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Mirtazapine exerts an anxiolytic-like effect through activation of the median raphe nucleus-dorsal hippocampal 5-HT pathway in contextual fear conditioning in rats

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Abstract

The functional role of serotonergic projections from the median raphe nucleus (MRN) to the dorsal hippocampus (DH) in anxiety remains understood poorly. The purpose of the present research was to examine the functional role of this pathway, using the contextual fear conditioning (CFC) model of anxiety. We show that intra-MRN microinjection of mirtazapine, a noradrenergic and specific serotonergic antidepressant, reduced freezing in CFC without affecting general motor activity dose-dependently, suggesting an anxiolytic-like effect. In addition, intra-MRN microinjection of mirtazapine dose-dependently increased extracellular concentrations of serotonin (5-HT) but not dopamine in the DH. Importantly, intra-DH pre-microinjection of WAY-100635, a 5-HT_{1A} antagonist, significantly attenuated the effect of mirtazapine on freezing. These results, for the first time, suggest that activation of the MRN-DH 5-HT_{1A} pathway exerts an anxiolytic-like effect in CFC. This is consistent with the literature that the hippocampus is essential for retrieval of contextual memory and that 5-HT_{1A} receptor activation in the hippocampus primarily exerts an inhibitory effect on the neuronal activity.

Keywords: Contextual fear conditioning; hippocampus; median raphe nucleus; serotonin; 5-HT_{1A} receptor

Abbreviations: 5-HT, serotonin; ANOVA, analysis of variance; AP, anterior/posterior; BLA, basolateral amygdala; CeA, central nucleus of the amygdala; CFC, contextual fear conditioning; DH, dorsal hippocampus; DV, dorsal/ventral; HPLC-ECD, high-performance liquid chromatography with electrochemical detection; ML,

medial/lateral; MRN, median raphe nucleus; NA, noradrenaline; PAG, periaqueductal gray; SEM, standard error of the mean; SSRIs, selective serotonin reuptake inhibitors

1. Introduction

Although the dorsal raphe nucleus and its projections (e.g., to the amygdala or the medial prefrontal cortex) have been well implicated in depression and anxiety, the role of the median raphe nucleus (MRN) and its projections (e.g., to the dorsal hippocampus [DH]) have been only recently investigated (Andrade et al., 2013; Deakin, 2013). It has been proposed that the MRN acts to inhibit the consolidation and retrieval of aversive memories through serotonergic projections to the DH (Deakin, 2013). This is in line with research regarding depression because the MRN-DH pathway in depression is hypoactive, which leads to ruminations, and successful antidepressants act to stimulate this pathway (Deakin, 2013). However, the role of the MRN-DH pathway in anxiety is still unclear.

Contextual fear conditioning (CFC) is a well-established model for generalized anxiety (Luyten et al., 2011). In a typical CFC experiment, animals are given unsignaled electrical footshocks in a conditioning chamber (i.e., contextual conditioning) and 24 hours later exposed to the same chamber again. Conditioned responses to the aversive context (i.e., freezing behavior) are used as a measure of anxiety. Acute systemic administration of selective serotonin (5-HT) reuptake inhibitors (SSRIs) and 5-HT_{1A} receptor agonists before re-exposure to the footshock chamber reduces the expression of CFC, an anxiolytic-like effect (for a review, see Inoue et al., 2011). Moreover, systemic administration of mirtazapine, a noradrenergic and specific serotonergic antidepressant, reduces the expression of CFC and increases extracellular 5-HT levels in the DH but not the prefrontal cortex (Kakui et al., 2009; Yamauchi et al., 2012; An et al., 2015). The anxiolytic-like effect of systemic mirtazapine was reversed by a selective 5-HT_{1A}

receptor antagonist, WAY-100635 (Kakui et al., 2009). Bilateral microinjection of a selective 5-HT_{1A} receptor agonist into the hippocampus or amygdala but not medial prefrontal cortex decreases the expression of CFC (Li et al., 2006; Matsuzaki et al., 2011). Furthermore, microinjection of mirtazapine into the MRN but not the DH or amygdala reduces the expression of CFC (An et al., 2013). Because the DH receives heavy 5-HT projections from the MRN (Hensler, 2006), and microinjection of mirtazapine into the MRN is believed to activate the MRN by $alpha_2$-adrenoceptor antagonism in the noradrenergic nerve terminals and subsequent $alpha_1$-adrenoceptor stimulation (see Kakui et al., 2009; An et al., 2013), we hypothesized that activation of the MRN-DH 5-HT_{1A} pathway is anxiolytic and underlies the therapeutic effect of mirtazapine.

In the present study, we examined the dose-dependent effects of intra-MRN microinjection of mirtazapine on the extracellular 5-HT level in the DH (using in vivo microdialysis) and on the expression of CFC. Furthermore, we examined whether the anxiolytic-like effect of intra-MRN microinjection of mirtazapine is abolished by bilateral pre-intra-DH microinjection of a 5-HT_{1A} receptor antagonist, WAY-100635.

2. Materials and methods

2.1. Animals

A total of 92 six-week-old male Sprague-Dawley rats from the Shizuoka Laboratory Animal Center (Shizuoka, Japan) were used. The rats were housed in polypropylene cages (3/cage) with wood shavings on the floor in a temperature-controlled environment (22 ± 2 °C) with unlimited access to food and water. They were maintained on a 12-h

light/dark cycle (light phase: 07:00–19:00). Experiments began after a 2-week period of acclimatization. Behavior experiments were performed between 09:00 and 14:00, and surgery and microdialysis were performed between 09:00 and 16:00. All experiments were approved by the Hokkaido University School of Medicine Animal Care and Use Committee and were in compliance with the Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Mirtazapine (Merck & Co, Inc., Whitehouse Station, NJ, USA) was suspended in 0.15% tartaric acid, and the selective 5-HT_{1A} antagonist, WAY-100635 maleate (Sigma-Aldrich, MO, USA) was dissolved in 0.9% saline. Based on our own (An et al., 2013) and others' (Dos Santos et al., 2008) studies, the doses of mirtazapine were chosen to be 0.15 or 1.5 μg/site, while the dose of WAY-100635 was chosen to be 0.925 μg/site. The vehicle alone was infused as a control.

