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Behavioral and Cross Sensitization after Repeated Exposure to Modafinil and Apomorphine in Rats

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Abstract

Repeated exposure to psychostimulant drugs has been known to produce behavioral sensitization, a phenomenon explicitly indexed by locomotion (LM) and stereotyped movements (SM). So far, no evidence has demonstrated that this phenomenon can be displayed following the administration of modafinil (MOD) in animal study. We, therefore, assessed the possibility of behavioral sensitization of MOD and a direct dopamine agonist, apomorphine (APO), and cross-sensitization of these two drugs with one other. Pretreatment with MOD (64 mg/kg) or APO (0.5 mg/kg or 1.0 mg/kg) for 10 consecutive days was followed by a short-term (3 days) or long-term (21 days) withdrawal. Rats were then challenged with the drug and reciprocally re-challenged with the counterpart drug. The results showed that following short-term and long-term washout periods, both MOD and APO successfully induced sensitization in LM and SM. There was no cross-sensitization; an even lesser magnitude in LM when MOD-sensitized rats were challenged with APO was observed. However, after both the short-term and long-term withdrawal periods, APO (1.0 mg/kg)-sensitized rats showed cross-sensitization in LM and SM to MOD (64 mg/kg) challenge. The magnitude of APO-MOD cross-sensitization was lesser than the behavioral sensitization induced by APO alone. Our results indicated behavioral sensitization could be induced in rats exposed to MOD. In addition, changes in dopaminergic receptor activities could be involved in cross-sensitization of APO to MOD but not vice versa.

Key Words: APO, behavioral sensitization, cross-sensitization, dopamine, MOD

Introduction

A phenomenon termed behavioral sensitization can be commonly found in rodents following repeated exposure to classical psychostimulants which is known to be involved in certain aspects of drug addiction, such as drug craving and compulsive drug-seeking behavior (27, 31). Another phenomenon called behavioral cross-sensitization is also contributive in drug addiction in which the sensitization caused by one stimulant can be established in others. In general, the neuroadaptations of dopaminergic substrates in brain reward areas have been attributed to the underlying mechanisms of those two modes of sensitization (1, 16, 27, 31, 37, 41). It has been shown that behavioral sensitization to psychostimulants may result from both direct pharmacological activity of the drugs and the experiences associated with the drugs (27).

Modafinil (2-[(diphenylmethyl)sulfinyl]

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acetamide) (MOD) is a novel vigilance-promoting agent approved by U.S. Food and Drug Administration (FDA) for treatment of daytime sleepiness in narcolepsy. MOD has been categorized as an atypical central stimulant agent (10), and is currently classified as a Schedule IV controlled substance. Since the use of MOD for nonmedical purposes is being expanded, we consider it necessary to have a further assessment of the abuse potential of this drug. Psycho-activity of MOD is higher than that of caffeine. However, the addictive potential of MOD is less than that of abused psycho-stimulants, for example, cocaine and amphetamine (6, 14, 15). Despite the expanding clinical indications, the precise mechanism of this compound is still unknown. Studies performed *in vivo* and *in vitro* have suggested several possible mechanisms for the action of this compound including modulation of multiple neurotransmitter systems such as catecholamines, serotonin, glutamate, GABA, orexin and histamine (2, 40). In terms of drug-induced neuronal activation, it is generally believed that MOD, rather than amphetamine, is more localized in the wakefulness areas (33).

Besides acting as a vigilance-promoting agent, MOD with its distinctive mechanisms makes it clinically helpful in minimizing the withdrawal symptoms of amphetamine- or cocaine-dependent patients with good tolerability (6, 15). However, there are still many disagreements in the reinforcing effects of MOD and the hidden low abuse potential of this compound is still a concern (2, 8, 19, 32, 36, 39). One of the concerns came from the evidences that this compound might produce typical psychoactive and euphoric effects as other abused psychostimulant drugs did (8, 18, 36, 39). Moreover, the vigilance-promoting and reinforcing properties of MOD have been reported to be related to the release of dopamine involving dopaminergic D1/D2 receptor family in the brain reward areas (12, 26, 36, 39, 42).

Given the fact that most drug addicts are multiple-drug abusers and may have cross-sensitization to several drugs, we were, therefore, interested in the possibility that MOD might induce poly-drug addiction based on the sensitization hypothesis. The present study was aimed at determining whether MOD elicited behavioral sensitization and cross-sensitization to APO, a direct agonist of dopaminergic D1/D2 receptors (24). The results may provide a better understanding of the addictive potential of MOD and the contribution of dopamine D1 and D2 receptors.

