Endogenous acetylcholine modulates impulsive action via α4β2 nicotinic acetylcholine receptors in rats

Iku Tsutsui-Kimura[1], Yu Ohmura[1, 2], Takeshi Izumi[1], Taku Yamaguchi[1], Takayuki Yoshida[1], and Mitsuhiro Yoshioka[1*]

[1]Department of Neuropharmacology, Hokkaido University Graduate School of Medicine, N15 W7 Kita-ku, Sapporo, 060-8638, Japan

[2]Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109-0632, USA

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*Corresponding author:

Mitsuhiro Yoshioka,

Department of Neuropharmacology, Hokkaido University Graduate School of Medicine, N15 W7 Kita-ku, Sapporo, 060-8638, Japan

Phone: 81+11-706-5904

Fax: 81+11-706-7872

E-mail: flute@med.hokudai.ac.jp

Abstract

Nicotine has been well established as an impulsive action-inducing agent, but it remains unknown whether endogenous acetylcholine affects impulsive action via nicotinic acetylcholine receptors. In the present study, the 3-choice serial reaction time task (3-CSRTT), a simple and valid assessment of impulsive action, was employed. Male Wistar/ST rats were trained to detect and respond to 1-s flashes of light presented in one of three holes until stable performance was achieved. Following training on the 3-CSRTT, rats received intracerebroventricular injections of the preferential α4β2 nicotinic acetylcholine receptor antagonist dihydro-β-erythroidine (DHβE; 0, 3, 10, and 30 μg) or the selective α7 nicotinic acetylcholine receptor antagonist methyllycaconitine (MLA; 0, 3, 10, and 30 μg) 5 min before test sessions. Injection of 10 μg of DHβE significantly suppressed premature responses, an index of impulsive-like action, without changing other behavioral parameters. On the other hand, MLA infusions failed to affect impulsive-like action at any dose. These results suggest that the central α4β2 nicotinic acetylcholine receptors that enable a provoking effect of endogenous acetylcholine play a critical role in impulsive action. Substances that modulate nicotinic acetylcholine receptors, especially the α4β2 subtype, may be beneficial for the treatment of

psychiatric disorders characterized by lack of inhibitory control.

Keywords

impulsivity, impulsive behavior, response inhibition, premature responding, attention-deficit/hyperactivity disorder, 5-choice serial reaction time task

1. Introduction

Activation of cholinergic pathways by nicotine has been associated with impulsive choice (Ohmura et al., 2005) and impulsive action (Yakir et al., 2007) in humans. Impulsive acts are often viewed as everyday normal behavior; however, excessive levels of impulsivity are associated with several psychiatric disorders, such as attention-deficit/hyperactivity disorder (ADHD: Solanto, 2002), schizophrenia (Enticott et al., 2008), and borderline personality disorder (Paris, 2005). In rodents, the impulsivity-provoking properties of nicotine have been well demonstrated in a delay discounting task (Dallery and Locey, 2005) and in a 5-choice serial reaction time task (5-CSRTT, Blondel et al., 2000; van Gaalen et al., 2006). Investigating central cholinergic function will contribute to further understanding of the neural correlates and neuropharmacological substrates of impulsivity.

The 5-CSRTT is one of the most prevalent animal models of impulsive action and is based on the human continuous performance test (Wilkinson, 1963; Robbins, 2002). In this task, a light in the aperture of one of five holes is briefly and randomly flashed, and animals are required to make a nose-poke into the flashed hole to get a food pellet. Responses that occur before the presentation of the stimulus light are described as

premature responses and result in a time-out period. They are regarded as impulsive-like action (Robbins, 2002). Thus, premature responses reflect a simple form of impulsive-like action in rodents, and the 5-CSRTT is suitable not only for screening novel treatments but also for revealing the neural basis of impulsive action.

The nicotinic acetylcholine receptor is a pentameric combination of α and β subunits. In the rat central nervous system, six α subunits (α2-α7) and three β subunits (β2-β4) have been described. Several combinations of these subunits have been detected, of which the α4β2 and α7 subtypes are the most widely distributed (Galzi and Changeux, 1995; Léna and Changeux, 1997; Cordero-Erausquin et al., 2000).

The effects of nicotine on impulsive action have been well established, though only a few studies have examined whether endogenous acetylcholine affects impulsive action via nicotinic acetylcholine receptors. Grottick and Higgins (2000) failed to detect any effects from systemic administration of nicotinic acetylcholine receptor antagonists by itself on impulsive-like action in the 5-CSRTT, though they blocked the effects of nicotine. It may not have been possible to observe a decreasing number of premature responses owing to floor effects; this study used Lister hooded rats, which rarely respond prematurely (< 10 per session, Broersen and Uylings, 1999) in the 5-CSRTT. Blondel et al. (2000) reported that systemic administration of nicotinic acetylcholine

receptor antagonists tended to decrease premature responses in the 5-CSRTT, but the effects were not significant. Generally speaking, systemically administered compounds have some difficulty in reaching the brain because of the blood-brain barrier, even if they are centrally acting drugs (Turek et al., 1995). Moreover, systemic administration often causes undesirable side effects due to peripheral actions (Curzon et al., 1996). Because of the disadvantages of systemic administration, the experiments by Blondel et al. (2000) may have failed to achieve significance. Direct infusions of nicotinic acetylcholine receptor antagonists into the brain may elicit effects on impulsive-like action.