2.3. Stereotaxic surgery

Rats were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneally) and then positioned in a stereotaxic apparatus. For microinjection experiments, rats were implanted with a 26-gauge stainless steel guide cannulae (Plastic One, Roanoke, VA, USA) directed toward to the MRN at anterior/posterior (AP) –7.8 mm, medial/lateral (ML) +0 mm, and dorsal/ventral (DV) –8.6 mm, or DH (bilateral) at AP –3.6 mm, ML ±1.9 mm, and DV –3.0 mm relative to the bregma, taken from the stereotaxic atlas (Paxinos and Watson, 2006). The guide cannulae for the MRN were inserted at a lateral

angle of 20° to avoid the sagittal sinus and cerebral aqueductal obstruction. For microdialysis experiments, AG-4 guide cannulae (Eicom, Kyoto, Japan) were implanted into the surface of the left DH unilaterally at AP –3.6 mm, ML +1.9 mm, and DV –2.2 mm relative to the bregma (Paxinos and Watson, 2006). After the surgery, rats were housed individually and allowed 7 days to recover from surgery. When not used for injection, the guide cannulae were occluded with obturators made of stainless steel wire.

2.4. Microinjection

A 33-gauge injection cannula, projecting 1.0 mm beyond the tips of the guide cannula (Plastic One), was inserted. The injection cannula was connected by polyethylene tubing to motor-driven microsyringes. The solution (0.5 µl) was infused through each injector at a rate of 0.5 µl/min. The injection cannula was left in position for an additional 60 s after the end of the drug infusion. All drug solutions were freshly prepared immediately before use. The exact placement of the injection cannula tips was verified at the end of the experiment (Fig. 1a-d). On the basis of the stereotaxic atlas of Paxinos and Watson (Paxinos and Watson, 2006), 11 rats that received injections outside the targeted area were excluded.

2.5. in vivo microdialysis

2.5.1. Perfusion

Under pentobarbital anesthesia (40 mg/kg intraperitoneally), microdialysis probes (membrane length 2 mm and 0.22 mm in outer diameter, A-I-4-02; Eicom) were inserted into the guide cannulae. The next day, microdialysis was performed in awake and freely

moving rats. Perfusion was started at a constant flow rate of 2 μl/min, using artificial cerebral spinal fluid (145 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, and 1.0 mM $MgCl_2$). Following initial perfusion for at least 2 h, dialysate samples were collected every 10 min in sample vials containing 20 μl of 0.05 M acetic acid; four baseline samples were collected. The exact placement of the probe tips was verified at the end of the experiment (Fig. 1e and f). On the basis of the stereotaxic atlas of Paxinos and Watson (Paxinos and Watson, 2006), nine rats that received probe placements outside the targeted area were excluded.

2.5.2. 5-HT and dopamine analysis

Extracellular 5-HT and dopamine levels were determined using high-performance liquid chromatography with electrochemical detection (HPLC-ECD) system (Eicom), as described previously (Kitaichi et al., 2010). For 5-HT and dopamine analysis, 20 μl of dialysate was injected into the HPLC system that used a 0.1 M phosphate buffer (pH 6.0) mobile phase containing 1% (v/v) methanol, 50 mg/l Na_2EDTA, and 500 mg/l sodium l-decanesulfonate. Separations were conducted at 25 °C with a flow rate of 0.5 ml/min. In the electrochemical detector, an oxidation potential was set at 400 mV. Standard solutions for 5-HT and dopamine were injected every working day, and the peak heights for the standards were used for comparison to determine the amount of 5-HT and dopamine in the samples. Microdialysis data were expressed as percentages of the corresponding baseline.

2.6. Fear conditioning and behavioral measures

As described previously (Inoue et al., 2004), for fear conditioning, the rats were individually exposed to a total of 2.5 min of inescapable electric footshocks [five footshocks (2.5 mA scrambled footshocks, pulse wave, 30 s duration) that were delivered at intershock intervals of 35–85 s (mean 60 s)] in a shock chamber with a grid floor (25 cm × 28 cm × 30 cm) (Modular Test Chamber, ENV-007, Med Associates Inc., St Albans, VT, USA). Electric shocks were administered using a Standalone Aversive Stimulator/Scrambler, ENV-414S (Med Associates Inc.). Twenty-four hours after footshocks, the rats were again placed in the shock chamber and were observed for 5 min without shocks. The behavior was videotaped and scored later. The duration of freezing behavior was recorded using a modified time-sampling procedure, as previously described (Inoue et al., 2004). Every 10 s, the behavior in which the animal was currently engaged was classified as either "freezing" or "activity." Freezing was defined as the absence of any observable movement of the skeleton and the vibrissae, except those related to respiration. All other behaviors were scored as activity. The percentage freezing score (freezing [%]) was computed as the proportion of 10-s periods during which the animal remained frozen all of the time.

2.7. Motor activity

Immediately after testing the expression of conditioned fear, rats were individually placed in a testing cage (38 × 33 × 17 cm) for the evaluation of spontaneous activity. Motor activity was automatically recorded, as described previously (Ohmori et al., 1994), using an apparatus with an infrared sensor that detects thermal radiation from animals over a

period of 10 min (Supermex; Muromachi, Japan). Horizontal movement was digitized and used as the measure of general motor activity.

2.8. Experimental design

2.8.1. Experiment 1: Effect of intra-MRN injection of mirtazapine on the expression of CFC

Seven days after surgery, rats were implanted with injection cannulae directed towards the MRN and then randomly divided into three groups. Each animal received an intra-MRN microinjection of mirtazapine at a dose of 0 (vehicle), 0.15, or 1.5 μg/site 10 min before the CFC testing to examine the dose-dependent effect.

2.8.2 Experiment 2: Effects of intra-MRN injection of mirtazapine on extracellular 5-HT and dopamine concentrations in the DH

In a separate group of rats that did not receive footshocks (i.e., CFC) a microdialysis guide cannula and probe was implanted. Twenty-four hours after implanting the probe, the rats were randomly divided into three groups. Following the collection of four baseline fractions, each group received an injection of mirtazapine at a dose of 0 (vehicle), 0.15, or 1.5 μg/site into the MRN.

2.8.3 Experiment 3: Effect of pre-intra-DH microinjection of WAY-100635 on the effect of mirtazapine on the expression of CFC

This experiment was exactly the same as experiment 1 except that rats were implanted with three injection cannulae directed towards the MRN and the bilateral DH. In this

experiment, the rats were randomly divided into three groups as follows: vehicle plus vehicle, vehicle plus mirtazapine (1.5 μg/site), and WAY-100635 (0.925 μg/site) plus mirtazapine (1.5 μg/site). Five minutes before the intra-MRN microinjection of mirtazapine (1.5 μg/site) or vehicle, WAY-100635 (0.925 μg/site) or vehicle was bilaterally injected into the DH.

