



HOKKAIDO UNIVERSITY

Title	Effects of methyl methacrylate on the excitability of the area postrema neurons in rats
Author(s)	Yoshizawa, Tomohiko; Funahashi, Makoto
Citation	Journal of oral biosciences, 62(4), 306-309 https://doi.org/10.1016/j.job.2020.09.003
Issue Date	2020-12
Doc URL	https://hdl.handle.net/2115/83415
Rights	© 2020. This manuscript version is made available under the CC-BY-NC-ND 4.0 license
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Type	journal article
File Information	Yoshizawa_JOB_manuscript_revise.pdf



**Effects of methyl methacrylate on the excitability of the area postrema
neurons in rats**

Tomohiko Yoshizawa^{1,2} and Makoto Funahashi²

¹Department of Physiology and Cell Biology, Graduate School of Medical and Dental
Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo
113-8510, Japan

²Oral Physiology, Department of Oral Functional Science, Division of Oral Medical
Science, Faculty of Dental Medicine and Graduate School of Dental Medicine,
Hokkaido University, Kita 13, Nishi 7, Kita-ku, Sapporo, 060-8586, Japan

Corresponding author: Tomohiko Yoshizawa

Department of Physiology and Cell Biology, Graduate School of Medical and Dental
Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo
113-8510, Japan

Tel: +81-3-5803-5156, E-mail: tomohiko.yoshizawa@gmail.com

20 **Abstract**

21 **Objectives**

22 The aim of the present study is to demonstrate the effects of inhaled methyl
23 methacrylate (MMA) on the excitability of neurons in the area postrema (AP). We also
24 investigated the relation between vagal afferent inputs and responding cells in the AP.

25 **Methods**

26 We set up two groups of experimental animals, such as rats inhaling MMA and rats
27 inhaling room air. To visualize the changes of AP neuron excitability after inhalation of
28 MMA for 90 min, c-Fos protein expression was identified and quantified by
29 immunohistochemical analysis. Some rats receiving ventral gastric branch vagotomy
30 were also subjected to the abovementioned experiment.

31 **Results**

32 The number of c-Fos-immunoreactive (Fos-ir) cells in the MMA group was more than
33 six times greater than that of the control group (statistically significant, $p < 0.01$). In
34 vagotomized rats inhaling MMA, markedly smaller number of Fos-ir cells was
35 identified in the AP compared to that of rats inhaling MMA without vagotomy.

36 **Conclusions**

37 These results indicate that inhalation of MMA increases neuronal excitability in the AP,

38 suggesting that vagal afferent inputs are involved in the induction mechanism of Fos-ir
39 cells by MMA.

40

41 Keywords: area postrema, methyl methacrylate, c-Fos, vagotomy

42

43

1. Introduction

The area postrema (AP) lacks the blood-brain barrier and is well known as the chemoreceptor trigger zone for emesis [1]. Previous studies have revealed the receptivity of the AP neurons to many chemical substances; however, the effects of methyl methacrylate (MMA) remained to be determined. Therefore, we hypothesized that MMA inhalation increases the excitability of AP neurons triggering nausea and/or emesis.

The MMA is a major component in some popular dental resin that is often used in dental medicine, such as dental composite resins, dentures, temporary crowns, etc. As the MMA vaporizes easily at room temperature, dentists or patients possibly inhale a small amount of MMA during dental treatment. Although safety has been established for small amounts of MMA inhalation, health hazards such as nausea and vomiting are a concern if inhaled in large quantities [2]. For example, toluene, an organic solvent, vaporizes easily and excites the AP neurons [3]. We also investigated the role of vagal afferent inputs in the effects of inhaled MMA on the excitability of AP neurons, because a previous study has reported that the vagal afferent inputs are involved in the induction mechanism of cisplatin-induced emesis [4].

The present study demonstrated the MMA-induced upregulation of the AP

neuronal excitability using immunohistochemical analysis of c-Fos expression and the possible role of abdominal vagal afferent inputs in the effects of MMA on AP neurons.