To determine whether endogenous acetylcholine affects impulsive action via nicotinic acetylcholine receptors and to determine the subtype of nicotinic acetylcholine receptors responsible, we assessed the effects of intracerebroventricular (i.c.v.) injections of selective α4β2 and α7 nicotinic acetylcholine receptor antagonists on impulsive action using a 3-choice serial reaction time task (3-CSRTT), which is a simplified version of the 5-CSRTT (Tsutsui-Kimura et al., 2009).

2. Materials and Methods

2.1. Subjects

Thirteen male Wistar/ST rats, supplied by Nippon SLC Co. Ltd. (Hamamatsu, Japan), were used. They were housed in groups of two to four rats under an alternating light-dark cycle (light from 7 p.m. to 7 a.m.) at approximately 21 °C and a relative humidity 40–50%. When the rats were 9 weeks old (270–290 g), we started to restrict their food intake. Thereafter their body weights were maintained at 85% of rats under free-feeding conditions. The daily food of rats in the home cages was purchased from CLEA JAPAN, Inc. (CE-2; Tokyo, Japan) and was given after each daily session. Food intake in the home cages was 10–15 g in the training period and 10–12 g (in addition to the daily food , another type of food 2–3 g in the operant box; see also *Three-choice serial reaction time task*) in the experimental period. Water was available ad libitum. The treatment of animals complied with the Guidelines for the Care and Use of Laboratory Animals of the Animal Research Committee of Hokkaido University.

2.2. Drugs

Dihydro-β-erythroidine hydrobromide (DHβE) and methyllycaconitine citrate (MLA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). DHβE is more selective for α3β2, α2β2, α4β2, and α4β4 (10–1000-fold) than α7-containing receptors (Harvey and

Luetje, 1996; Khiroug et al., 2004). MLA is > 1000-fold more selective for the α7 subtype than other subtypes (Ward et al., 1990). Both compounds were dissolved in 0.01 mol/l phosphate-buffered saline (PBS) to adjust the pH of resulting solutions to 6.9–7.0.

2.3. Apparatus

Aluminum operant chambers measuring 26 × 26 × 26 cm (Med Associates Inc., St. Albans, VT, USA) were used. The curved rear wall of each chamber contained nine 2.5-cm^2 holes, which were 2.2 cm deep and 2.3 cm above floor level. Each hole had an infrared photocell beam for detection of nose-poke responses and a 2.8-W bulb at its rear. Every other hole was sealed so that only the three centrally positioned holes were accessible. A food magazine was located on the opposite wall of the chamber, and a house light was located at the top of that wall. The apparatus was controlled by a computer program written in the MED-PC language (Med Associates Inc., St. Albans, VT, USA).

2.4. Three-choice serial reaction time task

The training procedure and the task sequence employed in the 3-CSRTT were as detailed in previous reports (Ohmura et al., 2009; Tsutsui-Kimura et al., 2009). Briefly,

when the task started, the house light was illuminated. After a fixed inter-trial interval (5 s), one of three holes was illuminated randomly and briefly (stimulus duration). Nose poking during the inter-trial interval was recorded as a premature response and resulted in the switching off of all lights (time-out: 5 s). The same trial was restarted immediately after finishing the time-out period. This parameter was regarded as an index of impulsive action. Nose poking into the lit hole while it was illuminated or within 5 s of limited hold was recorded as a correct response and was rewarded by the delivery of a food pellet (45 mg dustless precision pellets, Bio-serv, Frenchtown, NJ, USA). As an index of attentional function, accuracy (the percentage of correct responses) was calculated**.** Nose poking into another hole was recorded as an incorrect response and resulted in a 5 s time-out. The correct response latency, an index of motor function, and the reward latency, an index of motivation and/or appetite, were also measured. The reward latency was the time between a correct response and nose poking into the food magazine. When a rat failed to nose-poke within the limited hold, the trial was recorded as an omission and resulted in a 5 s time-out. This parameter was also regarded as an index of motivation and/or appetite. After a food pellet was delivered to and collected by a rat, the house light was switched off for 2 s to allow the rat to eat the pellet before the next trial was started. The start of the next inter-trial interval was

signaled by turning on the house light. Additional nose poking into any of the three holes prior to food collection was recorded as a perseverative response and resulted in a 5 s time-out. This parameter was regarded as an index of compulsive behavior. Because trials were initiated automatically, we did not set a time restriction for this task. Each session consisted of 100 trials. All rats in the present study finished 100 trials within 32 min. Training was conducted for one session per day and six sessions per week.