2.9. Data analysis

The results are expressed as mean ± standard error of the mean (SEM) of the individual value of the rats from each group. One-way analysis of variance (ANOVA) test was used to analyze the freezing behavior and motor activity data. If a significant difference was detected, subsequently a Bonferroni's post-hoc test was used for multiple comparisons. Analysis of in vivo microdialysis data (dose-response of intra-MRN injection of mirtazapine on extracellular 5-HT and dopamine concentrations in the DH) used two-way ANOVA with repeated measures for percentage of basal values. If the interaction effect was significant, subsequently a Bonferroni's post-hoc test was used for multiple comparisons. The baseline values of 5-HT and dopamine (the average of absolute values from four baseline samples) were compared by one-way ANOVA. The significance level was chosen at 0.05.

3. Results

3.1. Experiment 1: Effect of intra-MRN injection of mirtazapine on the expression of CFC

Microinjection of mirtazapine into the MRN caused a dose-dependent reduction in freezing (Fig. 2). One-way ANOVA analysis revealed a significant main effect of group [$F(2,24) = 3.5$; $P < 0.05$]. Post-hoc analysis showed that mirtazapine at 1.5 μg reduced the expression of CFC significantly when compared with controls ($P < 0.05$). Importantly, mirtazapine did not affect general motor activity of rats (Table 1).

3.2. Effects of intra-MRN injection of mirtazapine on extracellular 5-HT and dopamine concentrations in DH

3.2.1. For the extracellular 5-HT concentrations in DH

Microinjection of mirtazapine into the MRN similarly showed a dose-dependent effect on the extracellular concentration of 5-HT in the DH (Fig. 3). Two-way ANOVA with repeated measures of 5-HT from 0 min to 70 min showed significant effects of time [$F(7,112) = 13.94$, $P < 0.001$], group [$F(2,16) = 9.46$, $P < 0.01$], and time × group [$F(14,112) = 1.94$, $P < 0.05$]. Post-hoc analysis showed that mirtazapine at 1.5 μg, but not 0.15 μg, significantly increased 5-HT compared with controls at 10 min ($P < 0.05$), 20 min ($P < 0.05$), 30 min ($P < 0.01$), and 40 min ($P < 0.01$) after microinjection.

The mean of the basal values (from −30 min to 0 min) of the extracellular 5-HT concentrations in the controls, the mirtazapine 0.15 μg, and mirtazapine 1.5 μg groups were 14.62 ± 4.13, 12.59 ± 2.04, and 7.64 ± 1.57 pg/ml, respectively. No significant difference was detected between the groups.

3.2.2. For the extracellular dopamine concentrations in DH

There was no significant effect of mirtazapine on the extracellular concentration of

dopamine in the DH (Fig. 4). The mean of the basal values (from −30 min to 0 min) of the extracellular dopamine concentrations in the controls, the mirtazapine 0.15 μg, and mirtazapine 1.5 μg groups were 7.05 ± 0.93, 7.18 ± 1.18, and 6.45 ± 2.02 pg/ml, respectively. No significant difference was detected between the groups.

3.3. Effect of pre-injection of WAY-100635 into DH on the effect of mirtazapine into MRN on the expression of CFC

Pre-injection of WAY-100635 into the DH significantly attenuated the effect of mirtazapine (1.5 μg) into the MRN on the expression of CFC (Fig. 5). One-way ANOVA indicated a significant main effect [$F(2,23) = 4.627$, $P < 0.05$]. Post-hoc analysis showed a significant difference between the vehicle-mirtazapine 1.5-μg group and vehicle-vehicle group ($P < 0.05$) but not between other groups. Of note, WAY-100635 alone did not affect freezing; there was no difference between vehicle-vehicle and vehicle-WAY-100635 (data not shown).

4. Discussion

Extending our previous findings that intra-MRN microinjection of mirtazapine at the dose of 3 μg reduced freezing (An et al., 2013), microinjection of mirtazapine at the dose of 1.5 μg but not 0.15 μg significantly reduced freezing in CFC assessed 10 min later. In addition, intra-MRN microinjection of mirtazapine at the dose of 1.5 μg but not 0.15 μg significantly increased the 5-HT level in the DH to approximately 250% of baseline. Importantly, the anxiolytic-like effect of mirtazapine was abolished by pre-intra-DH

microinjection of a 5-HT_{1A} antagonist, WAY-100635, at a dose that did not affect freezing behavior in intra-MRN saline-treated rats. Consistent with this finding, our present study demonstrated that the anxiolytic-like effect of systemic mirtazapine was reversed by a selective 5-HT_{1A} receptor antagonist, WAY-100635 (Kakui et al., 2009).These results suggest that mirtazapine into the MRN exerts an anxiolytic-like effect in a dose-dependent manner, and for the first time, we suggest that activation of the MRN-DH 5-HT_{1A} pathway mediates the anxiolytic-like effect of mirtazapine into the MRN in the CFC.

Recently it has been shown that following footshock in the CFC, optical stimulation of MRN neurons impairs CFC 3 days later (Wang et al., 2015). Importantly, optical stimulation of MRN neurons diminishes, whereas inhibition increases, sharp wave-associated field oscillations of the hippocampus (Wang et al., 2015). These previous findings support our idea that the effect of MRN on CFC may be mediated by the hippocampus.

The role of the hippocampus, especially the DH, in context processing has been established (Rudy, 2009; Maren et al., 2013). The hippocampus is required for integrating contextual cues into a configural representation, which is conditioned to the footshock in the amygdala (Rudy, 2009; Maren et al., 2013), although DH-independent mechanisms may also exist. The hippocampus is also necessary for retrieving memory, such as the contextual memory associated with fear in CFC (Want and Morris, 2010; Nadel et al., 2012). The central nucleus of the amygdala (CeA)-to-periaqueductal gray (PAG) pathway is believed to drive the conditioned freezing response ultimately (LeDoux, 2007; Penzo et al., 2014). The basolateral amygdala (BLA)-to-CeA projection is responsible for

anxiety-related behaviors within the amygdala (Tye et al., 2011). The hippocampal CA1 and subiculum most heavily input to the basal amygdala (Pitkänen et al., 2000) and basal amygdala "fear neurons," which are activated during re-exposure to the context of CFC, receive input from the hippocampus (Herry et al., 2008). Thus, optogenetic reactivation of hippocampal dentate gyrus neurons that are activated during CFC induces freezing (Liu et al., 2012) because it sends contextual information to the CeA-to-PAG pathway. Therefore, electrolytic lesions placed in regions of the hippocampus that project to the BLA (Maren and Fanselow, 1995), lesion of CA1 (Ji and Maren, 2008; Hunsaker and Kesner, 2008), or optical inhibition of CA1 neurons (Sakaguchi et al., 2015) reduce freezing in the CFC.

