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Modification of 5-methoxy-*N,N*-dimethyltryptamine-induced hyperactivity by monoamine oxidase A inhibitor harmaline in mice and the underlying serotonergic mechanisms

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Abstract

Background—5-Methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) and harmaline are indolealkylamine (IAA) drugs often abused together. Our recent studies have revealed the significant effects of co-administered harmaline, a monoamine oxidase inhibitor (MAOI), on 5-MeO-DMT pharmacokinetics and thermoregulation. This study was to delineate the impact of harmaline and 5-MeO-DMT on home-cage activity in mouse models, as well as the contribution of serotonin (5-HT) receptors.

Methods—Home-cage activities of individual animals were monitored automatically in the home cages following implantation of telemetry transmitters and administration of various doses of IAA drugs and 5-HT receptor antagonists. Area under the effect curve (AUEC) of mouse activity values were calculated by trapezoidal rule.

Results—High dose of harmaline (15 mg/kg, *ip*) alone caused an early-phase (0–45 min) hypoactivity in mice that was fully attenuated by 5-HT_{1A} receptor antagonist WAY-100635, whereas a late-phase (45–180 min) hyperactivity that was reduced by 5-HT_{2A} receptor antagonist MDL-100907. 5-MeO-DMT (10 and 20 mg/kg, *ip*) alone induced biphasic effects, an early-phase (0–45 min) hypoactivity that was completely attenuated by WAY-100635, and a late-phase (45–180 min) hyperactivity that was fully suppressed by MDL-100907. Interestingly, co-administration of MAOI harmaline (2–15 mg/kg) with a subthreshold dose of 5-MeO-DMT (2 mg/kg) induced excessive hyperactivities at late phase (45–180 min) that could be abolished by either WAY-100635 or MDL-100907.

Conclusions—Co-administration of MAOI with 5-MeO-DMT provokes excessive late-phase hyperactivity, which involves the activation of both 5-HT_{1A} and 5-HT_{2A} receptors.

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Introduction

Indolealkylamine (IAA) drugs are 5-hydroxytryptamine (5-HT or serotonin) analogs that are able to modulate various physiological and psychological functions including body temperature, attention and behavior [1–3]. Many IAAs are found as psychoactive ingredients of a variety of plant and animal preparations used for medicine, religion and recreation purposes [4–8]. IAAs are also recognized as a major class of drugs of abuse, among which 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT or by the street name “5-MEO”) and 5-methoxy-N,N-diisopropyltryptamine (5-MeO-DiPT or “Foxy” and “Foxy methoxy”) were placed into Schedule I under the Controlled Substances Act in the United States by Drug Enforcement Administration (DEA) in 2011 and 2004, respectively [9, 10]. There are also many reports on IAA intoxications in recent years which include several fatal cases related to the abuse of 5-MeO-DMT or 5-MeO-DiPT [11–19].

5-MeO-DMT is metabolically inactivated by monoamine oxidase A (MAO-A), and thus it is often abused with MAO-A inhibitor (MAOI), e.g., harmaline, to achieve an improved hallucinogenic effect [20]. The pharmacokinetic drug-drug interactions among IAA compounds have been nicely demonstrated using 5-MeO-DMT and MAOI harmaline as model drugs [21–24]. In particular, co-administration of MAOI harmaline leads to a remarkably elevated and prolonged systemic and cerebral exposure to 5-MeO-DMT and an active metabolite bufotenine. In addition, MAOI harmaline greatly increases brain 5-HT levels in mice through the inhibition of 5-HT deamination metabolism [25]. Consequently, 5-MeO-DMT pharmacological effects may be significantly altered by co-administered MAOI harmaline [26–29]. Mechanistically, 5-MeO-DMT is rather a relatively less selective 5-HT receptor agonist and it is able to bind to 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors with modest to high affinities [5, 30–34]. Due to the presence of consistent pharmacokinetic and pharmacodynamic interactions [2, 24], co-administration of 5-MeO-DMT and MAOI may cause hyperserotonergic tone or even serotonin toxicity/syndrome, which exhibits a number of characteristic features in patients and animal models (e.g., neuromuscular excitation such as shivering and tremor, autonomic stimulation such as hyperthermia and tachycardia, and altered mental/behavioral status such as confusion, anxiety, and activity), and has become a more prevalent clinical issue [35–37].

Indeed our recent study has revealed the potentiation of 5-MeO-DMT-induced hyperthermia by MAOI harmaline, and defined the contribution of 5-HT_{1A} and 5-HT_{2A} receptors [28]. In this study, we aimed to delineate the effects of co-administered MAOI harmaline with 5-MeO-DMT on home-cage activities in mice maintained in home cages using an automated telemetry system. In addition, 5-HT_{1A} receptor antagonist WAY-100635 and 5-HT_{2A} receptor antagonists MDL-100907 were utilized to define the serotonergic mechanisms underlying home-cage activities altered by harmaline and 5-MeO-DMT. These results would advance the mechanistic understanding of IAA pharmacological effects on behaviors and the risks of interactions between IAA drugs of abuse.

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Material and Methods

Chemicals and Materials

Harmaline hydrochloride dihydrate, 5-MeO-DMT oxalate, and *N*-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride (WAY-100635) were bought from Sigma-Aldrich (St. Louis, MO). (R)-(+)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]-4-piperidyl]methanol (MDL-100907) was a generous gift from Sanofi-Aventis (Paris, France). Carprofen (Rimadyl) was purchased from Pfizer Inc. (New York, NY). Isoflurane (AErrane) was bought from Baxter Healthcare (Deerfield, IL). All drugs for injection were dissolved in saline and were administered as their free base weights. An injection volume of 10 ml/kg was used for both intraperitoneal (*ip*) and subcutaneous (*sc*) injections.

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee at University at Buffalo, The State University of New York. Age-matched male FVB/N mice (25–35 g; The Jackson Laboratory, Bar Harbor, ME, USA) were housed in an animal care facility maintained at 20 ± 2.0°C on a 12-h light/dark cycle (lights on from 6 AM to 6 PM) with *ad libitum* food and water.