2. Materials and Methods

2.1. Subjects

The Hokkaido University Animal Use Committee approved this study. Adult male Wister or SD rats (MMA, 5 rats; control, 5 rats; vagotomy, 3 rats; 150–600 g body weight) were housed individually under a 12/12 h light/dark cycle (lights on at 7 AM; off at 7 PM). Experiments were performed during the light phase.

2.2 Ventral gastric branch vagotomy

Ventral gastric branch vagotomy was performed as reported previously [5]. Briefly, rats were anesthetized and incised in the middle of the abdomen. The stomach was gently manipulated to pick out the ventral gastric branch of the subdiaphragmatic vagus. The ventral branch of the vagal nerve was transected, and pyloroplasty was performed to prevent pyloric stenosis. The pyloric sphincter was incised longitudinally (0.5–0.7 mm), and the incised part was sutured with silk sutures. After 10–14 days of recovery, the rats were subjected to the MMA inhalation experiment.

80

81 2.3 Administration of MMA

82 A rat was moved from the breeding cage to a desiccator and was habituated for
83 4 h before starting each experiment (Fig. 1). The rats without vagotomy were divided
84 into two experimental groups: one group breathes MMA for 90 min ($n = 5$), and the
85 other group breathes room air ($n = 5$). MMA liquid (3 mL) was dropped and volatilized
86 in the desiccator at room temperature (approximately $25 \pm 3^\circ\text{C}$). Three rats that
87 underwent vagotomy inhaled the MMA in the same manner as above. Immediately after
88 inhalation of MMA or room air for 90 min, animals were deeply anesthetized with
89 urethane and then perfused with 4% paraformaldehyde (PFA). Brains were carefully
90 removed from the skull and fixed in 4% PFA at 4°C overnight, and then transferred to a
91 30% sucrose/PBS solution at 4°C until the brains sank to the bottom.

92

93 2.4 Immunohistochemical analysis

94 Coronal sections including the AP (Fig. 2A) [6] were cut at a thickness of
95 $50\ \mu\text{m}$ in the freezing microtome (FX-801; Yamato, Saitama, Japan) and kept in wells
96 containing PBS at 4°C . Each section was rinsed thrice in PBS for 5 min, and sections
97 were immersed in 80% methanol containing 0.3% hydrogen peroxide (H_2O_2) for 1 h at

room temperature. After three rinses in PBS for 10 min and immersion in blocking buffer (1.5% normal goat serum and 0.4% Triton X-100 in PBS) for 1 h at room temperature, sections were incubated in primary rabbit anti-cFos antibody (sc-52; Santa Cruz, Dallas, United States) diluted 1:5000 in blocking buffer at room temperature overnight or at 4°C for 3–5 days. Each section was rinsed thrice in PBS for 10 min and incubated in biotinylated anti-rabbit secondary antibody (VECTASTAIN Elite ABC kit, Vector Laboratories, Burlingame, United States) for 1 h at room temperature. Sections were rinsed twice in PBS for 10 min and rinsed twice in TBS for 10 min and then immersed in TBS containing 0.01% DAB and 0.05% nickel ammonium sulfate for 10 min at room temperature. Each section was treated with 0.0006% H₂O₂ for 7 min, and then sections were rinsed in TBS for 5 min and in PBS for 10 min and mounted on glass slides and coverslipped with Entellan (Merck, Darmstadt, Germany). Microscopic images of each section were analyzed on a personal computer using the Cell Counter plugin of ImageJ (NIH, Bethesda, United States). The average value of Fos-ir cells was determined by counting the number of cells from three sections in each animal, which was used for analysis as a representative value.

2.5 Statistical analysis

Using MATLAB (Mathworks, Statistics and Machine Learning Toolbox,
Natick, United States), the number of Fos-ir cells between each experimental group was
statistically analyzed by Wilcoxon signed-rank tests. Data were represented by means $\pm$
SEM.

3. Results

3.1 c-Fos expression in the AP through MMA inhalation

As shown in Figure 2A and 2B, many Fos-ir cells were found in the AP of the
MMA group compared to the control group. The number of Fos-ir cells in the MMA
group (168 ± 46 cells, $n = 5$ rats) was more than six times greater than that in the control
group (26 ± 3 , $n = 5$, $p = 0.0079$, Wilcoxon signed-rank tests, Fig. 2C). This result
indicates that MMA inhalation increases neuronal excitability in the AP.