Consistent with the above logic, postsynaptic 5-HT_{1A} receptor activation is thought to primarily exert an inhibitory effect on neuronal activity (Hannon and Hoyer, 2008). Indeed, endogenous 5-HT inhibits the firing activity of hippocampal CA1 pyramidal neurons through 5-HT_{1A} receptors (Tada et al., 2004), and activation of the 5-HT_{1A} receptors in the DH decreases the expression of CFC (Almada et al., 2009).

Therefore, our results are in line with the evidence that was discussed earlier and suggest that mirtazapine activates the MRN-to-DH 5-HT_{1A} pathway and inhibits DH neurons that are necessary for constructing and retrieving fear-related contextual information. The failure in retrieving fear-related contextual memory will not activate the subsequent fear circuit that induces freezing behavior.

It should to be noted that although WAY-100635 has been widely employed as a 5-HT_{1A} antagonist in the scientific literature (e.g., Berrocoso and Mico, 2009; Horiguchi and Meltzer, 2012; Hunt et al., 2011; Vicente et al., 2008; Vinkers et al., 2010),

WAY-100635 also has dopamine D_4 receptor agonist activity (Chemel et al., 2006). However, a recent study demonstrated that D_4 agonism by WAY-100635 is much weaker (centesimal) than 5-HT_{1A} antagonism (Martel et al., 2007). More importantly, Falzone et al. (2002) showed that mice lacking dopamine D_4 receptors show normal contextual and tone-cued fear conditioning responses, which may allow us to exclude the involvement of potential dopamine D_4 receptor agonist activity. We note that research from another laboratory showed that inactivation of the MRN by either electrolytic lesion (Silva et al., 2004), neurotoxic lesion using NMDA (Borelli et al., 2005) or local delivery of 5-HT_{1A} agonists into the MRN (Silva et al 2004., Borelli et al., 2005; Almada et al., 2009) reduces the expression of CFC. Similar results seemingly supporting the hypothesis that activation of the MRN-DH 5-HT pathway is anxiogenic (Andrade et al., 2013) have been reported in rodent models of the elevated T-maze (Dos Santos et al., 2008) and the social interaction test (Andrews et al., 1994; Andrews et al., 1997). However, none of these studies directly measured 5-HT levels in the projection areas of the MRN, especially the DH. As noted earlier, the same laboratory also reported that activation of the 5-HT_{1A} receptors in the DH by locally delivering a 5-HT_{1A} agonist decreases the expression of CFC (Almada et al., 2009). These results suggest that inactivation of the MRN may have affected other unknown areas or may have induced contradictory effects and that the MRN-DH 5-HT pathway may play a different role in distinct models of anxiety, which deserves future in-depth investigation.

It has to be noted that a limitation of the present study is that we did not measure noradrenaline (NA) levels in the DH. Previous research has shown that systemic administration of mirtazapine promoted the release of NA in the DH and prefrontal cortex

of rats (Yamauchi et al., 2012). Whether intra-MRN microinjection of mirtazapine increases extracellular NA in the DH and whether NA in the DH is involved in the anxiolytic-like effect of mirtazapine remain to be investigated. Nevertheless, increased extracellular NA levels in the DH seem to be unassociated with the anxiolytic-like effect of the intra-MRN microinjection of mirtazapine because our previous studies showed that various drugs that increase extracellular NA levels do not have the anxiolytic-like effect in CFC (Inoue et al., 2011).

The hypothesis of the present study is that activation of the MRN-DH 5-HT pathway, which is produced by alpha$_2$-adrenoceptor antagonism in the MRN, exerts an anxiolytic-like effect. Therefore, we elected to use mirtazapine, which elevates extracellular 5-HT concentrations in the DH through alpha$_2$-adrenoceptor antagonism and subsequent alpha$_1$-adrenoceptor activation in the MRN, and showed that the anxiolytic-like effect of intra-MRN mirtazapine injection is blocked by intra-DH microinjection of a 5-HT$_{1A}$ antagonist, thereby supporting our hypothesis. However, mirtazapine blocks not only alpha$_2$-adrenergic autoreceptors and heteroreceptors but also 5-HT$_{2C/2A/3}$ and histamine$_1$ receptors (Millan, 2006). Therefore, another limitation of our study is that we did not examine selective antagonists for 5-HT$_{2C/2A/3}$ and histamine$_1$ receptors, with which mirtazapine shares common mechanisms. However, our previous studies reported that 5-HT$_{2C}$ and 5-HT$_{2A}$ antagonists were not effective at reducing expression of the CFC but that an alpha$_2$-adrenoceptor antagonist was effective (Masuda et al. 2013, 2014). Although the involvement of 5-HT$_{2C}$ and 5-HT$_{2A}$ antagonism in the anxiolytic-like effect of mirtazapine cannot be ruled out completely, alpha$_2$-adrenoceptor

antagonism is a strong candidate for the target mechanism of the anxiolytic-like effect of mirtazapine.

5. Conclusion

In conclusion, using microinjection and in vivo microdialysis, for the first time, we suggest that activation of the MRN-DH 5-HT_{1A} pathway reduces freezing in the CFC model of anxiety, an anxiolytic effect. This is consistent with the literature that postsynaptic 5-HT_{1A} receptor activation primarily exerts an inhibitory effect on the neuronal activity and that the hippocampus is essential for retrieval of contextual memory, activation of which contributes to freezing behavior in CFC.

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Figure legends

Figure 1. Photomicrographs of Nissl-stained sections showing the location of cannula tips in the MRN (a), DH (c), and microdialysis probes within the DH (e), as indicated by arrows. Schematic representation of the placements of the microinjection cannulae in the MRN (b) and DH (d) and the microdialysis probes within the DH (f). Rats with cannulae or probes outside of these target brain regions were excluded from the subsequent statistical analyses. Histological plates have been adapted from Paxinos and Watson (2006).

Figure 2. Dose-dependent effect of intra-MRN microinjection of mirtazapine (MTZ, 1.5 μg/site) on the expression of CFC. (a) Schematic illustration of the experimental procedure in this experiment. (b) The mean percentages ± S.E.M. for freezing were scored for the 5-min observation periods are given. Behavior was sampled in 10-s intervals. * $P < 0.05$ compared with vehicle. n = 9 per group.

Figure 3. Dose-dependent effect of intra-MRN microinjection of mirtazapine (MTZ, 1.5 μg/site) on the extracellular 5-HT in the DH. Values represent the mean ± S.E.M. (% of basal levels). The arrow indicates the timing of a drug injection. * $P < 0.05$ compared with vehicle; ** $P < 0.01$ compared with vehicle. Data are shown as mean ± SEM. n = 6–7 per group.