Surgical Preparations

All the surgery procedures were performed under aseptic conditions as described earlier [28]. A sterile Physiotel TA10TA-F20 telemetry transmitter (Data Sciences International, St. Paul, MN) was implanted into the peritoneal cavity. Mice were anaesthetized with isoflurane in oxygen (4%, reduced as necessary). Carprofen (5 mg/kg) as an analgesic was injected *sc* immediately after surgery and oral (*po*) dosed for 2 more days. After surgery, animals were individually housed for recovery and conditioned for 2 weeks before being used for activity studies in home cages (overall dimensions: mm 365×207×140 ht).

Experimental Procedures

All animals were tested in their home cages in an isolated and quiet room between 10:30 A.M. and 4:30 P.M.. On the afternoon before an experimental day, mice were weighted and returned to their home cages, which were placed on individual configured receivers (Data Sciences International, St. Paul, MN). The telemetry transmitter was activated for overnight stabilization and acquisition of baseline activities before experiments. Harmaline (0, 2, 5 or 15 mg/kg; N = 14 mice in each group), 5-MeO-DMT (0, 2, 10 or 20 mg/kg; N = 14 mice in each group) or their combination (0, 2, 5 or 15 mg/kg harmaline plus 2 mg/kg 5-MeO-DMT; N = 11 mice per group) were *ip* administered to the mice. In the combination studies, harmaline was injected 15 min before 5-MeO-DMT treatment. To define the role of 5-HT_{1A} and 5-HT_{2A} receptors in harmaline, 5-MeO-DMT and their combination elicited behavior changes, mice were pretreated *sc* with either 5-HT_{1A} receptor antagonist WAY-100635 (1 mg/kg; N = 7 mice per group) or 5-HT_{2A} receptor antagonist MDL-100907 (1 mg/kg; N = 7 mice in each group). For harmaline (15 mg/kg) treatment, antagonists were dosed 15 min before the administration of harmaline. For 5-MeO-DMT (20 mg/kg) alone and harmaline (2 m/kg) plus 5-MeO-DMT (2 mg/kg) treatments, antagonists were given 15 min before the

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administration of 5-MeO-DMT. Control animals were given drug vehicle (saline) by following the same injection protocols. After drug administration, the locomotor activities (counts) of individual animals were continuously recorded during the procedures using the same receivers (Data Sciences International, St. Paul, MN), which were controlled by the Ponemah software (Data Sciences International).

Data Acquisition and Analysis

Mouse home-cage activities (counts) were recorded every 10 seconds and the average values within a 15 min period were calculated and used for data analysis. Area under the effect curve (AUEC) of home-cage activity values were calculated by trapezoidal method (GraphPad Prism 5, GraphPad Software Inc., San Diego, CA). Because drug-induced changes of animal activities were biphasic, AUEC values were calculated for two periods, an early phase at 0–45 min and late-phase at 45–180 min which better indicated biphasic effects. Depending on the number of groups and variances, data were compared with one-way or two-way ANOVA followed by Bonferroni's *post-hoc* tests (GraphPad Prism 5). Difference was considered statistically significant when $p < 0.05$.

Results

High dose of harmaline is able to alter the home-cage activities of mice

Administration of vehicle (0 mg/kg harmaline) introduced some stress to the mice and led to a transient (0–45 min) increase in home-cage activities, which completely returned to the baseline levels later (45–180 min) (Figure 1A). Compared to the vehicle control treatment, lower doses of harmaline (2 and 5 mg/kg) had no significant effects on mouse home-cage activity (Figure 1A), which is also indicated by the lack of change in AUEC values (Figure 1B and 1C). Interestingly, a higher dose of harmaline (15 mg/kg) significantly reduced the activities of mice at early times (0–45 min) and slightly enhanced the home-cage activities at late phase (45–180 min) (Figure 1A). The impact of high dose of harmaline on mouse activities is also evidenced by a significant change of $AUEC_{0–45\ min}$ (Figure 1B) and $AUEC_{45–180min}$ values (Figure 1C).

5-MeO-DMT causes biphasic effects on mouse home-cage activities, a hypoactivity at early phase and hyperactivity at late phase

Compared with vehicle control treatment, administration of 5-MeO-DMT consistently induced biphasic effects on mouse home-cage activities (Figure 2). 5-MeO-DMT suppressed mouse activities at early phase (0–45 min) in a dose dependent manner, which became more obvious at 10 and 20 mg/kg dose levels (Figure 2A and 2B). Furthermore, 10 and 20 mg/kg of 5-MeO-DMT caused a significant increase in home-cage activity at the late phase (45–180 min), while 2 mg/kg of 5-MeO-DMT had no significant influence (Figure 2A and 2C). The biphasic effects of 5-MeO-DMT on home-cage activity are nicely demonstrated by a significantly decreased $AUEC_{0–45\ min}$ value (Figure 2B) and an increased $AUEC_{45–180min}$ value (Figure 2C).

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Co-administration of MAOI harmaline with a small dose of 5-MeO-DMT produces excessive hyperactivities in mice at late phase

We then chose the 2 mg/kg 5-MeO-DMT dose to examine whether MAOI harmaline alters 5-MeO-DMT pharmacological effects, given the above observation that this dose of 5-MeO-DMT had no or minimal effects on mouse home-cage activities (Figure 2) and our recent finding that the two drugs interact significantly at the pharmacokinetic level [21, 23]. Indeed the vehicle plus 2 mg/kg 5-MeO-DMT treatment did not cause any significant change in mouse activities, as compared with vehicle plus vehicle treatments (data not shown). However, co-administration of MAOI harmaline with 5-MeO-DMT led to a dose-dependent increase in late-phase hyperactivity in mice although there was no significant effect on early-phase home-cage activity (Figure 3). As indicated by the $AUEC_{45–180min}$ values, 2–15 mg/kg harmaline increased the home-cage activities by 2- to 3-fold, as compared with the 2 mg/kg of 5-MeO-DMT treatment alone (Figure 3C). The results demonstrate a prominent impact on animal home-cage activities when MAOI harmaline is co-administered with a small dose of 5-MeO-DMT. On the other hand, the small dose (2 mg/kg) of 5-MeO-DMT attenuated the early-phase hypoactivity and enhanced the late-phase hyperactivity that were induced by 15 mg/kg of harmaline alone (Figure 1), indicating the influence of 5-MeO-DMT on harmaline drug effects.