Materials and Methods

Animals

Male adult Sprague-Dawley rats weighing between 250 and 300 g were supplied by BioLASCO Taiwan Co., Ltd. The animals were housed in groups of three at a constant cage temperature (22 ± 1 °C) and humidity. The animals were allowed to adapt for 1 week to the new environment before any experiment was performed. They were kept under regular light-dark conditions (lights on at 07:00 a.m. and off at 19:00 p.m.) with food and water available *ad libitum* except during behavioral testing. There were 6~10 animals per treatment group. All procedures were approved by the research ethics review board of the National Defense Medical Center and, therefore, complied with the ethical standards laid down in the 1995 declaration of Helsinki.

Drugs

MOD used in the current study was obtained from Professor An-Rong Lee's laboratory at the School of Pharmacy, National Defense Medical Center (NDMC). Qualification of the drug was proved by Professor Lee's laboratory. Synthesis and functional analysis of MOD were one of the integrative projects funded by NDMC (with the approved code DOD97-10). The drug was suspended in a 0.5% gum arabic solution and administered intraperitoneally (i.p.) at 1.0 ml/kg. Gum arabic is a complex mixture of polysaccharides and glycoproteins used primarily as a stabilizer. APO hydrobromide (Sigma, St. Louis, MO, USA) were dissolved in 1% ascorbic acid and injected subcutaneously (s.c.) at 0.1 ml/kg. Escalating doses were adopted for MOD in Experiment 1 in order to choose a proper sub-threshold dosage for the later sensitization studies. In Experiments 2 and 3, doses for APO were chosen at 0.5 and 1.0 mg/kg since these dosages had successfully induced motor sensitivity in previous rat studies (4, 23, 38).

Motor Activity

Locomotor activity was recorded by using a computerized automated activity monitoring system (MED Associates, Inc., St. Albans, VT, USA). This system included four novel Plexiglas chambers (43.2 cm W × 43.2 cm L × 30.2 cm H) equipped with an I/R array of 16 photodetectors and corresponding light sources that emitted photobeams 3 cm apart and 4.5 cm above the chamber floor. Using the MED-Associates software, two behavioral variables, locomotion (LM) and stereotyped movements (SM, manifested mainly by repetitive oral movements, such as gnawing, biting, and sniffing) of motor activity, were recorded constantly at the assigned intervals for different drugs in each experiment.

During the experiments, rats were maintained in their home cages and then transferred to the test room

Table 1. Experimental design for Experiments 2 and 3

Pretreatment Groups	Baseline Day 1	Pretreatment Day 2-11	Sensitization Day 15	Sensitization Day 33	Cross Sensitization Day 18	Cross Sensitization Day 36
Experiment 2						
VEH Control	VEH	VEH	MOD	MOD	APO	APO
Repeated MOD	VEH	MOD	MOD	MOD	APO	APO
Experiment 3						
VEH Control	VEH	VEH	APO	APO	MOD	MOD
Repeated APO	VEH	APO	APO	APO	MOD	MOD

Motor activity was measured for 90 min (MOD) and 60 min (APO) beginning immediately after injection with the indicated vehicle or drugs (MOD, i.p.; APO, s.c.). Data following an injection were collected on day 1, days 2 and 11, days 15 and 18, and days 33 and 36. No tests were processed during the phase of drug withdrawal (days 12-14 and days 19-32). In Experiment 2, the dose was at 64 mg/kg for MOD and 0.5 mg/kg for APO. In Experiment 3, the dose was at 64 mg/kg for MOD and 0.5 and 1.0 mg/kg for APO.

where the test chambers for the behavior assessment were located. A 30-min period of habituation to the test chamber was preceded each session, and then, the rats were then injected with vehicle or drugs for evaluation of their behaviors.

Experiment Protocols

Experiment 1: Dose-Response Effects of MOD on Motor Activity

To study the effect of MOD on motor activity (LM and SM), an escalating doses were administrated to the rats (n = 8) in the whole course of this experiment. After habituation to the test chambers, motor activity was assessed in 5 sessions. The first session (vehicle 1.0 ml/kg, i.p.) marked the beginning of the experiments. Following this session, rats were injected (i.p.) with MOD at the dosages of 32, 64, 126 and 256 mg/kg in the following 4 sessions with an inter-session interval of 3 days.

Because the greatest MOD-induced changes in motor activity were usually found 60 min or later after MOD injection, we used a 90-min monitored session to ensure the capture of the stimulant effects of MOD. The effective dose of MOD would be selected after this experiment for the following experiments.

Experiment 2: Effects of MOD and APO in MOD-Pretreated Rats

This experiment investigated whether repeated treatment with MOD induced behavioral sensitization and whether such sensitization correlated to APO-induced changes of dopaminergic receptors. Treatments for inducing MOD sensitization and subsequent cross-sensitization to APO were scheduled as shown in the Table 1.