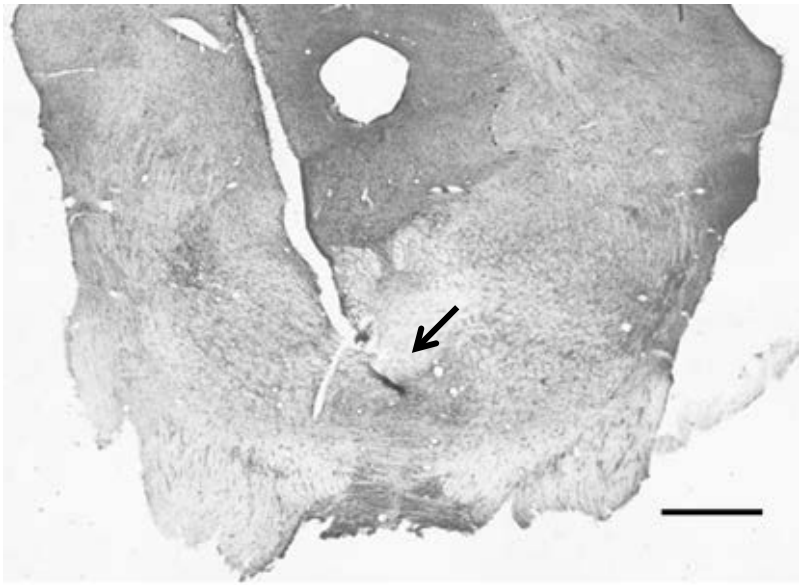
Figure 4. Effect of intra-MRN microinjection of mirtazapine (MTZ, 1.5 μg/site) on the

extracellular dopamine in the DH. Values represent the mean ± S.E.M. (% of basal levels). The arrow indicates the timing of a drug injection. Data are shown as mean ± SEM. n = 5 per group.

Figure 5. Anxiolytic effect of intra-MRN microinjection of mirtazapine (MTZ, 1.5 μg/site) was abolished by pre-intra-DH microinjection of a 5-HT_{1A} antagonist, WAY100635 (WAY, 0.925 μg/site). a) Schematic illustration of the experimental procedure in this experiment. b) The mean percentages ± S.E.M. for freezing that were scored for the 5-min observation periods are given. Behavior was sampled in 10-s intervals. * $P < 0.05$ compared with vehicle-vehicle. n = 8–9 per group.

Figure 1

a



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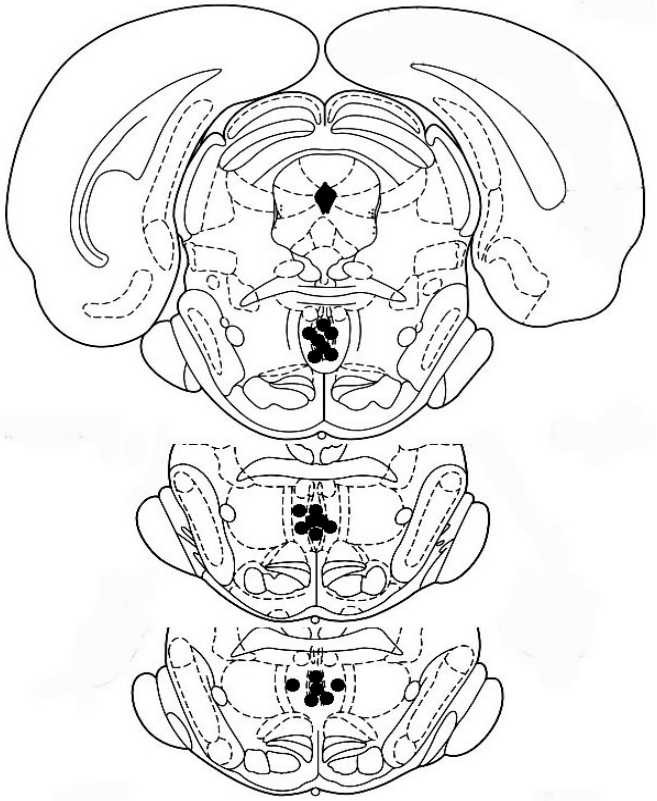

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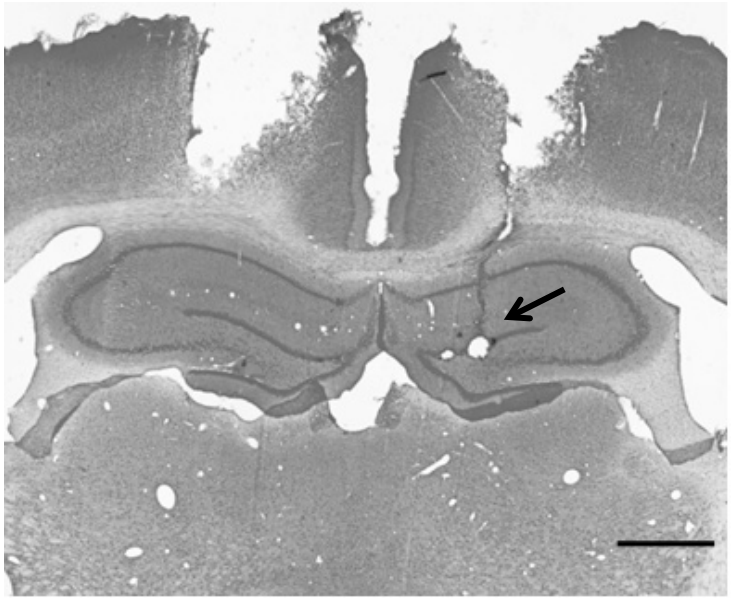