At the beginning of the training schedule, stimuli lasted 30 s. Depending on individual performance, the duration was progressively reduced to 1 s (by stepping to 15, 10, 5, 3, 2, 1.5, and 1 s). When a rat attained the criteria of > 80% accuracy (percentage of correct responses) and < 20 omissions in a session, the stimulus duration was reduced in the next session.

We used six behavioral parameters:

(a) Premature responses (no. per session)

(b) Accuracy (percentage of correct responses): [correct responses / (correct and incorrect responses)] ×100

(c) Omissions (no. per session)

(d) Perseverative responses (no. per session)

(e) Correct response latency (s)

(f) Reward latency (s)

Training was completed when rats reached the target phase (stimulus duration 1 s) and showed stable performance. After the completion of training, the stimulus duration was fixed at 1 s regardless of performance. We set the criteria for determining stable performance as follows: changes in premature responses stayed within ± 25%, accuracy stayed within ± 5%, and percent response omissions were less than 20 for at least three consecutive sessions.

2.5. Surgery

After completing the training, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and fixed in a stereotaxic frame (Narishige, Tokyo, Japan). Stainless-steel guide cannulas (24 gauge, 9 mm long) were unilaterally implanted 2 mm above the lateral ventricle with coordinates 0.8 mm posterior to the bregma, 1.5 mm lateral to the midline, and 2.2 mm ventral to the dura (Paxinos and Watson 1996). Dummy cannulas (30 gauge, 9 mm long) were also inserted, which penetrated to the tip of the guide cannulas. After surgery, rats were housed individually and allowed a 4-day recovery period prior to retraining.

2.6. Drug treatment schedule

Following the recovery period, rats were retrained until their performance was reestablished for at least three consecutive sessions. Microinjections were performed by gently restraining rats with a towel. Before the first microinjection was conducted, rats were subjected to three sham handling procedures prior to training sessions. They consisted of gently restraining the animal for approximately 2 min and removing the dummy cannula. Each drug session was separated by more than a 2-day interval. Twelve rats were divided into two groups. Group 1 (n = 6) received DHβE injections first and then were administered MLA injections one week after the last DHβE injection. On the other hand, Group 2 (n = 6) received MLA injections first and then were administered DHβE injections one week after the last MLA injection. As a result, all rats received both DHβE and MLA injections (n = 12).

2.6.1. Experiment 1: Effects of i.c.v. injection of DHβE on impulsive action as assessed by the 3-CSRTT

Rats were injected with DHβE (0, 3, 10, or 30 μg in 5 μl PBS) into the lateral ventricle according to a Latin Square design. Five minutes before the testing session, 5 μl of solution was injected with a Hamilton microsyringe using a 30-gauge stainless-steel injector (11 mm long) attached to a polyethylene tube. The solution was infused over a period of 2 min at contrast flow using a microinjection pump (CMA100,

Carnegie Medicine, Sweden), and the injector was left in place for 2 min after injection to allow diffusion.

2.6.2. Experiment 2: Effects of i.c.v. injection of MLA on impulsive action as assessed by the 3-CSRTT

Five minutes before the testing session, each rat was injected with MLA (0, 3, 10, and 30μg in 5 μl PBS) into the lateral ventricle according to a Latin Square design. Other procedures were the same as those of experiment 1.

2.7. Baseline performance assessment

We used data from the last 3 days of training to provide a preoperative baseline and data from the last 3 days of re-training to provide a postoperative baseline. Experimental baselines were assessed one day before the testing day.

2.8. Histology

Following completion of the experiments, rats were deeply anaesthetized with urethane (1 g/kg, i.p.), and 5 μl of Evans Blue was injected into the lateral ventricle. Rats were then sacrificed, and cannula placements were verified histologically according to the atlas of Paxinos and Watson (1996). Only data from rats with correct

injections were included in the analysis.

2.9. Data analysis

Six behavioral measures were analyzed (see *2.4. Three-choice serial reaction time task*). Before parametrical tests were conducted, we carried out Shapiro-Wilk tests for all six behavioral parameters. When we could not assume a normal distribution, the following data transformations were carried out to normalize data: accuracy was arcsine-transformed, premature responses were subjected to square-root transformations, and correct response latency was subjected to log 10 logarithm transformation. Current graphs were drawn by raw data.

For DHβE and MLA dose-response experiments, each measure was analyzed separately by two-factor ANOVA for repeated measures, with rank of dose injection as the between- and dose as the within-subject factor. Multiple comparisons with Bonferroni's correction were also conducted.

Baseline behavioral data were subjected to repeated-measures ANOVA.

The alpha level was set at 0.05 for all statistical procedures. All statistical procedures were conducted using SPSS (version 15.0 J).

3. Results

3.1. Histological analysis

Figure 1 shows coronal sections of the typical spread of Evans Blue. From 13 implanted rats, one rat was excluded after histological analysis, resulting in data analysis of 12 rats.