Harmaline-induced early-phase hypoactivity is mediated by the activation of 5-HT_{1A} receptor

To define the serotonergic mechanisms behind the change of animal activities by harmaline, we examined the impact of pretreatment of selective 5-HT_{1A} receptor antagonist WAY-100635 and 5-HT_{2A} receptor antagonist MDL-100907, respectively (Figure 4). Our data showed that WAY-100635 completely attenuated the early-phase hypoactivity elicited by 15 mg/kg of harmaline, whereas it had no effect on the late-phase hyperactivity (Figure 4A–4C). On the other hand, MDL-100907 exhibited no significant effects on harmaline-induced changes in home-cage activity (Figure 4D–4F), although MDL-100907 itself inhibited the home-cage activities in mice at early stage. These results point to an important role of 5-HT_{1A} receptor in harmaline-induced early-phase hypoactivity.

5-MeO-DMT-elicited early-phase hypoactivity is mainly mediated by the activation of 5-HT_{1A} receptor, whereas late-phase hyperactivity is determined by the activation of 5-HT_{2A} receptor

Likewise we employed the 5-HT_{1A} and 5-HT_{2A} receptor antagonists to evaluate serotonergic mechanisms underlying the alternation of mouse home-cage activities by a large dose (20 mg/kg) of 5-MeO-DMT (Figure 5). As indicated by the home-cage activity profiles (Figure 5A and 5D) and AUEC values (Figure 5B and 5F), pretreatment of WAY-100635 completely attenuated the early-phase hypoactivity induced by 20 mg/kg of 5-MeO-DMT, whereas MDL-100907 fully blocked the late-phase hyperactivity. In contrast, WAY-100635 had no impact on the late-phase hyperactivity and MDO-100907 showed only a minor effect on early-phase hypoactivity (Figure 5C and 5E). These results suggest that 5-MeO-DMT-induced biphasic effects on home-cage activity are mediated by 5-HT_{1A} and 5-HT_{2A} receptor at early and late phase, respectively.

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Both 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors contribute to the excessive, late-phase hyperactivity induced by co-administered harmaline and 5-MeO-DMT

The early-phase home-cage activities in mice treated with 2 mg/kg harmaline plus 2 mg/kg 5-MeO-DMT were not affected by pretreatment of WAY-100635 (Figure 6A–6B) but sharply reduced by MDL-100907 (Figure 6D–6E) as MDL-100907 itself suppressed mouse home-cage activities. Interestingly, both WAY-100635 and MDL-100907 was able to attenuate the excessive, late-phase (45–180 min) hyperactivity induced by 2 mg/kg of harmaline plus 2 mg/kg of 5-MeO-DMT treatment, as indicated by the home-cage activity profiles (Figures 6A and 6D) and AUEC values (Figure 6C and 6F). Together these results indicate that the late-phase hyperactivity induced by harmaline-5-MeO-DMT combination involves the actions of both 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors, which is different from the hyperactivity induced by 5-MeO-DMT alone.

Discussion

Our recent studies have demonstrated the remarkable effects of MAOI harmaline on 5-MeO-DMT pharmacokinetics [21, 23] besides the elevation of cerebral 5-HT levels in animal models [25]. As a result, co-administration of MAOI harmaline with 5-MeO-DMT may lead to an increased risk of hyperthermia in mice [28]. The present study revealed biphasic effects of 5-MeO-DMT on mouse home-cage activities, in which the early-phase hypoactivity was mediated by 5-HT$_{1A}$ receptor and late-phase hyperactivity was determined by 5-HT$_{2A}$ receptor. In addition, co-administration of MAOI harmaline with 5-MeO-DMT was able to dose-dependently induce excessive hyperactivity at late phase (45–180 min) that could be completely attenuated by either 5-HT$_{1A}$ or 5-HT$_{2A}$ receptor antagonist.

The biphasic effects of a higher dose of harmaline (15 mg/kg) on home-cage or locomotor activity revealed in mice (Figures 1 and 4) using the automated Telemetry system [38–40] is consistent with the very recent findings on biphasic effects of harmaline on mobility (locomotor activity) obtained from rats using fully automated Force Plate Actimeters [41]. However, different from a partial attenuation of the late-phase hyperactivity by Lu AF21934, a positive allosteric modulator of metabotropic glutamate receptor 4 (mGlu4) [41], the present study showed a complete attenuation of the early-phase hypoactivity by 5-HT$_{1A}$ receptor antagonist WAY-100635 (Figure 4). While harmaline interacts with various receptors [41–43], the contribution of 5-HT$_{1A}$ receptor to mouse home-cage activity may be explained by the facts that harmaline acts as a 5-HT$_{1A}$ receptor agonist [28, 42, 44], and harmaline-mediated inhibition of MAO-A leads to a remarkable increase of 5-HT level in central nervous system where 5-HT itself exhibits a higher affinity to 5-HT$_{1A}$ receptor than other 5-HT receptors [25, 45, 46]. In addition, the critical role of 5-HT$_{1A}$ receptor in hypoactivity, different from the transient hyperactivity caused by handling and injection stresses (Figure 1), is in agreement with previous findings on the importance of 5-HT$_{1A}$ receptor in the control of locomotor activities in rats [47].

The present study also revealed a clear biphasic effect for 5-MeO-DMT in the modulation of mouse home-cage activity, an early-phase hypoactivity (0–45 min) followed by hyperactivity (45–180 min) (Figures 2 and 5). This observation is consistent with previous findings on the biphasic effects (hypoactivity at 0–30 min and hyperactivity at 30–60 min) in rodents using

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the Behavior Pattern Monitor (BPM) method [26, 33, 47], supporting the characteristic effects of hallucinogens in the modulation of locomotor and exploratory behavior. Further investigation for the serotonergic mechanisms behind home-cage activity revealed that not 5-HT_{2A} receptor antagonist MDL-100907 but 5-HT_{1A} receptor antagonist WAY-100635 blocked 5-MeO-DMT-induced initial phase hypoactivity (Figure 5), which agrees with the role of 5-HT_{1A} receptor [33, 47]. However, our study also showed that, as time progressed to the late stage (e.g. 45–180 min post-treatment), 5-HT_{2A} receptor became a determinant factor because the late-phase hyperactivity was fully suppressed by MDL-100907 whereas the impact of WAY-100635 was minimal (Figure 5). Therefore, the mechanistic actions of 5-MeO-DMT are similar as nonselective 5-HT agonist hallucinogens lysergic acid diethylamide (LSD) and 3,4,5-trimethoxyphenethylamine (mescaline), which both exert an initial attenuation of crossings that is abolishable by 5-HT_1 antagonist propranolol, and a late-phase elevation in crossings that may be attenuated by 5-HT_2 antagonist ritanserin [48, 49].

