Lee et al., 2011), suggesting that nAchR might play independent roles in production of MS-related GI symptoms. The present study found that both MEC and HEX can sufficiently inhibit rotation-induced nausea-related conditioned gaping in rats and emesis in cats, suggesting a potent peripheral mechanism for MEC in inhibiting nausea and vomiting. It is believed that nausea might be the result of low-intensity emetic activation that fails to reach sufficient intensity to activate the reflex, and nausea and vomiting is two consecutive responses during the activation of the emetic system (Horn, 2014). Although the mechanism of nausea is still unclear and in debate, it is reasonable to propose that the nausea perception or visceral malaise might be related to the awareness of the GI responses, including decrease in GI motility, relaxation of the proximal stomach, and the retrograde giant contraction in the small intestine, that commonly develop prior to vomiting (Balaban and Yates, 2017; Muth, 2006). Previous studies showed that HEX can block the gastric relaxation and jejunoileal motor responses that correlate of vomiting (Krowicki and Hornby, 2000; Lang and Marvig, 1989; Nakamura et al., 1995). HEX can also suppress the reduction in intragastric pressure induced by electrical stimulation of AP neurons in rats (Kawachi et al., 2008), abolish the cardiovascular responses prior to retching induced by mechanical stimulation of stomach, and elongate the latency of retching in *Suncus murinus* (Uchino et al., 2002), and significantly inhibited GI motility disorders-induced by chemical stimulators such as capsaicin and copper sulfate (Holle et al., 1992; Makale and King, 1992; Qin et al., 1993; Shibata et al., 1999). As excitatory cholinergic nicotinic synapses mediate fast synaptic transmission in autonomic ganglia (Cooper, 2001; Del Signore et al., 2004), it strongly suggested that alleviation of motion-induced nausea by MEC and HEX might be attributed to their inhibition of efferent autonomic neural transmission which play key roles in regulation of GI motility (Hasler, 2013; Hurst et al., 2013). In addition, it has been reported that both MEC and HEX can dose-dependently reduce tetanic peak muscle tension induced by high frequency electrical stimulation of the phrenic nerve in rat isolated phrenic-nerve hemidiaphragms (Faria et al., 2003). Given that the motor act of vomiting is mostly accomplished through the synchronized contractions of diaphragm and abdominal muscles (Andrews, 1992), MEC and HEX might attenuate motion-induced vomiting possibly through effective inhibition of nAchR mediated neurotransmission in the neuromuscular junction of the muscles engaged in breathing.