Insert Figure 1 About Here

3.2. Experiment 1: Effects of i.c.v. injection of DHβE on impulsive action as assessed by the 3-CSRTT

Figure 2 shows the effects of DHβE on behavioral parameters in the 3-CSRTT. Injections of DHβE produced a main effect of dose on premature responses ($F_{3, 33} = 5.36$, $P < 0.05$) and omissions ($F_{3, 33} = 6.14$, $P < 0.05$) but not on accuracy ($F_{3, 33} = 0.43$, NS), perseverative responses ($F_{3, 33} = 0.95$, NS), correct response latency ($F_{3, 33} = 1.35$, NS), or reward latency ($F_{3, 33} = 0.97$, NS). There was no main effect of rank of dose injection or significant dose × rank of dose injection interaction in any of the six

parameters. A multiple-comparison test with Bonferroni's correction revealed that 10 μg of DHβE significantly decreased the number of premature responses compared to those in vehicle control ($P < 0.05$). A multiple comparison also revealed that 30 μg of DHβE significantly increased the number of omissions compared to a 3 μg dose ($P < 0.05$).

Insert Figure 2 About Here

3.3. Experiment 2: Effects of i.c.v. injections of MLA on impulsive action assessed by the 3-CSRTT

Figure 3 shows the effects of MLA on behavioral parameters in the 3-CSRTT. The ANOVA revealed a main effect of dose on omissions ($F_{3, 33} = 5.77$, $P < 0.05$) but not on premature responses ($F_{3, 33} = 1.43$, NS), accuracy ($F_{3, 33} = 0.31$, NS), perseverative responses ($F_{3, 33} = 1.93$, NS), correct response latency ($F_{3, 33} = 2.56$, NS), or reward latency ($F_{3, 33} = 0.75$, NS). There was no main effect of rank of dose injection or significant dose × rank of dose injection interaction in any of the six parameters. A multiple-comparison test with Bonferroni's correction revealed that 30 μg of DHβE significantly increased the number of omissions compared to vehicle ($P < 0.05$).

Insert Figure 3 About Here

3.4. Baseline performance assessment

Figure 4 shows the preoperative (Pre-ope), postoperative (Post-ope), and experimental (Pre-infusion) baseline and control vehicle injection (Vehicle) performances as assessed by premature responses, accuracy, and omissions for all rats, assessed over 16 different sessions. Repeated-measures ANOVA revealed no significant effects of day on premature responses ($F_{15, 165} = 0.80$, NS), accuracy ($F_{15, 165} = 1.26$, NS), or omissions ($F_{15, 165} = 0.88$, NS). This analysis indicated that baseline performance remained stable throughout the duration of the experiment.

Insert Figure 4 About Here

4. Discussion

The major findings of the present studies are as follows: (1) i.c.v. injections of 10 μg of DHβE decreased impulsive-like premature responses without changing any other behavioral parameters in the 3-CSRTT, and (2) i.c.v. injections of MLA failed to alter impulsive-like action at any dose in the 3-CSRTT. Therefore, the present study demonstrates that endogenous acetylcholine provokes impulsive action via its action on DHβE-sensitive nicotinic acetylcholine receptors.

Several nicotinic acetylcholine receptor subtypes, including α4β2, α4β4, α3β2, and α2β2 nicotinic acetylcholine receptors, are antagonized by DHβE (Harvey and Luetje, 1996; Khiroug et al., 2004). Given that α4β2 nicotinic acetylcholine receptors are the subtype most sensitive to DHβE and are one of the most prominent nicotinic acetylcholine receptors in the rat brain (Galzi and Changeux, 1995; Harvey and Luetje, 1996), α4β2 nicotinic acetylcholine receptors could be the critical receptor subtype through which endogenous acetylcholine produces its effects on impulsive-like action in the 3-CSRTT.

Only the intermediate of three tested doses of DHβE caused a significant decrease in impulsive-like premature responses (Fig. 2A). At this juncture, we could interpret this in at least three ways. First, the highest doses of DHβE might disrupt motivation and/or

appetite due to excessive blockade of α4β2 and/or other DHβE-sensitive nicotinic acetylcholine receptors. Second, the highest doses of DHβE might induce cognitive impairment; Curzon et al. (1996) showed that high-dose i.c.v. injections of DHβE disrupted spatial memory. Third, moderate stimulation of α4β2 nicotinic acetylcholine receptors might be required to suppress impulsive behavior. If the highest doses of DHβE had decreased motivation and/or appetite, it would have also decreased the number of premature responses. However, it did not affect premature responses (Fig. 2A). Moreover, accuracy, an index of attentional function, was not significantly impaired by 30 μg of DHβE (Fig. 2B). Considering these results, the former two interpretations seem unlikely. It is likely that moderate stimulation of α4β2 nicotinic acetylcholine receptors is required to suppress impulsive behaviors; previous studies of ADHD patients have demonstrated that nicotine-patch application can suppress impulsive behaviors (Potter and Newhouse, 2004, 2008), and we demonstrate in the present study using normal animals that an α4β2 nicotinic acetylcholine receptor antagonist suppressed impulsive-like action. It has been hypothesized that ADHD patients have cholinergic dysfunction (Beane and Marrocco, 2004), and some studies have demonstrated that polymorphisms of the α4 nicotinic acetylcholine receptor subunit gene are modestly associated with ADHD (Todd et al., 2003; Brookes et al.,

2006; Guan et al., 2009; but see Kent et al., 2001).