When MAOI harmaline (2, 5 and 15 mg/kg) was co-administered with the small dose of 5-MeO-DMT (2 mg/kg), mice showed excessive late-phase hyperactivities but no change of early-phase activities (Figures 3 and 6), although higher dose of harmaline (15 mg/kg) itself suppressed mouse home-cage activities (Figure 1). This observation may indicate an antagonism between 5-MeO-DMT and harmaline at these dose combinations, and thus it is different from the biphasic effects on rodent locomotor activities (initial decrease at 10–20 min, and an increase at 40–70 min) induced by distinct dose combinations of MAOI plus 5-MeO-DMT [26, 29]. In addition, the present study revealed that the late-phase hyperactivity induced by harmaline-5-MeO-DMT combination was completely attenuated not only by 5-HT_{1A} receptor antagonist but also 5-HT_{2A} receptor antagonist (Figure 6), which is different from previous findings on the attenuation of late-phase hyperlocomotion in rodents only by 5-HT_{2A} receptor antagonist [26, 29]. This could be attributed to the differences in experimental settings, doses of drugs, animal models, and methods to monitor animal activities.

In vitro studies have revealed that cytochrome P450 2D6 (CYP2D6), one of the most important polymorphic Phase I drug-metabolizing enzymes, metabolizes harmaline and 5-MeO-DMT [50, 51]. *In vivo* studies with wild-type and *CYP2D6*-humanized (Tg-*CYP2D6*) mice have also demonstrated significant effects of CYP2D6 status on harmaline pharmacokinetics and the production of active metabolite from 5-MeO-DMT [21, 23, 28, 52], which might consequently influence drug response or pharmacodynamics. In our studies we found that, at current dose levels, harmaline plus 5-MeO-DMT treatments caused the same degrees of change in home-cage activities among wild-type and Tg-*CYP2D6* mice (data not shown). This observation agrees with our recent findings using stimulus control assay [27] and it may be associated with relatively high variability in animal behavioral studies that could mask rather a limited impact of CYP2D6 status on the behavioral effects of IAA drugs.

In summary, the present study demonstrated that 5-MeO-DMT followed the trait of many hallucinogenic drugs to induce biphasic effects on mouse home-cage activities, among which the early-phase hypoactivity was mediated by 5-HT_{1A} receptor and the late-phase

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hyperactivity was determined by 5-HT_{2A} receptor. Furthermore, co-administration of MAOI harmaline with 5-MeO-DMT caused excessive activities at late phase, which could be fully attenuated by either 5-HT_{1A} or 5-HT_{2A} receptor antagonist. These findings may improve the understanding of hazards of combined use of IAA agents and provide insights into developing effective means to treat IAA intoxications.

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Highlights

- High dose of MAOI harmaline reduces mouse home-cage activity at early phase that is mediated by 5-HT_{1A} receptor, and increases mouse mobility at late-phase that is controlled by 5-HT_{2A} receptor.
- 5-MeO-DMT has biphasic effects on mouse home-cage activities. The early-phase hypoactivity is mediated by 5-HT_{1A} receptor, and late-phase hyperactivity is determined by 5-HT_{2A} receptor.
- Co-administration of MAOI harmaline with 5-MeO-DMT provokes excessive late-phase hyperactivity, which may be attenuated by either 5-HT_{1A} or 5-HT_{2A} receptor antagonist.

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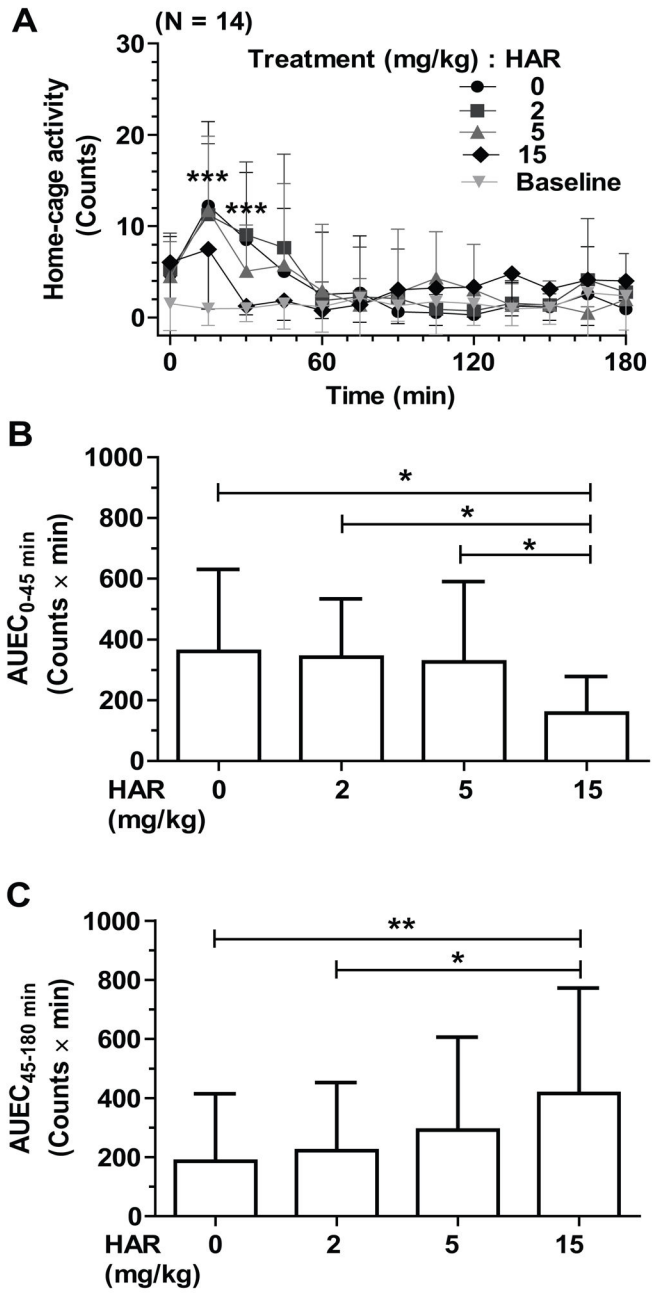

Figure 1.