Rats were firstly randomly assigned to two groups. One was the vehicle control (VEH control, n = 8) that received daily injection of 0.5% gum Arabic solution (1.0 ml/kg/day, i.p.) in the pretreatment phase (days 2-11) and followed by MOD (64 mg/kg, i.p.) challenge for the assessment of sensitization on days 15 and 33, respectively. The rats were also challenged with APO (0.5 mg/kg, s.c.) for the assessment of cross-sensitization on days 18 and 36, respectively. The other group (Repeated MOD, n = 8) received daily injection of MOD (64 mg/kg/day, i.p.) in the pretreatment phase, followed by MOD challenge for assessment of sensitization on days 15 and 33, respectively. These rats were also challenged with APO (0.5 mg/kg, s.c.) for the assessment of cross-sensitization on days 18 and 36, respectively. Throughout the whole experiment, motor activity was assessed on days 1, 2, 11, 15, 18, 33 and 36.

Experiment 3: Effects of APO and MOD in APO-Pretreated Rats

This experiment investigated whether MOD challenge caused cross-sensitization in APO-sensitized rats. Two doses of APO (0.5 or 1.0 mg/kg, s.c.) were chosen based on previous reports showing motor activation in rats (4). Similar to Experiment 2, rats were firstly randomly assigned to four groups. The first two groups were the vehicle controls (VEH control, n = 14) that received daily injection of 0.5% gum Arabic solution (1.0 ml/kg/day, i.p.) in the pretreatment phase (days 2-11) and followed by two doses of APO (0.5 or 1.0 mg/kg, s.c., n = 6 or 8 respectively) challenge for the assessment of sensitization on days 15 and 33. The rats were also challenged with MOD (64 mg/kg, i.p.) for the assessment of cross-sensitization on days 18 and 36, respectively. The

other two groups (Repeated APO, n = 16) received two doses of APO (0.5 mg/kg, n = 6 or 1.0 mg/kg, n = 10, s.c.) repeatedly in the pretreatment phase (days 2-11), followed by the same dose of APO challenge for assessment of sensitization on days 15 and 33. These rats were also challenged with MOD (64 mg/kg, i.p.) for the assessment of cross-sensitization on days 18 and 36, respectively. Throughout the whole experiment, motor activity was assessed on days 1, 2, 11, 15, 18, 33 and 36.

Statistical Analysis

Statistical analyses were conducted using SPSS 14.0 for Windows (SPSS, Chicago, Ill., USA). Total travel distance of LM (cm) and numbers of SM (counts) were analyzed by analysis of variance (ANOVA). Data in Experiment 1 were analyzed using one-way repeated measures ANOVA. Data from the sensitization and cross sensitization phases in Experiments 2 and 3 were analyzed using two-way repeated measures ANOVA consisting of between-subject factor of treatment (drug or saline) and within-subject factor of repeated measurement (time points). Multiple within-subject comparisons with Bonferroni post hoc test were taken when the main effects of time points were significant. Behavioral changes induced by one drug (for example, MOD) in the short-term sensitization (on day 15) and by the other drug (for example, APO) in the short-term cross-sensitization (on day 18) were analyzed by the paired *t*-test. The same test was also applied for the assessment of differences between long-term sensitization to one drug (on day 33) and long-term cross-sensitization to the other drug (on day 36). Independent t-test was conducted to determine between-group differences at a given time point. All data are presented as means ± SEM. A difference with $P < 0.05$ was considered statistically significant.

Results

Experiment 1: MOD Dose-Responses

ANOVA revealed that injections of MOD elicited behavioral changes in LM ($F(4,28) = 15.439$, $P < 0.001$) (Fig. 1A) and SM ($F(4,28) = 8.594$, $P < 0.001$) (Fig. 1B). Compared with the vehicle controls, multiple comparisons with t-test followed by Bonferroni post hoc test showed that MOD at the dosages of 128 and 256 mg/kg caused significant effects on LM and SM (all *P*-values < 0.05). Since the dose-effect curve was more or less linear starting from 64 mg/kg which was relative to responding after administration of vehicle or 32 mg/kg MOD, we selected the dose 64 mg/kg for subsequent experiments.