Although α4β2 nicotinic acetylcholine receptors seem to play a role in provoking impulsive action by endogenous acetylcholine, α7 nicotinic acetylcholine receptors do not seem to be critical. In the present study, i.c.v. injection of the selective α7 nicotinic acetylcholine receptor antagonist MLA failed to alter impulsive-like action. This result is consistent with previous studies on systemic administration of MLA, which reported that α7 receptors do not play a role in the impulsive action-inducing effects of nicotine observed in the 5-CSRTT (Blondel et al., 2000; Grottick and Higgins, 2000).

One may wonder if we failed to detect the effects of MLA on impulsive-like action due to the unfixed duration of sessions. However, the average time taken to finish the task in drug-testing days was only 23.9 min. Moreover, the half-life of MLA in the rat brain is about 30 min when intraperitoneally injected (Turek et al., 1995). Thus, it is highly likely that rats were under the effects of drugs during the entirety of the task because of the task's short duration, even though somewhat-faster elimination might be expected for i.c.v. injection. Indeed, 30 μg of MLA did significantly affect a behavioral parameter (Fig. 3C).

Which brain sites are responsible for impulsive action modulated by activation of α4β2 nicotinic acetylcholine receptors by endogenous acetylcholine? From previous

neuropharmacological studies, we can identify at least two possible brain regions.

First, endogenous acetylcholine may provoke impulsive action via activation of α4β2 nicotinic acetylcholine receptors in the ventral tegmental area and dopamine terminals in the nucleus accumbens (NAc) (Xi et al., 2009). It is well known that systemic administration of nicotine increases dopamine levels in NAc (e.g., Liang et al., 2008; for a review, Xi et al., 2009). Dopamine depletion in the NAc enhances inhibitory control in the 5-CSRTT (Cole and Robins, 1989). A more recent study indicates that activation of dopamine D_1, rather than dopamine D_2, receptors in the NAc appears to be important in regulating inhibitory control in the 5-CSRTT (Pattij et al., 2007).

Second, endogenous acetylcholine may increase impulsive-like premature responses by acting on α4β2 nicotinic acetylcholine receptors in the infralimbic cortex (IL), the ventral portion of the medial prefrontal cortex. Selective excitotoxic IL lesions increase impulsive-like premature responses (Chudasama et al., 2003). Moreover, local application of N-methyl-D-aspartate (NMDA) receptor antagonists in the IL disrupted inhibitory control in the 5-CSRTT (Murphy et al., 2005). The fact that the prefrontal cortical α4β2 nicotinic acetylcholine receptors are expressed not only on pyramidal cells but also on interneurons in rats (Court and Clementi, 1995; Alkondon and Albuquerque, 2004) supports this hypothesis.

Additional approaches involving local administration of a nicotinic receptor antagonist are required to elucidate the brain sites responsible for effects of endogenous acetylcholine on impulsive action.

One of the most prominent disorders involving lack of inhibitory control is ADHD. Symptoms of ADHD are characterized by three main components: inattention, lack of inhibitory control, and hyperactivity. Only two substances, methylphenidate and atomoxetine, have been proposed as effective pharmacological treatments for ADHD. For additional treatment, use of nicotinic acetylcholine receptor agonists rather than antagonists has become an issue (Potter and Newhouse, 2004, 2008). We used normal rats, while other previous studies employed ADHD patients who might have cholinergic dysfunction (for a review, Beane and Marrocco, 2004). It is possible that dysfunction of the cholinergic system alters the effects of nicotinic acetylcholine receptor agonists and/or antagonists, as discussed above. The reasons for these contradictory findings should be addressed in future studies.

Taken together, the present study provides the first evidence for a role of endogenous acetylcholine in impulsive action via its effects on α4β2 nicotinic acetylcholine receptors. This finding will extend the understanding of neural mechanisms underlying impulsive action and will help explorations for novel therapeutic agents for psychiatric

diseases characterized by lack of inhibitory control.

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Figure Legends

Fig. 1. Representative photographs of coronal sections showing sites of the typical spread of Evans blue (indicated by arrows). Tips of injectors were not observed below or above the lateral ventricles.

Fig. 2. The effects of i.c.v. injection of DHβE (0, 3, 10, and 30 μg) on (A) premature responses, (B) accuracy, (C) omissions, (D) perseverative responses, (E) correct response latency, and (F) reward latency.