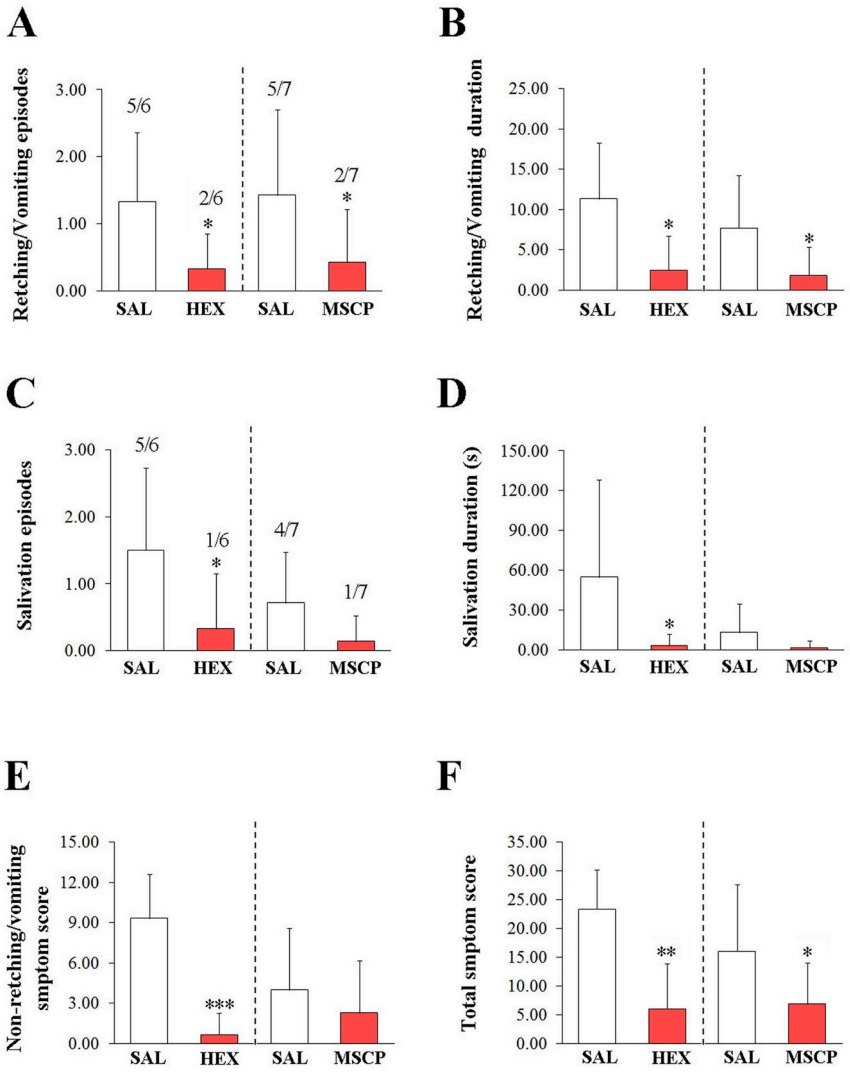


**Fig. 7. Effects of hexamethonium chloride (HEX) and methyl scopolamine (MSCP) pretreatment on rotation-induced MS symptoms in cats.** The number of retching/vomiting and salivation episodes (A and C), the sum of duration for each retching/vomiting and salivation episode (B and D) as well as the non-retching/vomiting score and total symptom score (E and F) were analyzed. The occurrence of retching/vomiting and salivation is indicated above the column in A and C, respectively. STH, starch; Rot, rotation; Sta, static. Data are represented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 compared with the data of the corresponding SAL control treatment.

Clinical investigations have demonstrated that SCOP had prophylactic effects against motion-induced and postoperative nausea and vomiting (Antor et al., 2014; Gil et al., 2012; Spinks and Wasiak, 2011), but could not control chemotherapy-induced emesis (Longo et al., 1982). As expected, the present study showed that SCOP and MSCP effectively prevented MS-induced nausea-related behavioral responses in the rats and retching/vomiting in the cats. We also found that SCOP but not MEC slightly attenuated rotation-induced hypothermia which is believed to be the consequence of enhanced sweating and skin vasodilatation during MS (Ngampramuan et al., 2014; Nobel et al., 2006). These results are consistent with the findings that SCOP can elevate core temperature in rats (Gordon and Grantham, 1999), while the muscarinic receptor agonist and the acetylcholinesterase inhibitors led to hypothermia in mice and rats (Gordon and Grantham, 1999; Patel and Hutson, 1994; Sanchez and Lembol, 1994; Smith et al., 1997). SCOP in combination of dexamphetamine is also effective in alleviating core temperature reduction in humans exposed to Coriolis acceleration (Cheung et al., 2011), while MSCP can reverse hyperthermia caused by handling, cage switch, open field stress (Johnson Rowsey et al., 2002; Rowsey and Gordon, 2000). Moreover, our previous study showed that the increased afferent vagal activity correlated with emesis in humans during the voyage at sea (Wang et al., 2016), suggesting that MS-related decline in gastric motility and frequent tachygastria in electrogastrogram could be attributed to enhanced activity of the vagus nerve (Jokerst et al., 1999; Muth, 2006). Given these facts, we proposed that SCOP might regulate autonomic responses of MS partly through inhibition of peripheral mAchR signaling transduction most possibly in the postganglionic fibers of the parasympathetic system during MS. The finding that neuromuscular presynaptic muscarinic receptor inhibition significantly enhanced rocuronium-induced tetanic fade in diaphragm muscles after high frequency electrical stimulation of the phrenic nerve also suggests that the anti-emesis effects of SCOP and MSCP in cats could be partly attributed to presynaptic modulation of the release of acetylcholine in neuromuscular junction of diaphram during MS stimulation (Kim et al., 2017). Nevertheless, the precise molecular mechanism of the therapeutic effect on MS-induced vomiting of the peripheral mAchR inhibitor still need to clarify in further investigation.

Human studies have demonstrated that nicotine nasal spray can increase sensitivity to MS, while short-term smoking deprivation can enhance tolerance to MS in humans (Golding et al., 2011; Zingler et al., 2007), indicating that central nAchR may play a role in MS development. Previous studies have demonstrated that the CVN serves as the central part of the neural networks that contribute to vestibulo-autonomic manifestations, such as retching, excessive salivation, defecation and urination during galvanic vestibular stimulation and MS (Aleksandrov et al., 1998; Balaban, 1996; Balaban and Beryozkin, 1994; Balaban et al., 2014; Miller et al., 2008; Mori et al., 2005). Anatomic studies have confirmed that the CVN have direct and indirect connections with autonomic brain regions (Balaban, 2004; Balaban et al., 2002; Balaban and Porter, 1998). The projection originated from the