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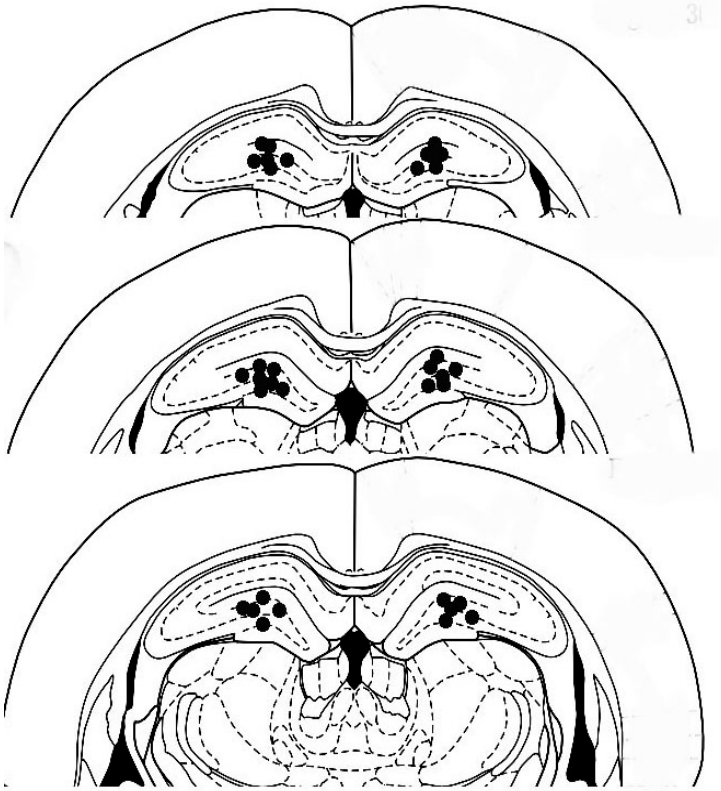

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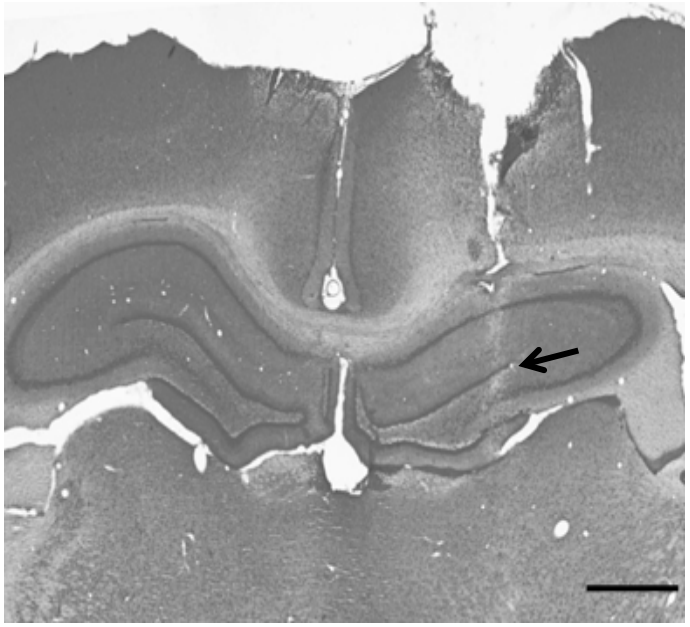

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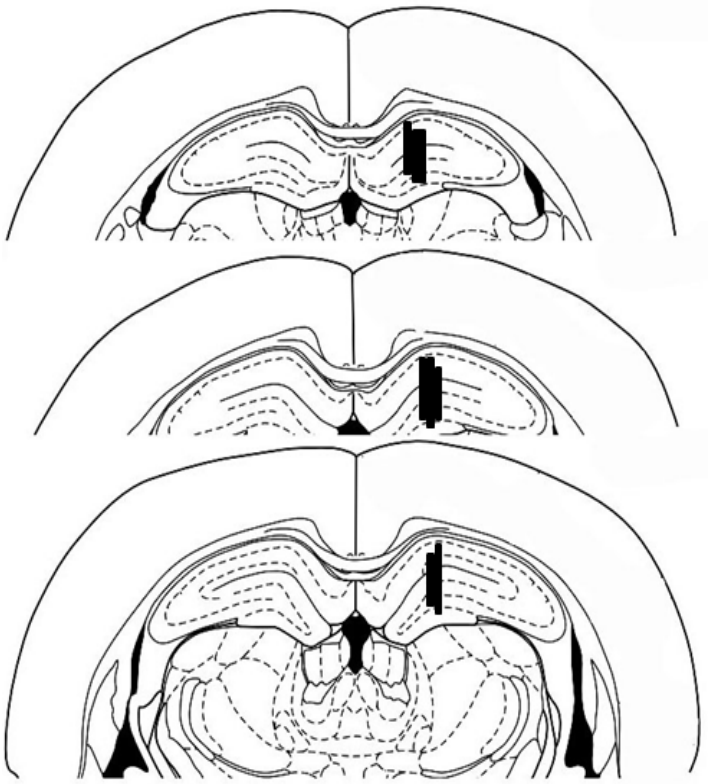
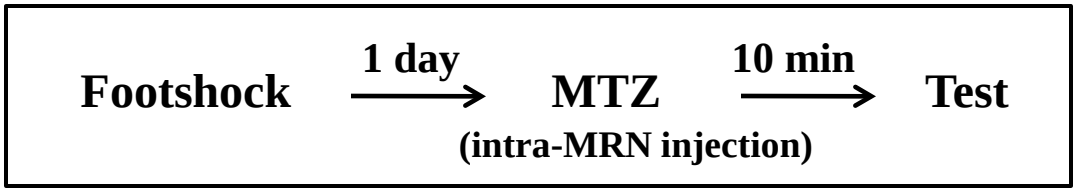