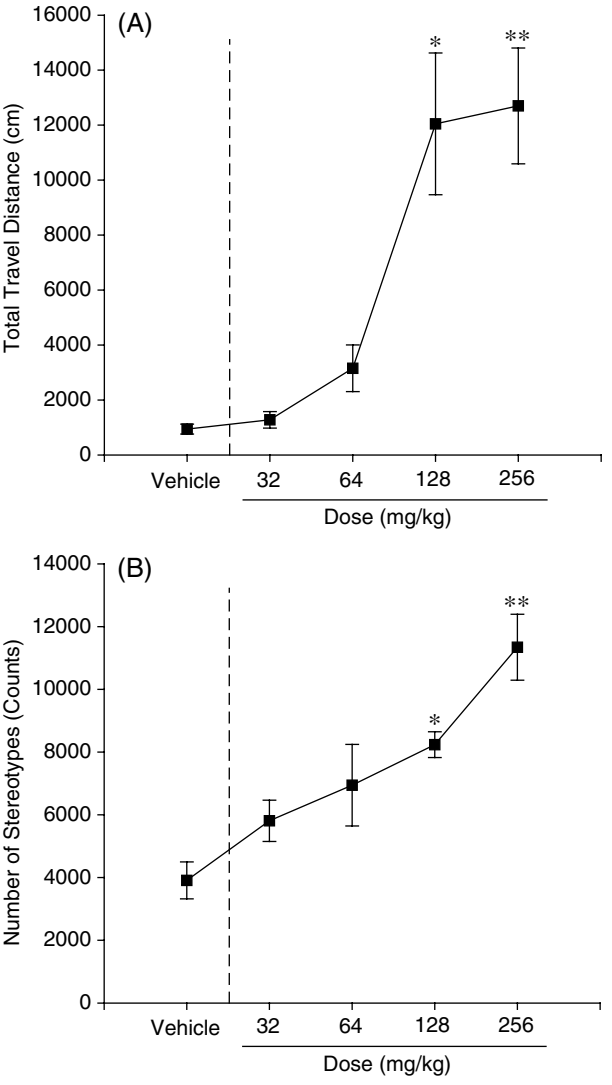

Fig. 1. Effects of the dose MOD administration (32 up to 256 mg/kg/day, n = 8) on (A) total travel distance and (B) stereotyped movements (stereotypes). Total travel distance and stereotyped movements were recorded for 1.5 h after MOD administration. The administration of MOD significantly increased stereotyped movements and traveled distance between the dose range of 128 and 256 mg/kg/day. Multiple comparisons with LSD test *$P < 0.05$, **$P < 0.01$ compared to saline administration. Results are expressed as means ± SEM.

Experiment 2: Effects of MOD and APO in MOD-Pretreated Rats

Repeated MOD (Repeated MOD) and vehicle (VEH Control) treatments resulted in different patterns of motor activity (LM (Fig. 2A) or SM (Fig. 2B)) across days (1, 2, 11, 15, 18, 33 and 36). As shown in the figures, ANOVA revealed significant main effects of treatment (LM: $F(1,14) = 4.515$; $P = 0.052$ and SM: $F(1,14) = 4.923$; $P < 0.05$) and time point (LM:

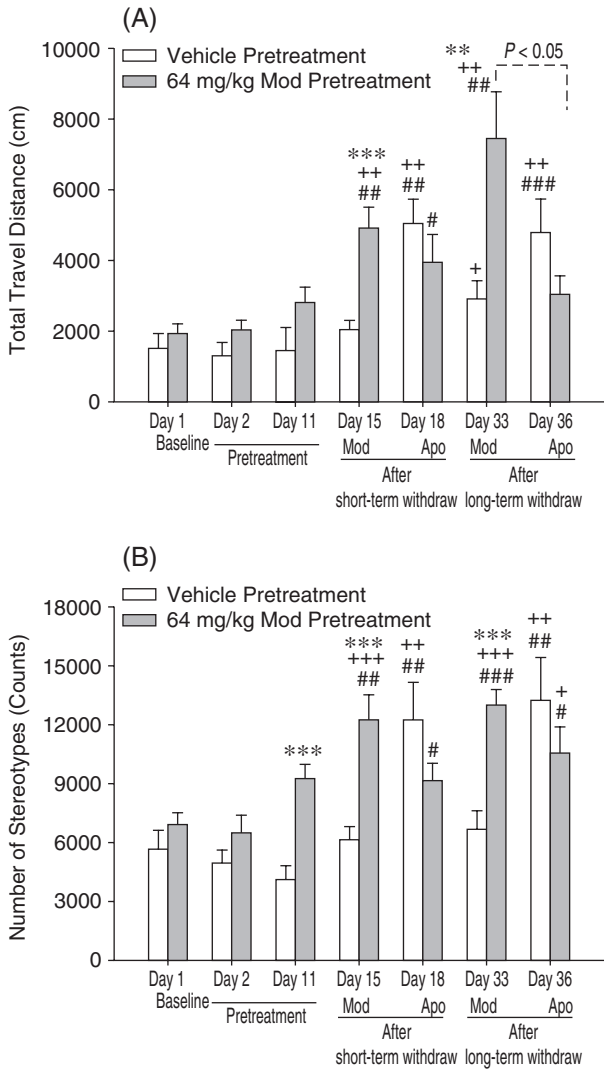

Fig. 2. Effects of a challenged dose of MOD (Mod, 64 mg/kg) or APO (Apo, 0.5 mg/kg) after short- or long-term washout periods in rats pretreated with MOD (64 mg/kg, n = 8) or vehicle (n = 8). Total travel distance (A) and stereotyped movements (stereotypes) (B) were recorded for 1.5 h in MOD test, 1 h in APO test, and an identical span as the challenged drug at the indicated time point in saline test. Repeated treatments with MOD-induced sensitization to MOD but not cross-sensitization to APO both in total travel distance and stereotyped movements. $^{\#}P < 0.05$, $^{\#\#}P < 0.05$ and $^{\#\#\#}P < 0.05$ compared to Day 1, $^{+}P < 0.05$, $^{++}P < 0.01$ and $^{+++}P < 0.001$ compared to Day 2 at the indicated test day, and there was a significantly lower effect of APO relative to MOD in total travel distance ($P < 0.05$, Day 36 *vs*. Day 33) following multiple comparisons with LSD method. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared at the indicated time point of the group of rats by Independent sample *t*-test. Results are expressed as means ± SEM.