Higher dose (15 mg/kg) of harmaline (HAR) significantly reduced the early-phase (0–45 min) home-cage activity and elevated late-phase (45 – 180 min) activity in mice (Two-way ANOVA with Bonferroni's *post-hoc* test: $p < 0.0001$ for drug treatment, time and interaction; ** *$p < 0.001$ at indicated time points, compared to vehicle control), whereas lower doses (2 or 5 mg/kg) of harmaline showed no effect on home-cage activity (A). This is clearly indicated by the AUEC values (B and C). Values are mean ± SD (N = 14 in each group). Baseline represents mouse home-cage activity without any interference. Harmaline or vehicle was injected *ip* at 0 min. AUEC values were calculated by trapezoidal rule. One-

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way ANOVA with Bonferroni's *post-hoc* Multiple Comparison Test for AUEC values: F = 4.462, $R^2 = 0.2711$, and $p = 0.0092$ for the four groups of $AUEC_{0–45min}$ values (B), and F = 3.134, $R^2 = 0.1942$, and $p = 0.0363$ for $AUEC_{45–180min}$ values (C); $^*p < 0.05$ as compared with the specified groups.

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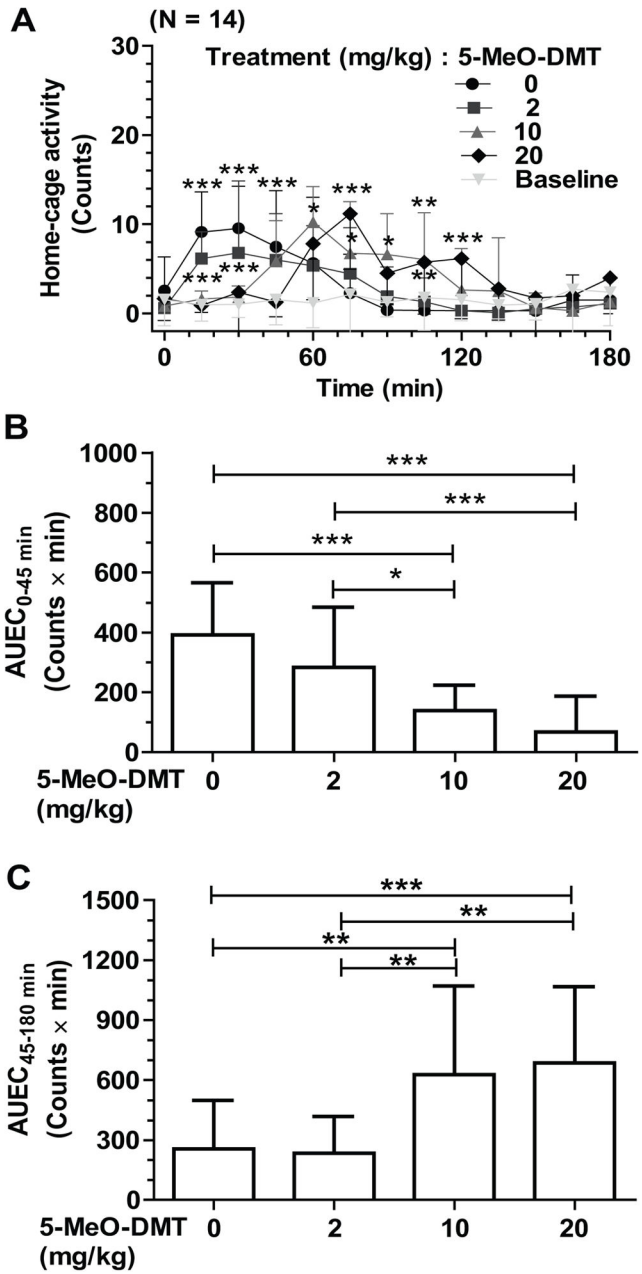

Figure 2.

5-MeO-DMT caused biphasic effects on mouse home-cage activity (A) in a dose dependent manner. As indicated by the AUEC values (B and C), higher doses (10 and 20 mg/kg) of 5-MeO-DMT led to hypoactivity at early phase (0–45 min) and hyperactivity at late phase (45–180 min). Values are mean ± SD (N = 14 in each group). Baseline represents the activity without any interference. 5-MeO-DMT or vehicle was injected *ip* at 0 min. AUEC values were calculated by trapezoidal rule. Two-way ANOVA with Bonferroni's *post-hoc* test for the activity data (A): $p < 0.0001$ for drug treatment, time and interaction; $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ at indicated time points, when the 10 and 20 mg/kg dose was compared to vehicle control. One-way ANOVA with Bonferroni's *post-hoc* Multiple

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Comparison Test for AUEC values: F = 15.82, $R^2 = 0.5490$, and $p < 0.0001$ for the four groups of $AUEC_{0–45min}$ values (B), and F = 10.31, $R^2 = 0.4423$, and $p < 0.001$ for $AUEC_{45–180min}$ values (C); $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ as compared with the indicated groups.