caudal MVe and SpVe to the NTS and other autonomic regions, such as dorsal motor nucleus of the vague, rostral ventrolateral medulla and nucleus ambiguous, might constitute the direct vestibulo-autonomic descending pathways, while the CVN connections with the caudal part of PBN form an indirect vestibulo-autonomic pathway linking to the NTS and the rostral ventrolateral medulla (Balaban, 2004; Balaban et al., 2002; Balaban and Porter, 1998). The present study showed that both MEC and SCOP moderately decreased rotation-induced Fos expression in the rat CVN and NTS, suggesting that MEC and SCOP might significantly but insufficiently inhibit the direct vestibulo-autonomic neural pathways during MS. In the meantime, we also found that MEC seemed to be more efficient than SCOP on inhibiting Fos expression in the CVN and NTS, which symphonized with the behavioral observations that MEC was more effective than SCOP on alleviating rotation-induced nausea and vomiting. Furthermore, the PBN also has reciprocal connections with the hypothalamus, amygdala and the infralimbic cortex, indicating that vestibular information might exert some influence on the ascending pathways involved in endocrine response and anxiety during MS (Balaban, 2004; Krukoff et al., 1993). However, neither MEC nor SCOP alleviated rotation-induced Fos expression in the PBN, the MeA and CeA, or the LC, suggesting that these drugs might have no effect on vestibular stimulation-induced anxiety or stress responses (Balaban, 2002).

Finally, the limitation of the present study is that our results failed to clarify whether MEC and SCOP affected the function of the vestibular end organs during MS. It has been reported that efferent modulation of vestibular hair cell function was partly achieved through α9 containing nAchR (Elgoyhen et al., 2001; Rabbitt and Brownell, 2011). Knockout of α9 subnuit of nAchR attenuated hypothermia elicited by provocative motion in mice (Tu et al., 2017). Nevertheless, although SCOP (but not MEC) slightly relieved rotation-induced hypothermia in rats, neither MEC nor SCOP impaired the spontaneous locomotion and balance performance in un-rotated static animals, and the rotation-induced Fos expression in the CVN still remained remarkable after MEC or SCOP administration. Based on these facts, we argue that the prophylactic effects of anticholinergics against nausea and vomiting could not be attributed to their potential of modulating vestibular end organs during MS. The precise role of vestibular expressed nAchR during MS-induced nausea and vomiting still remained to be clarified. In addition, clinical and preclinical studies have demonstrated that nicotinic cholinergic activation is beneficial for alleviating gait and balance deficits in many disorders such as L-dopa-induced dyskinesias and intracerebral hemorrhage (Bordia et al., 2008; Hijioka et al., 2012). Daily administration of nAchR agonist varenicline improved balance deficits on the rotorod and balance beam performance in rats with olivocerebellar lesions, and such effects were prevented by MEC pretreatment (Wecker et al., 2013). The present study showed that MEC at a high dose aggravated balance impairments in MS rats, while HEX did not show such effects, suggesting a central nAchR mechanism of high-dose MEC on worsening of balance deficits during MS. Nevertheless, we surprisedly found that HEX and MSCP significantly improved balance performance in rotated animals. The relative contribution of the central and peripheral nAchR and mAchR to the balance disorders induced by MS merits further investigation in the future.

## 5. Conclusions and implications

Nowadays, there are about 9 different categories of drugs that have been demonstrated to be effective against MS (Zhang et al., 2016). The non-selective mAchR antagonist SCOP is known to be the most effective MS prophylactics but with apparent side effects including drowsiness, blurred vision, dry mouth, and dizziness (Murdin et al., 2011). The present study found that non-selective nAchR antagonist MEC seems to be more effective than SCOP in preventing MS-induced GI symptoms in both rats and cats, and in inhibiting rotation-induced Fos expression in the rat vestibule-autonomic pathways. Meanwhile, HEX and MSCP were also effective in preventing MS in these two species, indicating that MEC and SCOP may exert their anti-MS activity via both central and peripheral actions. The reported privileges of MEC are its rapid and complete absorption from the GI tract, and a much longer duration of action than scopolamine (22 h vs. 6 h) (Nickell et al., 2013). The anti-MS activity of MEC is demonstrable at much lower doses than those induced hypotension associated with the inhibition of sympathetic activity in rats (1 mg/kg vs. 4 mg/kg) (Jutkiewicz et al., 2013). Our results suggest the potential of MEC to be used as a new candidate for management of GI symptoms induced by MS and other vestibular disorders. Further investigation should focus on verifying anti-MS effects of MEC on human subjects and finding out whether MEC also had similar side effects of SCOP such as memory and cognitive impairment, anxiety, and depression (Bagci et al., 2016; Silveira et al., 2015) (Weerts et al., 2015; Zhang et al., 2016).

## Conflicts of interest

None.

## Acknowledgements

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2018.12.006.

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