$F(6,84) = 11.14$; $P < 0.001$ and SM: $F(6,84) = 10.88$; $P < 0.001$), as well as significant treatment-by-time point interactions (LM: $F(6,84) = 6.71$; $P < 0.001$ and SM: $F(6,84) = 7.34$; $P < 0.001$).

Simple main effect analyses followed by multiple comparisons with Bonferroni post hoc test revealed that the group of Repeated MOD-rats expressed significant behavioral sensitization on day 15 as compared with day 1 ($P < 0.01$, for both LM and SM) and day 2 ($P < 0.01$, for both LM and SM). The similar sensitization could also be significant in day 33. In addition, this group of rats showed a significant difference between day 33 and day 36 in LM ($P < 0.05$). On the other hand, there were significant changes in LM ($P < 0.05$) of VEH Control-rats after MOD challenge on day 33 compared with day 2. But there was no change in SM. In addition, both LM and SM were significantly enhanced in MOD-pretreated rats on day 11 (SM: $P < 0.001$), 15 (LM: $P < 0.001$; SM: $P < 0.001$) and day 33 (LM: $P < 0.01$; SM: $P < 0.001$).

On days 18 and 36, 3 days following the last MOD challenge, the rats received APO challenges. After the short-term withdrawal period, MOD challenge on day 15 caused higher behavioral activity in LM ($P < 0.05$) and SM ($P < 0.05$) compared with APO challenge on day 18. A similar phenomenon was observed after the long-term withdrawal period in which MOD and APO challenges were taken on days 33 and 36, respectively.

In addition, data showed that there was no significant difference between the Repeated MOD- and VEH Control-rats after APO challenges on days 18 and 36.

Experiment 3: Effects of APO and MOD in APO-Pretreated Rats

Following APO (Repeated APO) or vehicle (VEH Control) treatment, the effects of changes in motor activity across days are shown in Fig. 3 (APO dose: 0.5 mg/kg) or Fig. 4 (APO dose: 1.0 mg/kg). ANOVA for each APO dosage (0.5 mg/kg or 1.0 mg/kg) was performed separately in this experiment. Patterns of motor activity (LM (panel A) or SM (panel B)) across days (days 1, 2, 11, 15, 18, 33 and 36) varied depending on the treatment groups: APO-0.5 mg/kg (LM: $F(1,10) = 4.238$; $P = 0.067$ and SM: $F(1,10) = 15.504$; $P < 0.01$), day points (LM: $F(6,60) = 8.816$; $P < 0.001$ and SM: $F(6,60) = 8.960$, $P < 0.001$); APO-1.0 mg/kg (LM: $F(1,16) = 25.798$; $P < 0.001$ and SM: $F(1,16) = 64.56$; $P < 0.001$), day points (LM: $F(6,96) = 10.531$; $P < 0.001$ and SM: $F(6,96) = 16.834$, $P < 0.001$), and an interaction effect between factors (LM: $F(6,96) = 2.322$; $P < 0.05$; SM: $F(6,96) = 3.725$, $P < 0.01$). In addition, Repeated APO (0.5 mg/kg)-rats expressed escalating LM (Fig. 3A) and SM (Fig. 3B) on day 15 compared with day 1 (LM: $P < 0.05$; SM: $P < 0.001$) and day 2 in SM ($P < 0.05$). A similar phenomenon