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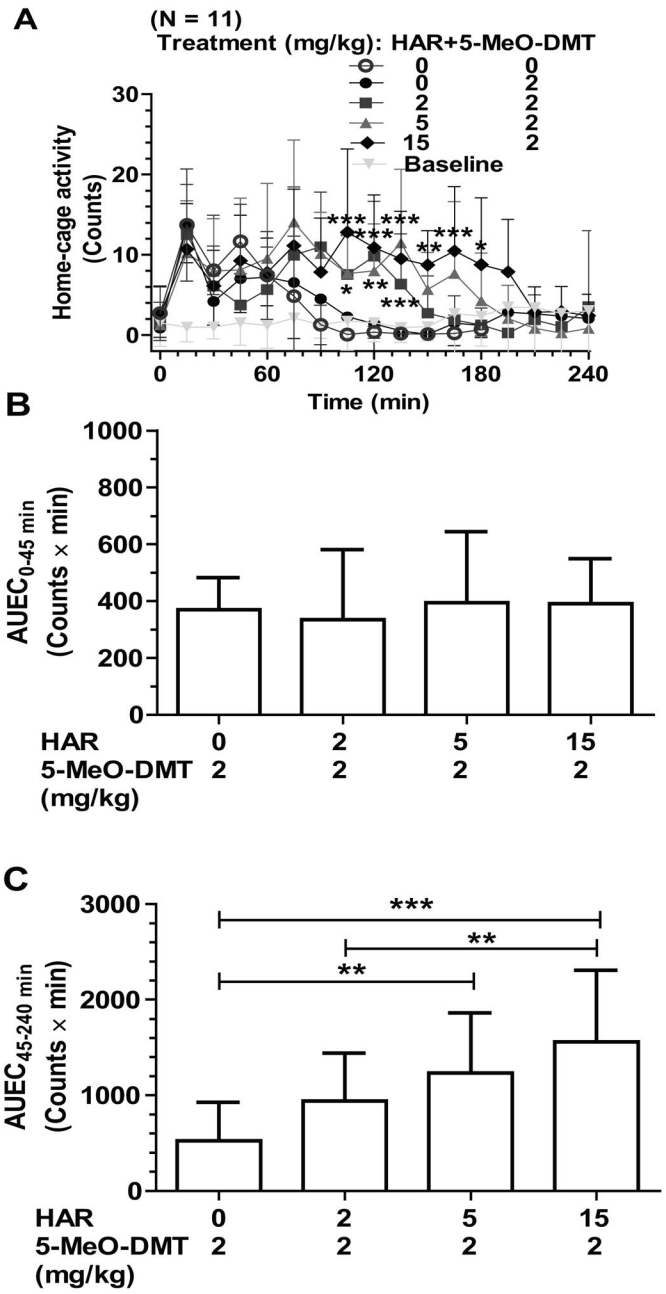

Figure 3.

Co-administration of harmaline (HAR) with a small dose of 5-MeO-DMT (2 mg/kg) induced excessive hyperactivity at late phase (45–180 min) in a dose dependent manner (A). This is clearly manifested by the calculated AUEC values (B and C). 5-MeO-DMT was administered *ip* 15 min after the *ip* treatment with harmaline (at 0 min). Values are mean ± SD (N = 11 in each group). Baseline represents the home-cage activity without any interference. AUEC values were calculated by trapezoidal rule. Two-way ANOVA with Bonferroni's *post-hoc* test for the activity data (A): $p < 0.0001$ for drug treatment, time and interaction; $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ for the specified time points, when 5 or

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10 mg/kg harmaline plus 5-MeO-DMT treatment was compared to vehicle plus 5-MeO-DMT treatment. One-way ANOVA with Bonferroni's *post-hoc* Multiple Comparison Test for the AUEC values: F = 0.5779, $R^2 = 0.05463$, and $p = 0.6340$ for the four groups of $AUEC_{0–45min}$ values (B), and F = 22.58, $R^2 = 0.6724$, and $p < 0.0001$ for $AUEC_{45–180min}$ values (C); * $p < 0.01$ and ** $p < 0.001$ as compared with the specified groups.

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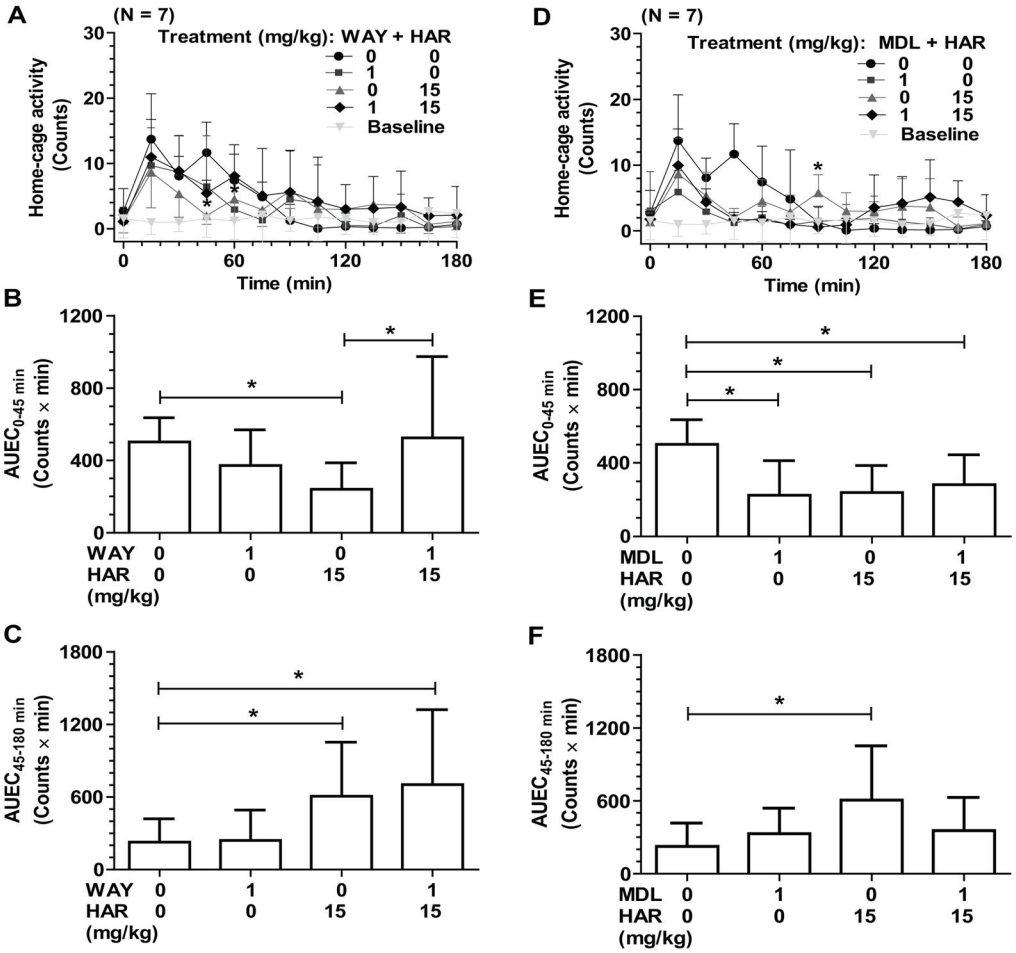

Figure 4.