3.2 Effects of vagotomy on the expression of c-Fos in the AP

In vagotomized rats ($n = 3$), the average number of Fos-ir cells in the AP was 37 ± 1 (Fig.
3A, B). This value was markedly smaller than that of the MMA group (168 ± 46 , $n = 5$).
This result suggests that vagal afferent inputs are involved in MMA-induced c-Fos
expression of the AP neurons.

4. Discussion

In the present study, we investigated the effects of MMA on the excitability of neurons in the AP. The major findings in the present study were (1) MMA inhalation induced c-Fos expression in the AP, and 2) ventral gastric branch vagotomy suppressed c-Fos expression in the AP. These results revealed the sensitivity of AP neurons to MMA, suggested that vagal afferent inputs are involved in MMA-induced upregulation of neuronal excitability in the AP.

The c-Fos protein is often used as a marker of neuronal activity [7-10], because its expression indicates the facilitation of neuronal activity associated with the depolarization of membrane potentials, reaching a peak after 1.5–2 h from excitation of the neuron [11]. Some studies have reported that administration of cisplatin, which is an anticancer drug that causes nausea and vomiting as a side effect, introduces expression of the c-Fos protein in the AP of rodents [12, 13]. Another study has examined that c-Fos protein expression is related with the intake of kaolin, which is a measurement of nausea [14].

Two possible pathways of the effects of MMA on AP neuronal excitability are (1) circulating MMA induced upregulation of the vagal afferent inputs to AP neurons,

and 2) AP neurons may directly respond to circulating MMA. There are vagal afferent nerves that show the direct projection to AP neurons [15] and the indirect projection to AP neurons via the nucleus tractus solitarius (NTS) [16].

The present study suggests that vagal afferent inputs are involved in the excitation of the AP neurons by the MMA. Whether the MMA in the blood directly excites the AP neurons remains unexplained. To answer this question, we will perform another experiment using electrophysiological methods [17-24].

Considering the functional role of AP in triggering nausea and/or emesis, animals may feel nauseous during exposure to MMA in the present study. A study on humans has reported that inhalation of MMA caused respiratory tract irritation, weakness, fever, dizziness, nausea, headache, and sleepiness [2]. An animal study using rats has reported that the main clinical signs of acute MMA inhalation were depression, ataxia, and excessive salivation [25]. Some previous studies have reported MMA toxicity to nasal mucosal epithelial cells in rats [26, 27]. Raje et al. have subjected rats to MMA inhalation (100 ppm) for up to 4 hours, and they have reported several symptoms such as interalveolar congestion/hemorrhage, pulmonary vasodilatation, and edema; however, no histopathologic change was seen in the brain [28]. When rats inhaled a relatively high concentration of MMA (3000 ppm), extensive cerebellar

congestion and hemorrhage occurred in the cerebellar peduncles [29]. Increase of Fos-ir cells in the AP after MMA inhalation may occur because of MMA toxicity; however, further work is required for its elucidation.

Some previous studies have reported that intestinal motor activities and smooth muscle tonus were reduced by MMA inhalation (116 ppm) [30, 31]. In the present study, MMA-induced gastrointestinal motility disorder possibly increased afferent vagal nerve activity, resulting in the increased number of Fos-ir cells in the AP.

5. Conclusion

The present study revealed that AP neurons could respond to the inhalation of extreme amounts of MMA. Increases in the vagal nerve activity by MMA are possibly involved in the upregulation of neuronal excitability in the AP.

Ethical statement

The Hokkaido University Animal Use Committee approved this study.

Conflicts of interest

The authors declare no competing financial interests.

CRedit authorship contribution statement

Tomohiko Yoshizawa: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing (original draft), writing (review and editing), and visualization. **Makoto Funahashi:** conceptualization, methodology, investigation, resources, writing (original draft), writing (review and editing), visualization, and funding acquisition.