Figure 2

a


Footshock
1 day
MTZ
(intra-MRN injection)
10 min
Test

b

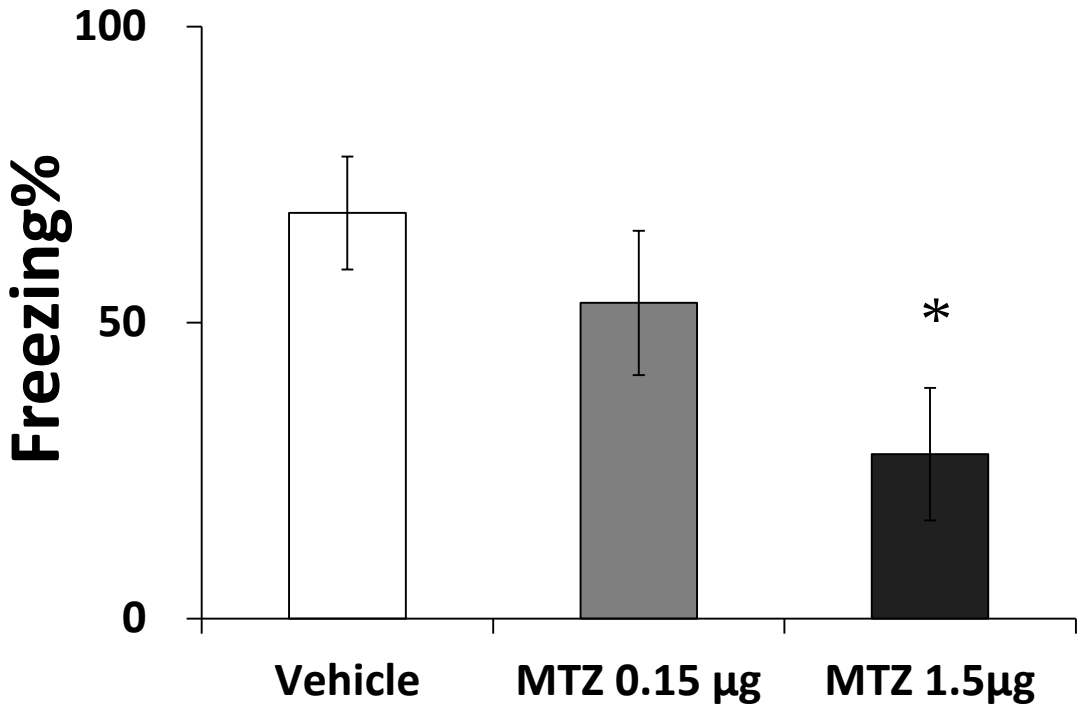

100
50
0
Freezing%
*
Vehicle
MTZ 0.15 µg
MTZ 1.5µg

Figure 3

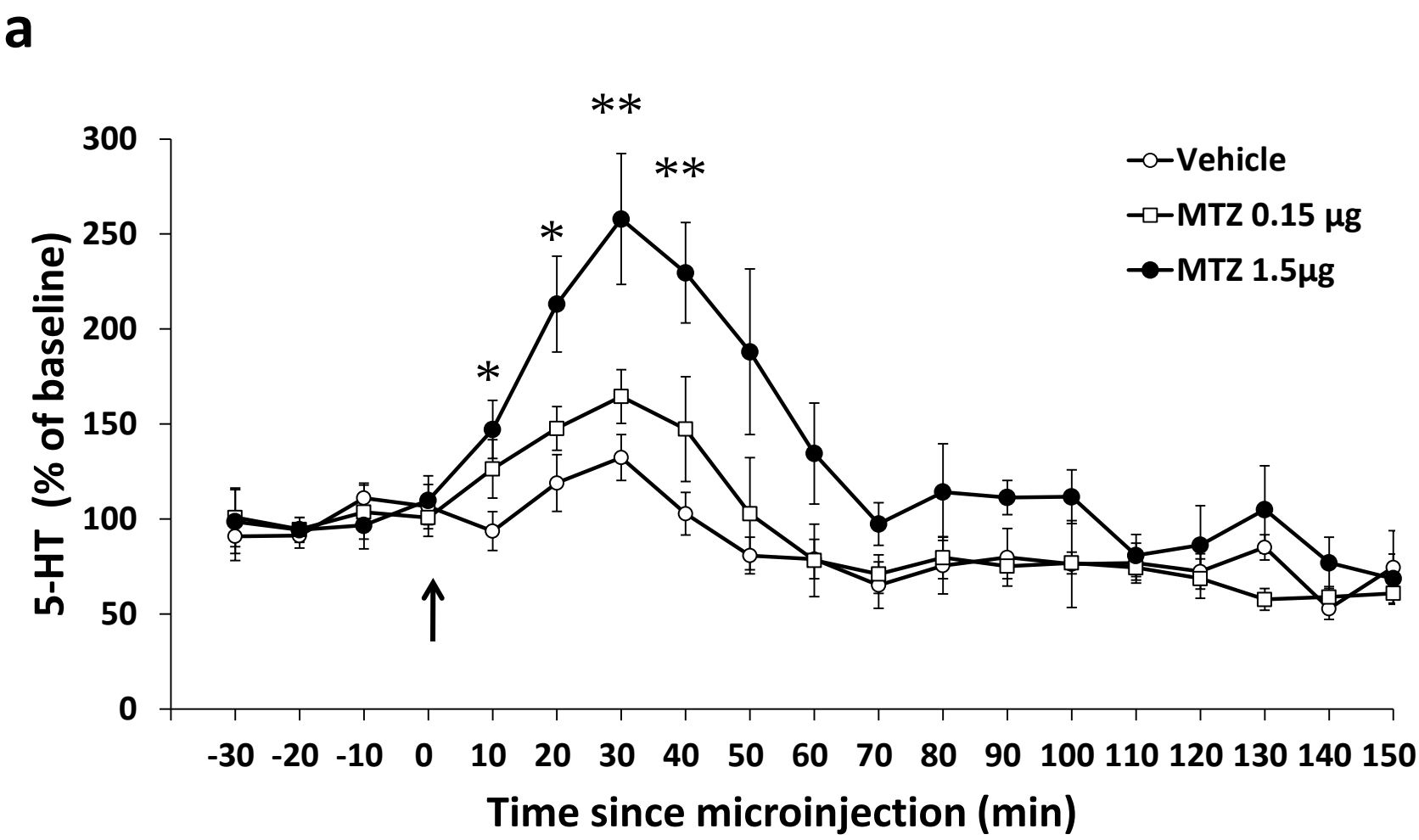

a
5-HT (% of baseline)
300
250
200
150
100
50
0
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Time since microinjection (min)
Vehicle
MTZ 0.15 µg
MTZ 1.5µg
*
*
**
**