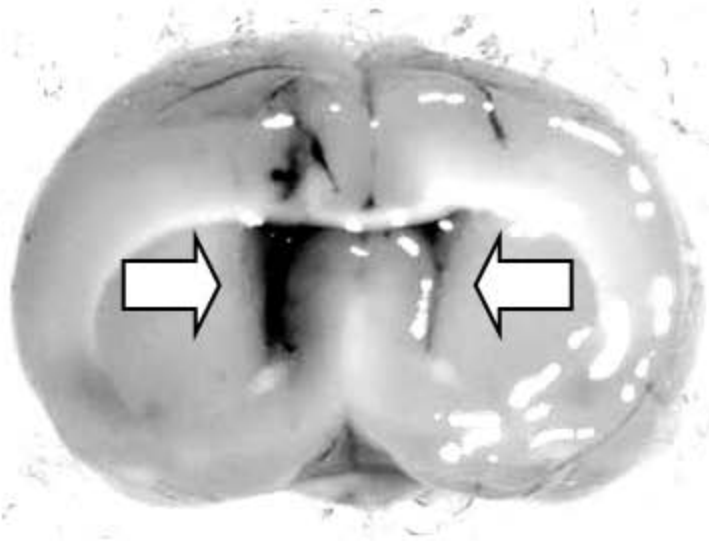
Data are presented as the mean ± S.E.M. (n = 12). * $P < 0.05$ (with Bonferroni's correction).

Fig. 3. The effects of i.c.v. injection of MLA (0, 3, 10, and 30 μg) on (A) premature responses, (B) accuracy, (C) omissions, (D) perseverative responses, (E) correct response latency, and (F) reward latency.

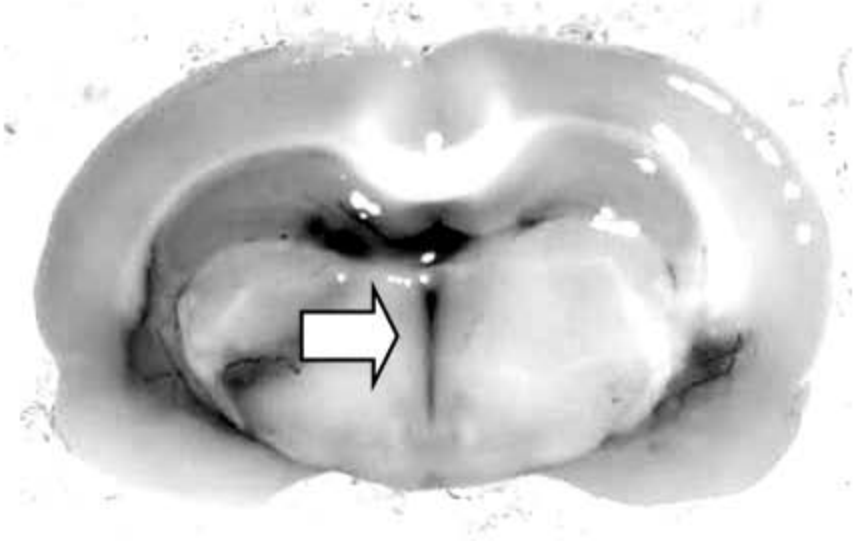
Data are presented as the mean ± S.E.M. (n = 12). * $P < 0.05$ (with Bonferroni's correction).

Fig. 4. Baseline performance assessment

Three preoperative (Pre-ope), three postoperative (Post-ope), and eight experimental (Pre-infusion) baseline session and two control-vehicle injection (Vehicle, left side; DHβE experiment, right side; MLA experiment) performance levels of premature responses, accuracy, and omissions in all twelve rats [■: accuracy (%); ♦: premature responses (no.); ▲: omissions (no.)]. The data are presented as the mean ± S.E.M. No significant differences were found using repeated-measures ANOVA for each variable.