Funding

This work was supported by the JSPS KAKENHI Grant Numbers JP19K10039 and JP19K16299 and by generous research support of Hokkaido University for Oral Physiology.

Acknowledgements

We thank the members of the Oral Physiology laboratory in Hokkaido University for helpful comments and discussion.

206 **References**

- 207 [1] Borison HL, Wang SC. Physiology and pharmacology of vomiting. Pharmacol
208 Rev 1953;5:193-230.
- 209 [2] Karpov B. Methyl methacrylate from the viewpoint of labor hygiene. Gig Sanit
210 1954;10:25-8.
- 211 [3] Perit KE, Gmaz JM, Caleb Browne JD, Matthews BA, Dunn MB, Yang L, et
212 al. Distribution of c-Fos immunoreactivity in the rat brain following abuse-like toluene
213 vapor inhalation. Neurotoxicol Teratol 2012;34:37-46.
- 214 [4] Endo T, Nemoto M, Minami M, Yoshioka M, Saito H. Changes in the afferent
215 abdominal vagal nerve activity induced by cisplatin and copper sulfate in the ferret.
216 Biogenic Amines. 1995;11:399-407.
- 217 [5] Okusha Y, Hirai Y, Maezawa H, Hisadome K, Inoue N, Yamazaki Y, et al.
218 Effects of intraperitoneally administered L-histidine on food intake, taste, and visceral
219 sensation in rats. J Physiol Sci 2017;67:467-74.
- 220 [6] Paxinos G, Watson C. The rat brain in stereotaxic coordinates: hard cover
221 edition: Elsevier; 2006.
- 222 [7] Morgan JJ, Cohen DR, Hempstead JL, Curran T. Mapping patterns of c-fos
223 expression in the central nervous system after seizure. Science 1987;237:192-7.

224 [8] Sheng M, Greenberg ME. The regulation and function of c-fos and other
 225 immediate early genes in the nervous system. *Neuron* 1990;4:477-85.

226 [9] Guzowski JF, McNaughton BL, Barnes CA, Worley PF. Environment-specific
 227 expression of the immediate-early gene Arc in hippocampal neuronal ensembles. *Nat*
 228 *Neurosci* 1999;2:1120-4.

229 [10] Okuno H. Regulation and function of immediate-early genes in the brain:
 230 beyond neuronal activity markers. *Neurosci Res* 2011;69:175-86.

231 [11] Yamamoto T, Sawa K. c-Fos-like immunoreactivity in the brainstem following
 232 gastric loads of various chemical solutions in rats. *Brain Res* 2000;866:135-43.

233 [12] Horn CC, Ciucci M, Chaudhury A. Brain Fos expression during 48 h after
 234 cisplatin treatment: neural pathways for acute and delayed visceral sickness. *Auton*
 235 *Neurosci*. 2007;132:44-51.

236 [13] Horn CC. Brain Fos expression induced by the chemotherapy agent cisplatin in
 237 the rat is partially dependent on an intact abdominal vagus. *Auton Neurosci*
 238 2009;148:76-82.

239 [14] Horn CC, De Jonghe BC, Matyas K, Norgren R. Chemotherapy-induced kaolin
 240 intake is increased by lesion of the lateral parabrachial nucleus of the rat. *Am J Physiol*
 241 *Regul Integr Comp Physiol* 2009;297:R1375-82.

242 [15] Altschuler SM, Bao XM, Bieger D, Hopkins DA, Miselis RR. Viscerotopic
243 representation of the upper alimentary tract in the rat: sensory ganglia and nuclei of the
244 solitary and spinal trigeminal tracts. J Comp Neurol 1989;283:248-68.

245 [16] van der Kooy D, Koda LY. Organization of the projections of a
246 circumventricular organ: the area postrema in the rat. J Comp Neurol 1983;219:328-38.

247 [17] Hay M, Lindsley KA. Membrane properties of area postrema neurons. Brain
248 Res 1995;705:199-208.

249 [18] Jahn K, Bufler J, Weindl A, Arzberger T, Hatt H. Patch-clamp study on
250 membrane properties and transmitter activated currents of rabbit area postrema neurons.
251 J Comp Physiol A. 1996;178:771-8.