Figure 4

a

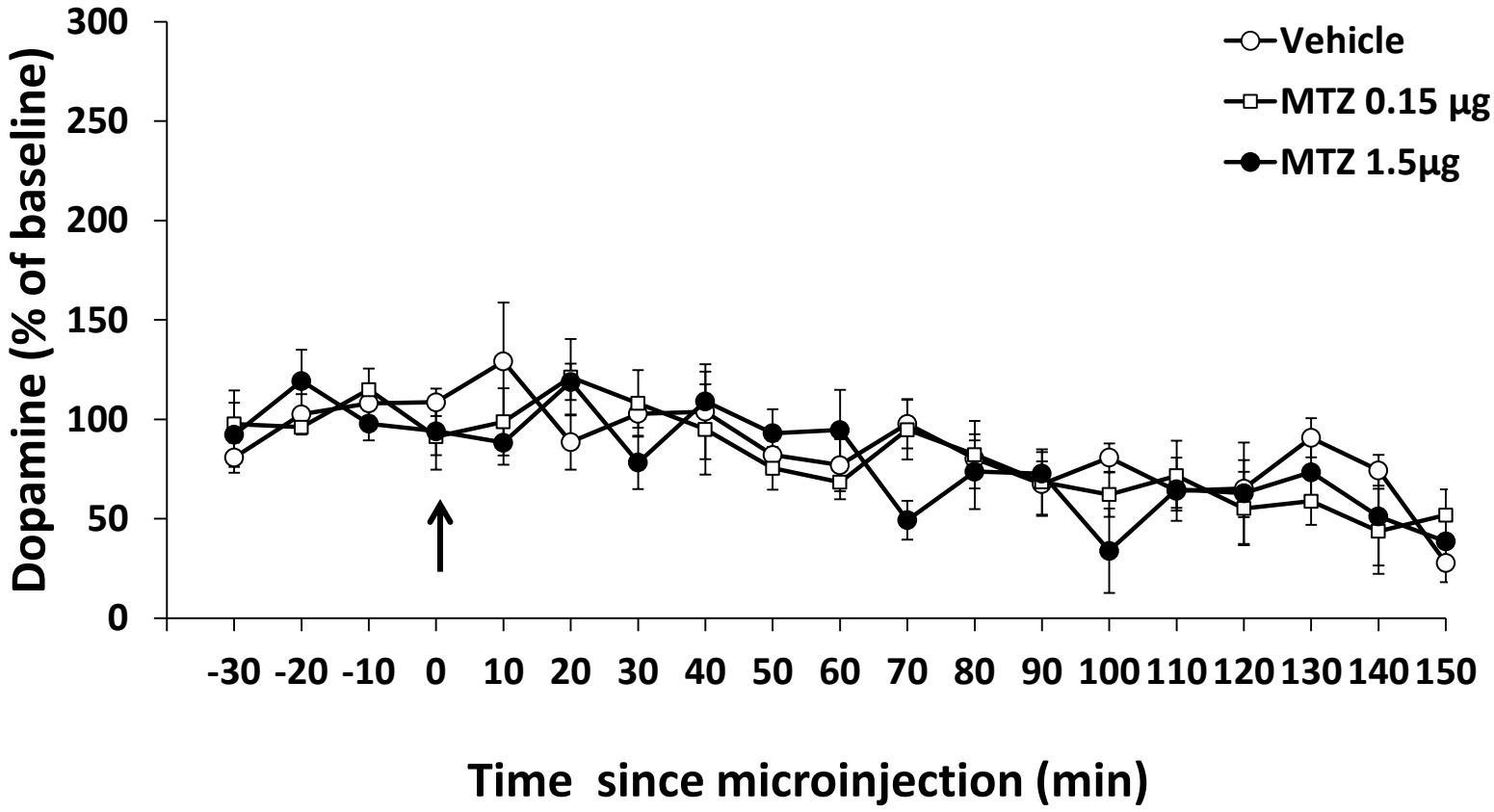
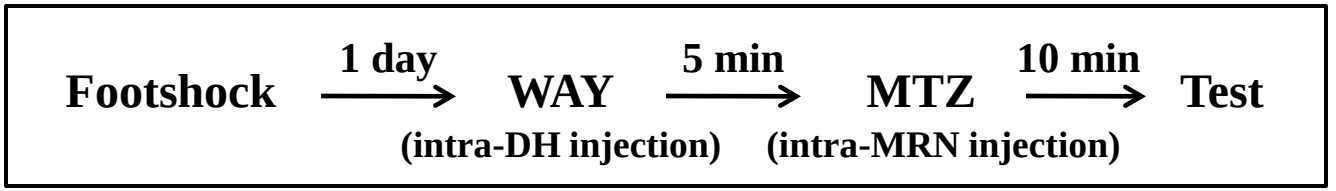
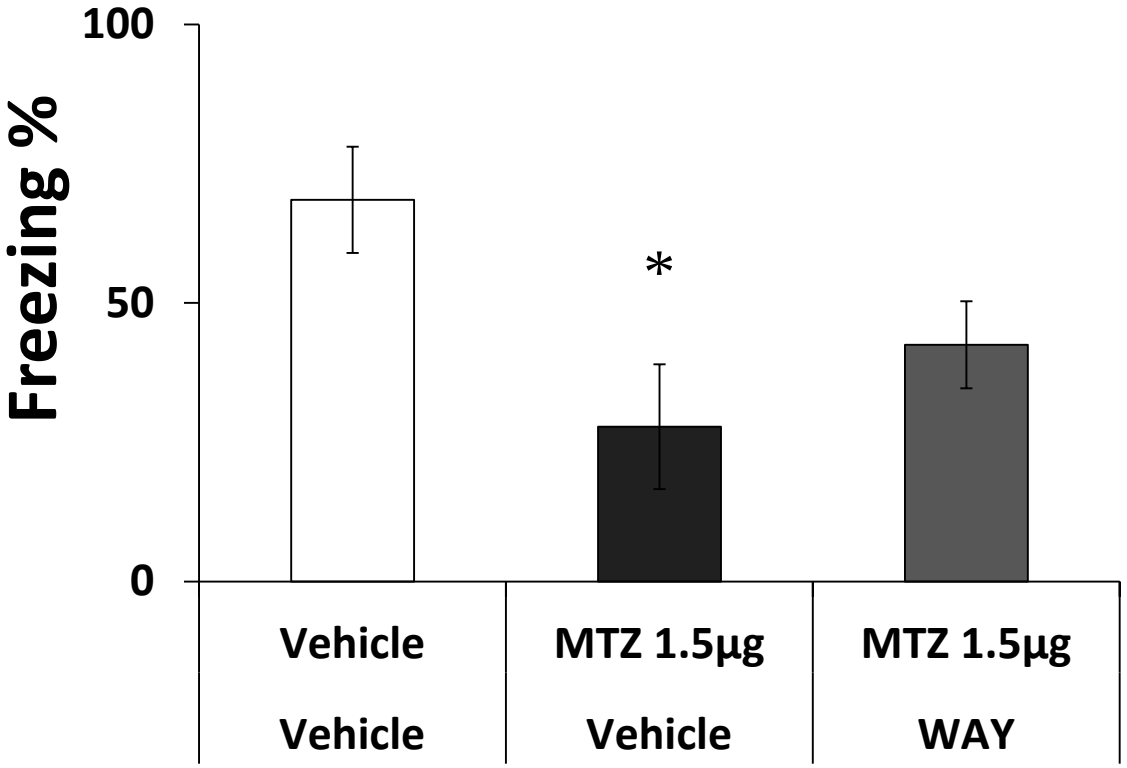

Vehicle
MTZ 0.15 µg
MTZ 1.5µg
Dopamine (% of baseline)
300
250
200
150
100
50
0
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Time since microinjection (min)

Figure 5

a


Footshock
1 day
WAY
(intra-DH injection)
5 min
MTZ
(intra-MRN injection)
10 min
Test

b


Freezing %
100
50
0
*
Vehicle
Vehicle
MTZ 1.5µg
Vehicle
MTZ 1.5µg
WAY