Bregma -0.40 mm



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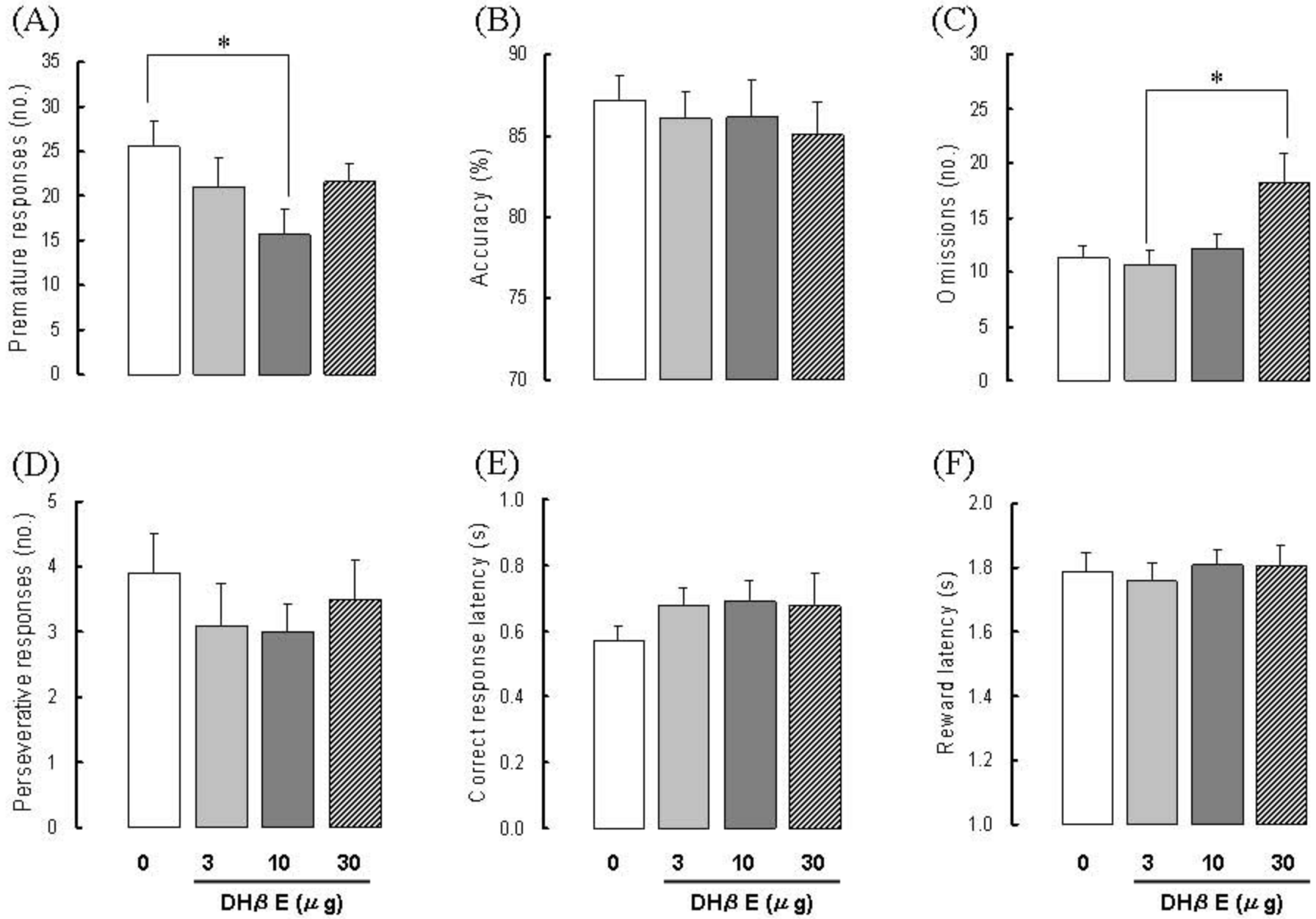
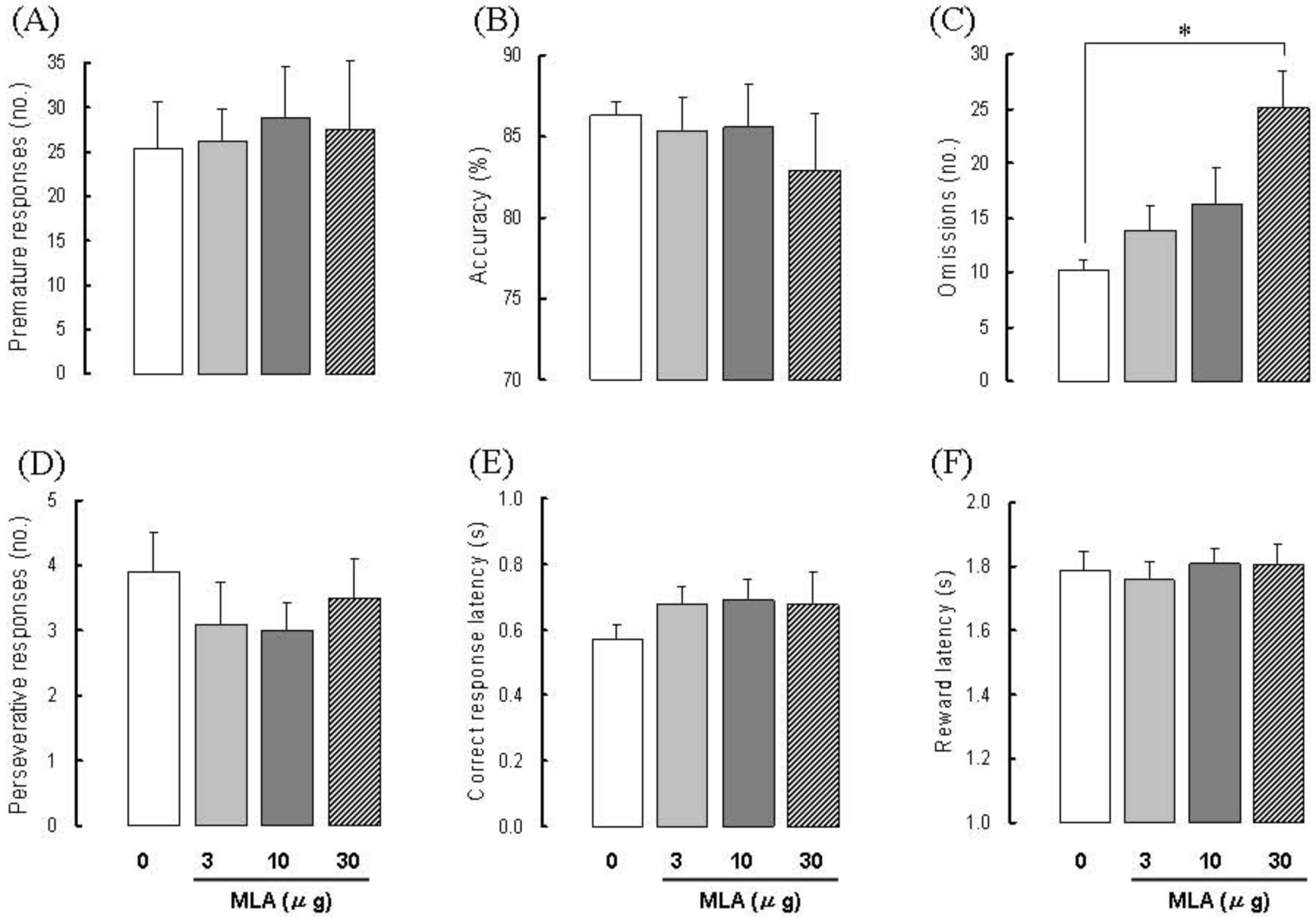
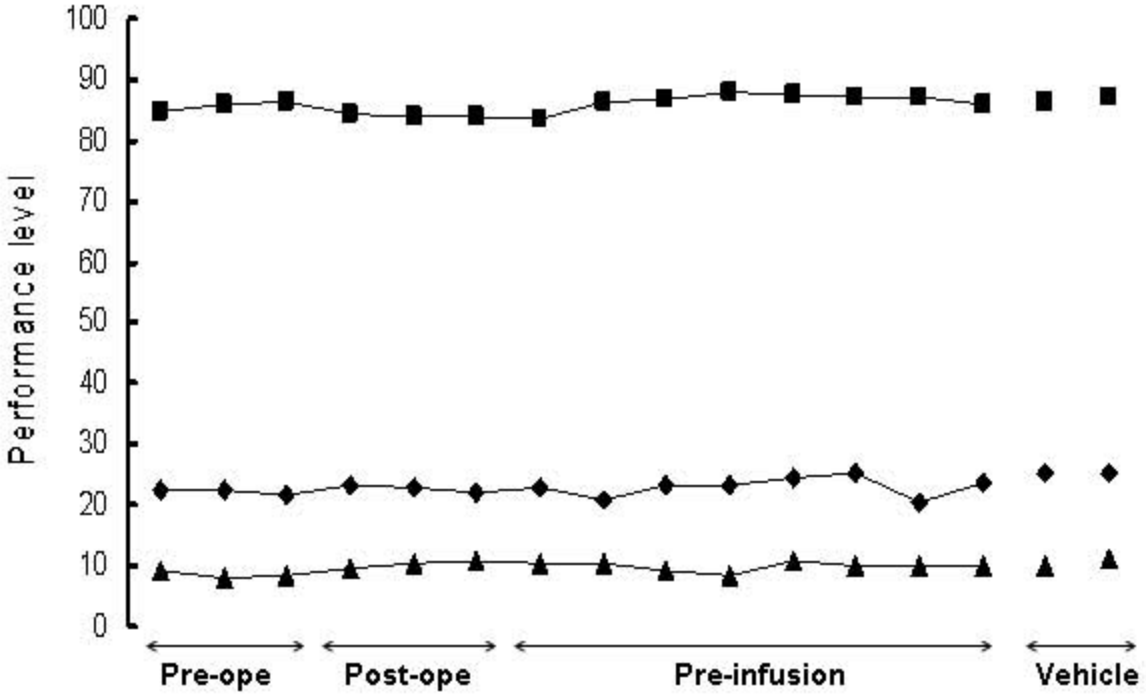