252 [19] Funahashi M, Mitoh Y, Matsuo R. Two distinct types of transient outward
253 currents in area postrema neurons in rat brain slices. Brain Res 2002;942:31-45.

254 [20] Funahashi M, Mitoh Y, Matsuo R. Electrophysiological properties of the rat
255 area postrema neurons displaying both the transient outward current and the
256 hyperpolarization-activated inward current. Brain Res Bull 2002;58:337-43.

257 [21] Funahashi M, Mitoh Y, Kohjitani A, Matsuo R. Role of the hyperpolarization-
258 activated cation current (I_h) in pacemaker activity in area postrema neurons of rat brain
259 slices. J Physiol 2003;552:135-48.

260 [22] Shinpo K, Hirai Y, Maezawa H, Totsuka Y, Funahashi M. The role of area
261 postrema neurons expressing H-channels in the induction mechanism of nausea and
262 vomiting. *Physiol Behav* 2012;107:98-103.

263 [23] Fukuda T, Hirai Y, Maezawa H, Kitagawa Y, Funahashi M.
264 Electrophysiologically identified presynaptic mechanisms underlying amylinergic
265 modulation of area postrema neuronal excitability in rat brain slices. *Brain Res*
266 2013;1494:9-16.

267 [24] Sugeta S, Hirai Y, Maezawa H, Inoue N, Yamazaki Y, Funahashi M.
268 Presynaptically mediated effects of cholecystokinin-8 on the excitability of area
269 postrema neurons in rat brain slices. *Brain Res* 2015;1618:83-90.

270 [25] Spealman C, Main R, Haag H, Larson P. Monomeric Methyl Methacrylate-
271 Studies on Toxicity. *Ind Med Surg* 1945;14:292-8.

272 [26] Lomax L, Krivanek N, Frame S. Chronic inhalation toxicity and oncogenicity
273 of methyl methacrylate in rats and hamsters. *Food Chem Toxicol* 1997;35:393-407.

274 [27] Chan PC, Eustis SL, Huff JE, Haseman JK, Ragan H. Two-year inhalation
275 carcinogenesis studies of methyl methacrylate in rats and mice: inflammation and
276 degeneration of nasal epithelium. *Toxicology*. 1988;52:237-52.

277 [28] Raje RR, Ahmad S, Weisbroth SH. Methylmethacrylate: tissue distribution and

pulmonary damage in rats following acute inhalation. Res Commun in Chem Pathol

Pharmacol 1985;50:151-4.

[29] Program NT. Toxicology and carcinogenesis studies of methyl methacrylate in

F344/N rats and B6C3F1 mice (Inhalation studies). NIH Publ; 1986.

[30] Tansy MF, Kendall FM, Benhayem S, Hohenleitner FJ, Landin WE, Gold M.

Chronic biological effects of methyl methacrylate vapor: I. Body and tissue weights,

blood chemistries, and intestinal transit in the rat. Environ Res 1976;11:66-77.

[31] Tansy M, Landin W, Perrong H, Kendall F. Acute and chronic intestinal motor

effects of methyl-methacrylate vapor. J Dent Res 1976;55:B240.

Figure legends

Fig. 1. Experimental design

Immediately after administration of MMA or room air for 90 min in the desiccator, rats

are fixed with 4% paraformaldehyde (PFA)

Fig. 2. Fos-ir cells in the AP after MMA inhalation

A, A schematic diagram of the coronal section of the AP. The AP is located at the

bottom of the fourth ventricle and lacks the blood-brain barrier, called the

chemoreceptor trigger zone

B, Example photomicrographs of a coronal section of the area postrema region. Each

lower panel is an enlarged view of the part surrounded by a solid line in the upper panel.