WAY-100635 (WAY, a selective 5-HT_{1A} receptor antagonist) fully attenuated 15 mg/kg of harmaline (HAR)-induced early-phase (0–45 min) hypoactivity, whereas it had no impact on late-phase (45–180 min) hyperactivity (A–C). In contrast, MDL-100907 (MDL, a selective 5-HT_{2A} receptor antagonist) showed no effect on harmaline-induced changes in home-cage activity (D–F). WAY or MDL (1 mg/kg) or vehicle was injected *sc* at 0 min, and harmaline (15 mg/kg) or vehicle was injected *ip* at 15 min. Values are mean ± SD (N = 7 per group). AUEC values were calculated by trapezoidal rule. Two-way ANOVA with Bonferroni's *post-hoc* test for the activity data (A & D): $p < 0.0001$ for drug treatment, time and interaction; $*p < 0.05$ for the specified time points, when WAY or MDL plus harmaline treatment was compared to vehicle plus harmaline treatment. One-way ANOVA with Bonferroni's *post-hoc* Multiple Comparison Test for AUEC values: F = 2.113, $R^2 = 0.2605$, and $p = 0.1342$ for the four groups of WAY-treated $AUEC_{0–45min}$ values (B), and F = 3.936, $R^2 = 0.3599$, and $p = 0.0225$ for WAY-treated $AUEC_{45–180min}$ values (C); F = 4.085, $R^2 = 0.4051$, and $p = 0.0224$ for the four groups of MDL-treated $AUEC_{0–45min}$ values (E), and F = 2.079, $R^2 = 0.2290$, and $p = 0.1336$ for MDL-treated $AUEC_{45–180min}$ values (F); $*p < 0.05$ as compared with the indicated groups.

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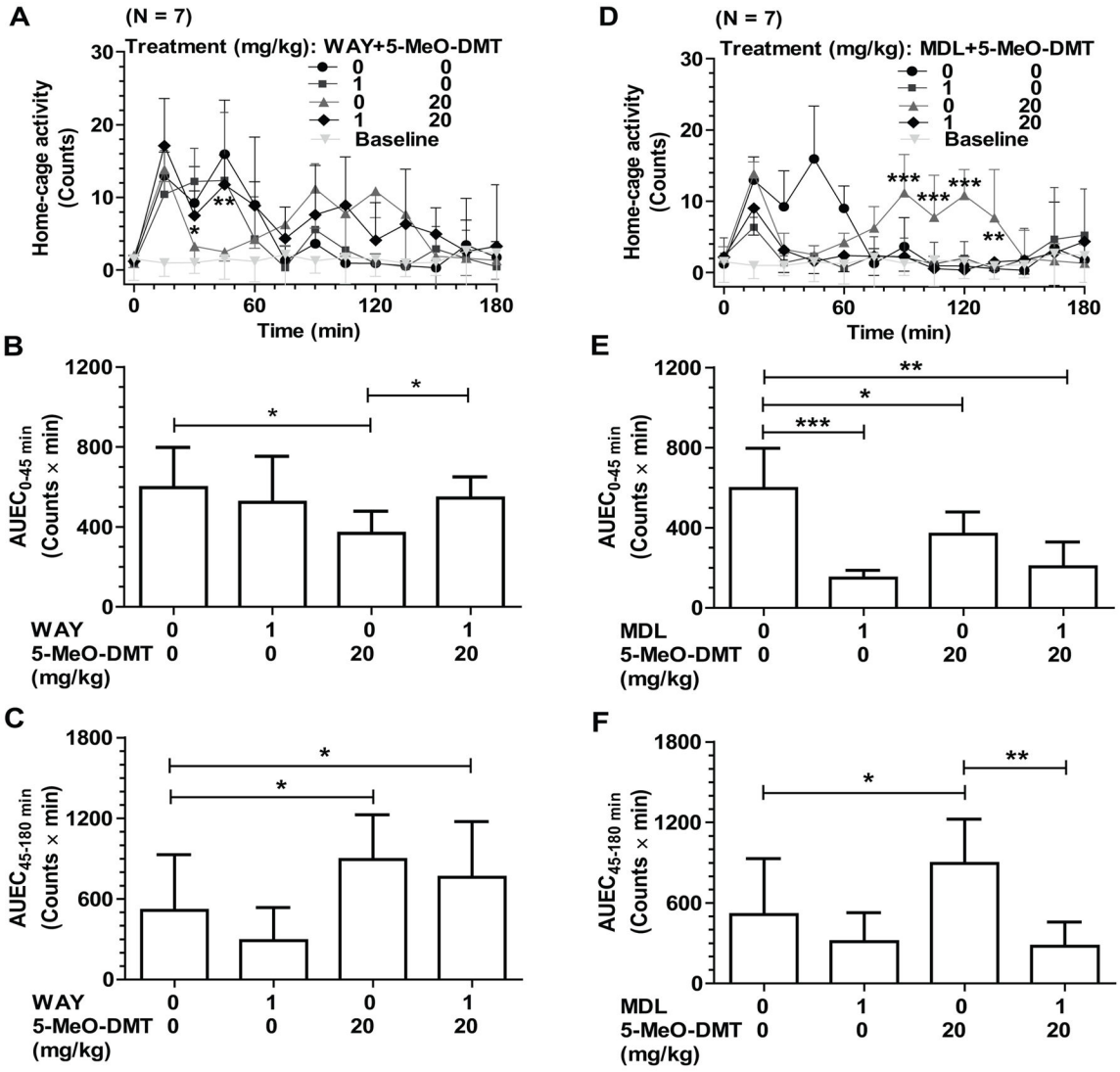

Figure 5.

WAY-100635 (WAY) completely attenuated 5-MeO-DMT-induced early-phase (0–45 min) hypoactivity (A–C), while MDL-100907 (MDL) fully suppressed 5-MeO-DMT-elicited late-phase (45–180 min) hyperactivity (D–F). WAY or MDL (1 mg/kg) or drug vehicle was injected *sc* at 0 min, and 5-MeO-DMT (20 mg/kg) or vehicle was injected *ip* at 15 min. Values are mean ± SD (N = 7 per group). AUEC values were calculated according to trapezoidal rule. Two-way ANOVA with Bonferroni's *post-hoc* test for the activity data (A & D): p≤ 0.001 for drug treatment, time and interaction; $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ for the specified time points, when WAY or MDL plus 5-MeO-DMT treatment was compared to vehicle plus 5-MeO-DMT treatment. One-way ANOVA with Bonferroni's *post-hoc* Multiple Comparison Test for the AUEC values: F = 4.490, $R^2 = 0.4280$, and $p = 0.0161$ for the four groups of WAY-treated $AUEC_{0–45min}$ values (B), and F = 4.599, $R^2 = 0.4339$, and $P = 0.0147$ for WAY-treated $AUEC_{45–180min}$ values (C); F = 15.37, $R^2 = 0.7192$, and $p < 0.0001$ for the four groups of MDL-treated $AUEC_{0–45min}$ values (E), and F = 6.745, R^2 =

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0.5292, and $p = 0.0030$ for MDL-treated $AUEC_{45–180min}$ values (F); $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ as compared with the specified groups.