(A)
Premature responses (no.)
35
30
25
20
15
10
5
0
*
(B)
Accuracy (%)
90
85
80
75
70
(C)
Omissions (no.)
30
25
20
15
10
5
0
*
(D)
Perseverative responses (no.)
5
4
3
2
1
0
0
3
10
30
DHβ E (μg)
(E)
Correct response latency (s)
1.0
0.8
0.6
0.4
0.2
0.0
0
3
10
30
DHβ E (μg)
(F)
Reward latency (s)
2.0
1.8
1.6
1.4
1.2
1.0
0
3
10
30
DHβ E (μg)


(A)
Premature responses (no.)
35
30
25
20
15
10
5
0
(B)
Accuracy (%)
90
85
80
75
70
(C)
*
Omissions (no.)
30
25
20
15
10
5
0
(D)
Perseverative responses (no.)
5
4
3
2
1
0
0
3
10
30
MLA (μ g)
(E)
Correct response latency (s)
1.0
0.8
0.6
0.4
0.2
0.0
0
3
10
30
MLA (μ g)
(F)
Reward latency (s)
2.0
1.8
1.6
1.4
1.2
1.0
0
3
10
30
MLA (μ g)


100
90
80
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60
50
40
30
20
10
0
Performance level
Pre-ope
Post-ope
Pre-infusion
Vehicle