A lot of Fos-ir cells (white arrows) were found in the AP of the MMA group rats,
whereas few cells in the control group rats. Scale bar: 100 μ m (upper) and 50 μ m
(lower)

C, The average number of Fos-ir cells in the MMA group is significantly larger than that
in the control group. **: $p < 0.01$, Wilcoxon signed-rank tests

Fig. 3. Effects of vagotomy on the c-Fos expression of the AP neurons

A, An example photomicrograph of a coronal section of the area postrema region in
vagotomized rats. Scale bar: 100 μ m

B, The average number of Fos-ir cells in the vagotomy group ($n = 3$) is markedly
smaller than that in the MMA group ($n = 5$, same value as shown in Fig. 2C)

Fig.4. Schematic illustration of the possible mechanism of the MMA effects on the AP
neurons

The present study suggests that MMA absorbed into the blood from the lungs increases
abdominal vagal nerve activity, and the excitability of the AP neurons receiving
synaptic inputs from the vagus nerve increased (solid arrows). Further, MMA may
directly affect the AP neurons; however, it remains to be determined (dotted arrow)

Fig. 1

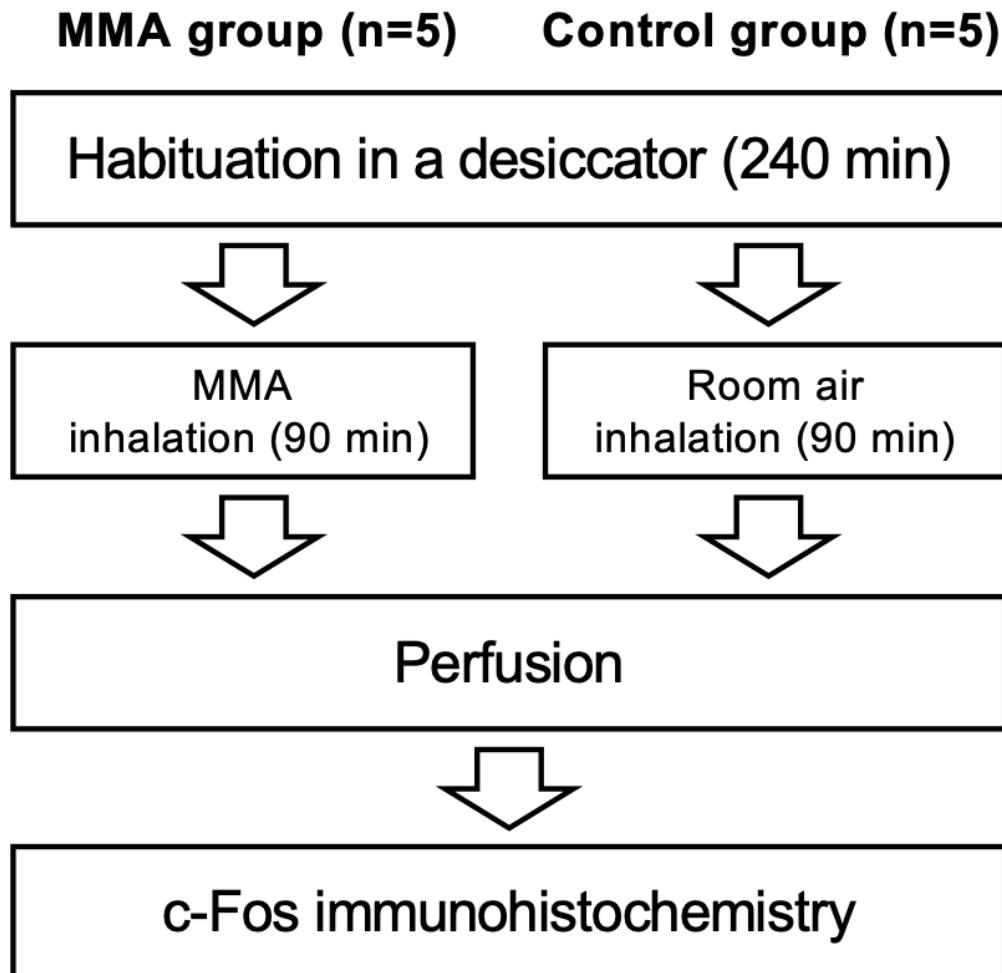


Fig. 2

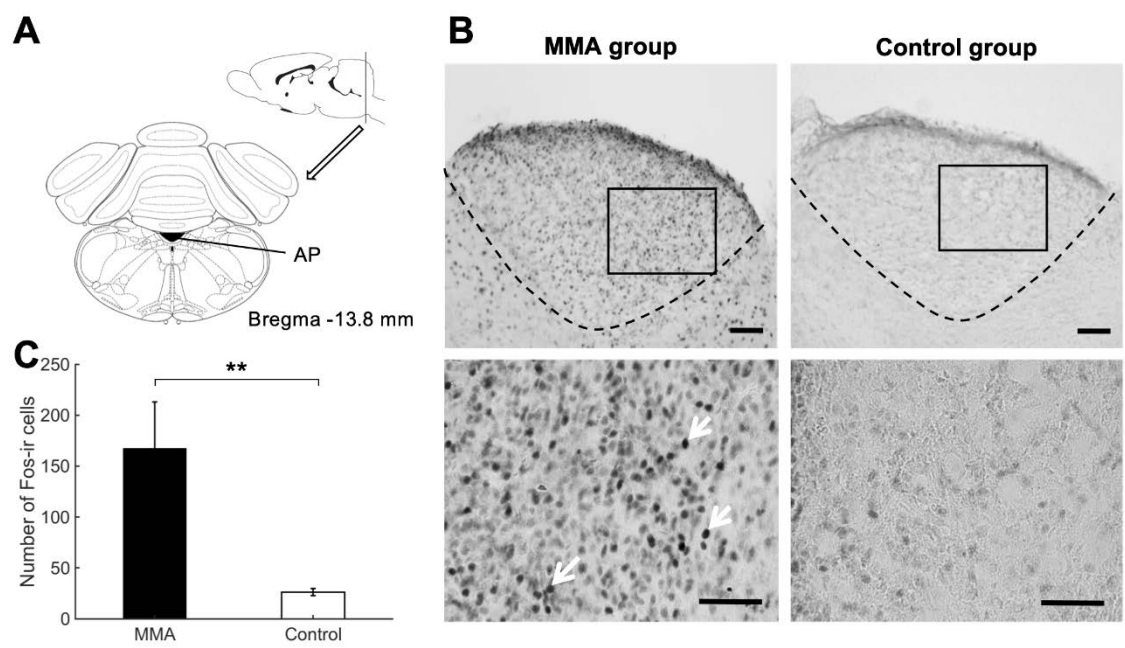


Fig. 3

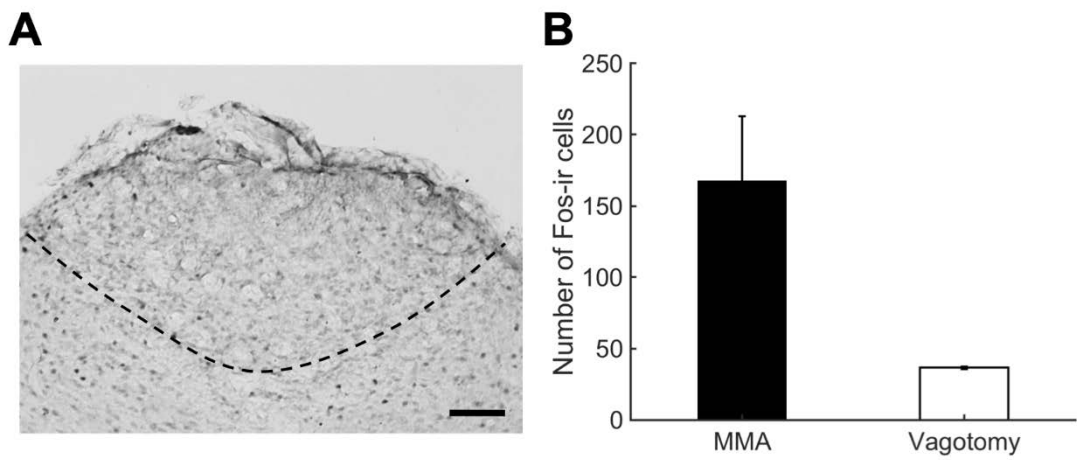


Fig. 4