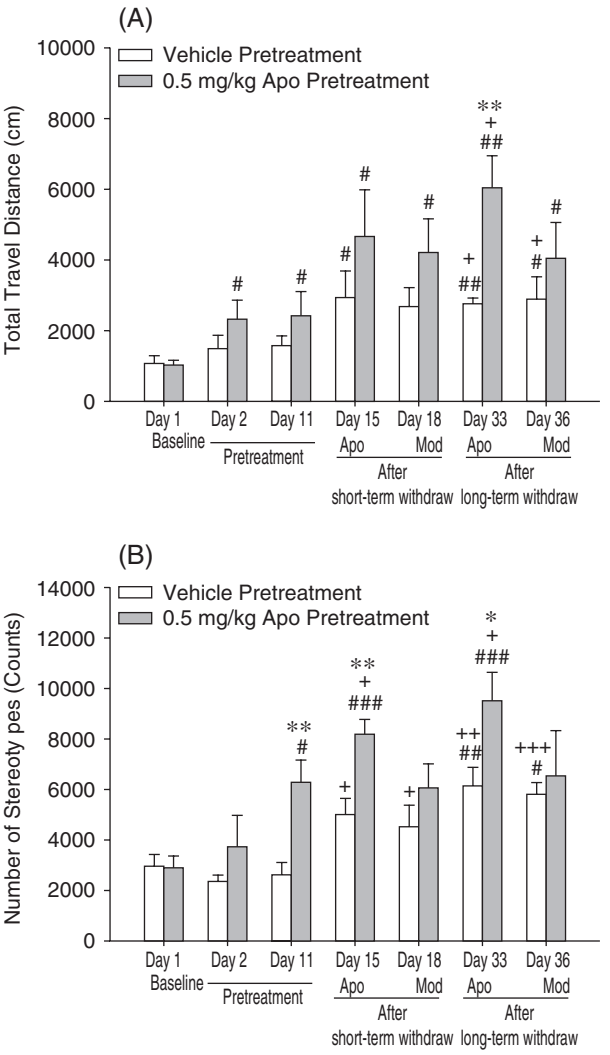

Fig. 3. Effects of a challenged dose of APO (Apo, 0.5 mg/kg) or MOD (Mod, 64 mg/kg) after short- or long-term washout periods in rats pretreated with APO (0.5 mg/kg, n = 6) or vehicle (n = 6). Total travel distance (A) and stereotyped movements (stereotypes) (B) were recorded for 1 h in APO test, 1.5 h in MOD test, and an identical span as the challenged drug at the indicated time point in saline test. Repeated treatments with APO induced sensitization to APO and had a tendency to induce cross-sensitization to MOD but no statistical significance in both total travel distance and stereotyped movements. For other details of statistical analysis, see Fig. 2. Results are expressed as means ± SEM.

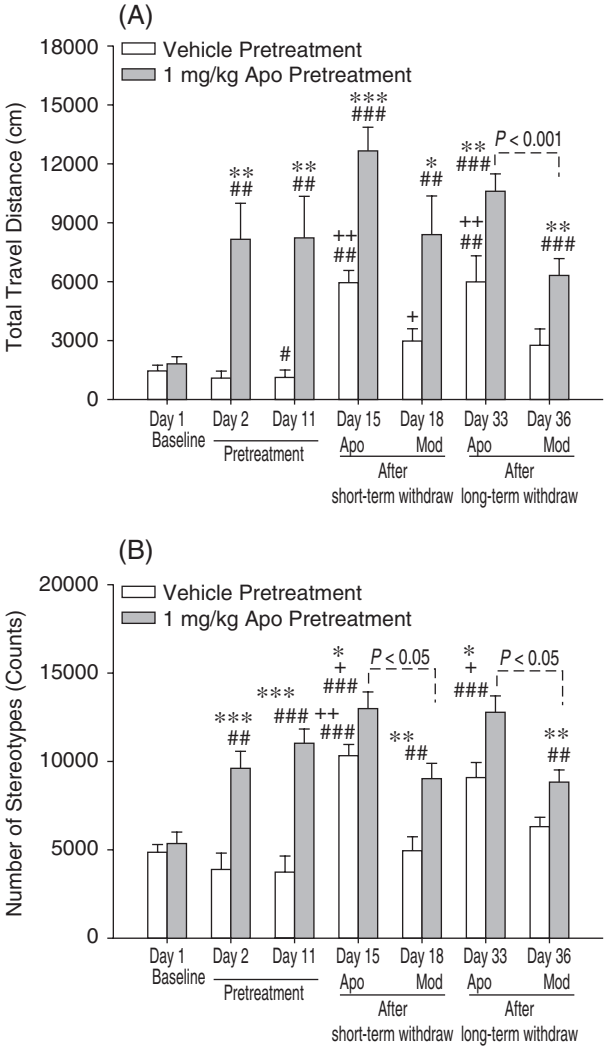

Fig. 4. Effects of a challenged dose of APO (Apo, 1.0 mg/kg) or MOD (Mod, 64 mg/kg) after short- or long-term washout periods in rats pretreated with APO (1.0 mg/kg, n = 10) or vehicle (n = 8). Total travel distance (A) and stereotyped movements (stereotypes) (B) were recorded for 1 h in APO test, 1.5 h in MOD test, and an identical span as the challenged drug at the indicated time point in saline test. Repeated treatments with APO induced sensitization to APO and cross-sensitization to MOD in both total travel distance and stereotyped movements in general. There was a significantly lower effect of MOD relative to APO in total travel distance ($P < 0.001$, Day 36 *vs*. Day 33) as well as in stereotyped movements ($P < 0.05$, Day 18 *vs*. Day 15 and Day 36 *vs*. Day 33) following multiple comparisons with LSD method. For other details of statistical analysis, see Fig. 2. Results are expressed as means ± SEM.

was also be found significant in day 33. Repeated APO (1.0 mg/kg)-rats also expressed escalating LM (Fig. 4A) and SM (Fig. 4B), particularly on days 15 and 33 compared with day 1 (LM: all $P < 0.001$; SM: all $P < 0.001$) and on day 33 compared with day 2 (LM: $P < 0.05$ and SM: $P < 0.05$). Nonetheless, the APO (1.0 mg/kg)-pretreated rats showed a significantly decreasing level of motor activity on day 33 *vs*. day 36 in LM ($P < 0.001$), day 15 *vs*. day 18, and day 33 *vs*. day 36 in SM (all $P < 0.05$).