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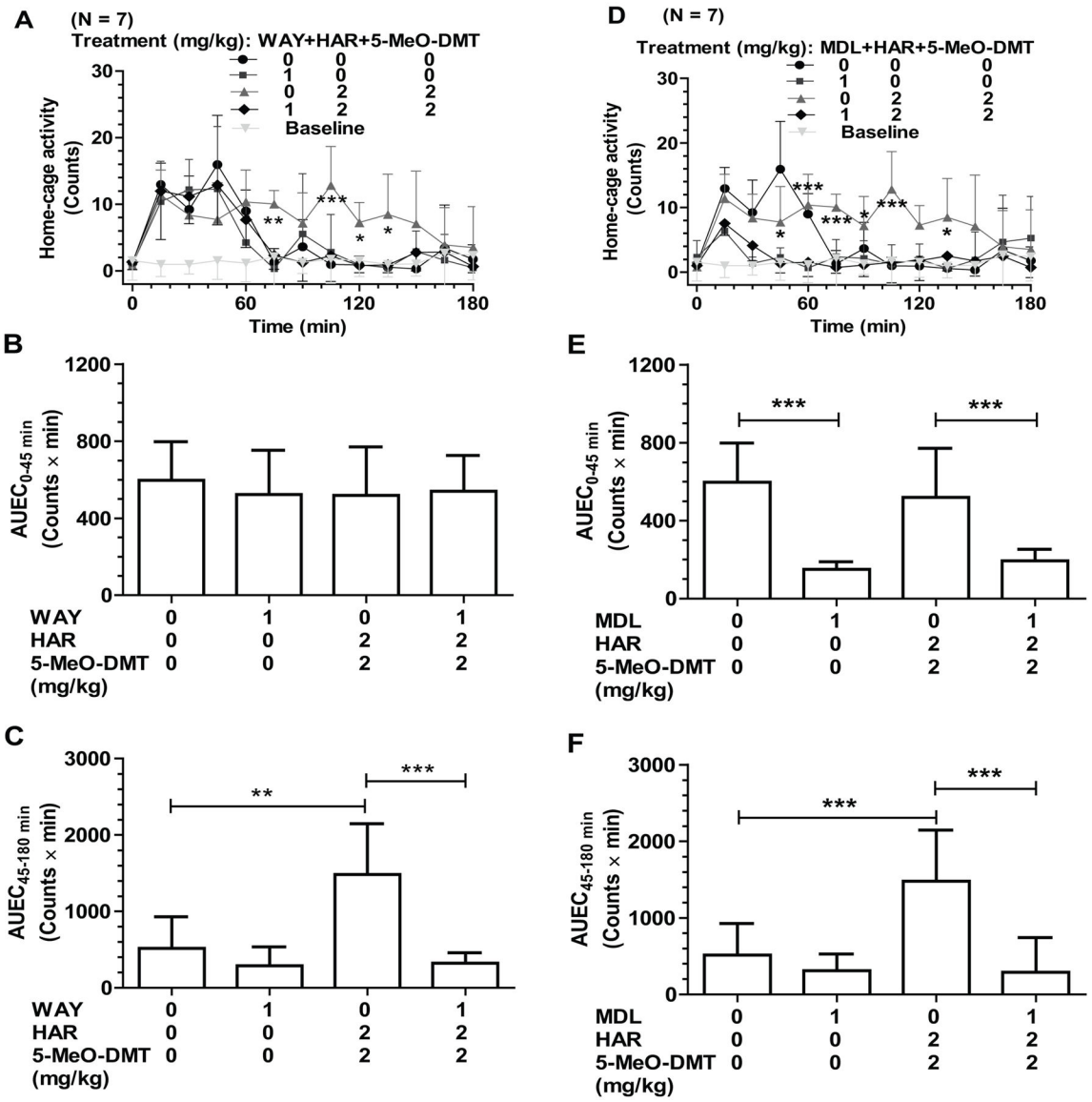

Figure 6.

Both WAY-100635 (WAY) (A–C) and MDL-100907 (MDL) (D–F) completely attenuated the excessive, late-phase (45–180 min) hyperactivity induced by co-administered harmaline and 5-MeO-DMT. In addition, MDL suppressed the early-phase (0–45 min) hyperactivity. WAY or MDL (1 mg/kg) or vehicle was injected *sc* at 0 min, and harmaline (2 mg/kg) and 5-MeO-DMT (2 mg/kg) or corresponding vehicles were injected *ip* at 0 and 15min, respectively. Values are mean ± SD (N = 7 per group). Baseline represents the home-cage activity without any intervention. AUEC values were calculated according to trapezoidal rule. Two-way ANOVA with Bonferroni's *post-hoc* test for the activity data (A & D): $p < 0.0001$ for drug treatment, time and interaction; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ for the specified time points, when WAY or MDL plus harmaline-5-MeO-DMT treatment was compared to vehicle plus harmaline-5-MeO-DMT treatment. One-way ANOVA with Bonferroni's *post-hoc* Multiple Comparison Test for the AUEC values: F = 0.2957, R^2 =

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0.04696, and $p = 0.8280$ for the four groups of WAY-treated $AUEC_{0–45min}$ values (B), and F = 15.10, $R^2 = 0.7156$, and $p < 0.0001$ for WAY-treated $AUEC_{45–180min}$ values (C); F = 14.41, $R^2 = 0.6070$, and $p < 0.0001$ for the four groups of MDL-treated $AUEC_{0–45min}$ values (E), and F = 9.276, $R^2 = 0.6072$, and $P = 0.0006$ for MDL-treated $AUEC_{45–180min}$ values (F); $**p < 0.01$, and $***p < 0.001$ as compared with the specified groups.