On the other hand, these data showed that there was a significant change of VEH Control-rats after APO 0.5 mg/kg challenge on day 15 and day 33 compared with days 1 and 2 (all $P < 0.05$) in LM, and

also that on days 15 and 33 compared with day 1 (all $P < 0.001$), and on days 15 and 33 compared with day 2 ($P < 0.01$ and $P < 0.05$) in SM. The VEH Control-rats after APO 1.0 mg/kg challenge expressed similar changes. In addition, compared with the VEH Control-rats, APO pretreated at 0.5 and 1.0 mg/kg performed similarly in baseline activity for their LM and SM (all $P > 0.05$). The Repeated APO (0.5 mg/kg)-rats compared to VEH Control-rats had significantly elevated LM on day 33 ($P < 0.01$) and also SM on days 11 ($P < 0.01$), 15 ($P < 0.01$) and 33 ($P < 0.05$). Similar results were found in the group of Repeated APO (1.0 mg/kg)-rats except that this group of rats had higher activity levels in both LM and SM on days 2 to 33 ($P < 0.05 \sim P < 0.001$). These results indicated that early exposure to both doses of APO might induce behavioral sensitization after both short- or long-term withdrawal.

On days18 and 36, 3 days following the last APO challenge, rats from each of the VEH Control- and Repeated APO-group were cross-sensitized into MOD challenges (64 mg/kg). Follow-up analyses showed that only the larger-dose (Repeated Apo 1.0 mg/kg) rats compared with the VEH Control-rats on day 18 expressed a greater activity in LM and SM after MOD treatment (LM: $P < 0.05$; SM: $P < 0.01$). On day 36, the similar phenomenon still remained significant (all $P < 0.05$). After a short-term withdrawal period, APO challenge on day 15 caused higher behavioral activity in LM ($P < 0.05$) and SM ($P < 0.05$) compared with MOD challenge on day 18. A similar phenomenon was observed after the long-term withdrawal period where APO and MOD challenges were taken on days 33 and 36, respectively. The results supported that cross-sensitization was established between APO (1.0 mg/kg) and MOD. However, these data also indicated that subsequent MOD challenges following repeated early exposures of APO might reduce the magnitude of sensitization (LM and SM).

Discussion

The hallmark finding of the present study suggested that MOD effect was liable to sensitization following repetitive activation of dopaminergic D1/D2 receptors. MOD, like other central stimulants, may share a common mechanisms of action. We examined the possibility whether enhanced sensitivity to one drug (behavioral sensitization) could be cross-sensitized to the other. The results indicated that MOD in vigilance-promoting dosages enabled motor activity of the rat to be sensitized and this was different from the conventional dopaminergic D1/D2 receptor agonist such as APO. MOD enhancing motor activities in LM and SM were dose-dependent. Repeated administration of both MOD and APO caused sensitization. Cross-sensitization was not observed in APO challenges in rats that had shown sensitization to MOD. Interestingly, cross-sensitization occurred in MOD challenges in rats already sensitized to APO. This was more pronounced when a larger dose of APO (1.0 mg/kg) was used no matter such challenge was performed following a short- or long-term withdrawal period.

Our findings in Experiment 1 were consistent with previous literature demonstrating that a single injection of MOD was sufficient to elicit the assumed stimulant effects on LM (Fig. 1). However, the effect on SM shown in the current study was reported at high dosages in studies with mice (9, 35). Species differences, therefore, were worthy of consideration in describing MOD effects. The findings in Experiment 2 indicated that behavioral sensitization was induced after repeated exposures to MOD (Fig. 2). After repeated administrations of MOD, significant behavioral sensitization in LM and SM was observed after both short-term and long-term washout periods. When challenged acutely with APO on days 18 and 36, however, the Repeated MOD-group showed a lesser magnitude of motor activity compared with VEH Controls.

To our knowledge, these findings are the first to demonstrate that MOD is able to induce behavioral sensitization as other psychostimulants do. Since behavioral sensitization is one of the animal models for drug addiction (27, 31), our data suggest that after repeated exposures, MOD might have the potential to elicit dependency. Interestingly, literature has reported that MOD has only minimal potential for abuse (8, 19, 32). This discrepancy may be due to different dosing, single or repeated, used. It could also be due to relative small numbers of test animals used in those studies, and the less addictive potential was referred to naive individuals used in those studies, which was rather different to what the subjects experienced in the current experiments.

Neuronal adaptation may play a crucial role in behavioral sensitization. Psychostimulant-induced behavioral sensitization may be accompanied with increased reactivity of dopaminergic receptors (1, 16, 20, 27, 31, 37, 41). Cross-sensitization between psychostimulants may result from common neuronal functions. We, therefore, further examined whether similar mechanisms were involved in MOD-induced sensitization and cross-sensitization to APO. Our results showed that there was no cross-sensitization to APO in the rats that had been sensitized by repeated treatments of MOD (Fig. 2). Since the APO-induced motor stimulation is closely related to the dopamineergic D1/D2 receptors (20), the results suggested that dopaminergic system might not be the main

substrate involved in the motor effects of MOD.

MOD has been known for its wake-promoting effects. This appeared less relevant to the dopamine systems (2, 35) as indexed by its low affinity for dopamine reuptake sites (40), less contribution to catechol release on nigro-striatal function at the presynaptic level (7), and minor disturbance of the metabolic activity in dopamine rich regions (11). APO-induced motor sensitization was chiefly obtained *via* the activation of central DA system. The use of APO paradigm in the present study provided a well contrast between DA and non-DA systems. For the latter, for example, the hypocretin/orexin protein has recently been found as a pharmacological target of MOD (29, 33). By using the MOD-APO cross sensitization paradigm, the obtained result suggested that MOD sensitization was distinguishable with that of APO. Experiment 3 demonstrated that cross-sensitization occurred under the reciprocal conditions where the rats that had been sensitized by APO were challenged with MOD. The effects of cross-sensitization into MOD were found detectably less than induced sensitization by APO alone. These results provide further support to our ideas that repeated treatments of MOD and APO led to different neuronal adaptations and, on the other hand, this distinction could also be manifested in quantity level, *i.e.*, the associated reactivity could be changed when the established sensitization crossed to other neurosubstrate. Taken together; APO sensitization only partially explained the MOD effect. This was probably due to sharing with MOD the same DA contribution. In other words, the failure of using APO sensitization to describe MOD motor effects indicated that the non-DA mechanism was also responsible for MOD sensitization.

The specific non-DA systems involved in this phenomenon remained unclear. Literature has revealed that both NE and DA are involved in the behavioral effects of modafinil in rodents; however, the requirement for NE is very much indirect because the NE involvement has to be bypassed by hypersensitive DA signaling (25). On the other hand, although adrenergic alpha-1 receptor was found to be responsible for the antiepileptic effect of MOD (5), this was theoretically opposite to sensitization mechanism. Taken together, while the agonist activity of MOD on adrenergic alpha-1 receptor should be taken into account, it seemed unlikely to play a major role in the MOD sensitization phenomenon.

APO-induced motor stimulant effects were often employed to approach the background of behavioral sensitization to dopaminergic agonists. Previous works have demonstrated that repeated administrations of APO may cause behavioral sensitization in LM like other psychostimulants do (4, 17, 20, 22). Behavioral sensitization to APO in SM has only been observed at doses where postsynaptic dopaminergic D2 receptors were proposed to be involved (22). However, in the present study, SM such as gnawing, biting and sniffing were frequently manifested following repeated administrations of APO. The discrepancy could be partly owing to differences in the experimental procedures. In the present studies, rats for all the testing periods were transported from the home cage to the test room, injected with APO, and immediately placed in the test chambers for behavioral evaluation. The procedure possibly caused the occurrence of context-depended APO sensitization which was worthy of taking into account (3, 21).

Given the evidence that cross-sensitization into MOD was detectably less than APO alone-induced LM and SM sensitization, it might be clinically valuable. Firstly, it is known that behavioral arousal is a key component of the stress response. Stress increases the reinforcing effects of drugs and plays an important role in determining vulnerability to developing drug addiction (13). MOD-modified brain areas or connections are known to be important in anxiety and stress response (13). For example, MOD overdose is clinically manifested as over-arousal and stress-related syndromes including headache, nervousness, insomnia, anxiety and nausea (19, 28). Secondly, the potency of a drug to induce sensitization may be an index of the potency of this drug to develop addiction or seeking behavior. The lowered magnitude of cross-sensitization of MOD may indicate that this drug may have a potential in replacement treatment for psychostimulant dependency (6, 15, 34).

In summary, we demonstrated in this study that the development of MOD-induced behavioral sensitization seemed to be independent on direct neuroadaptive changes in D1 and D2 dopaminergic receptors. The application of MOD needs to be with caution because this drug may have the potential of addiction. APO-pretreated rats showed cross-sensitization to MOD challenges. However, the behavioral stimulation of MOD was less than that induced by APO challenges indicating that MOD may be useful for medication of addiction or withdrawal symptoms induced by other psychostimulants.

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