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Methamphetamine plus scopolamine potentiates behavioral sensitization and conditioning ☆

Kunio Yui [a,*], Takao Miura [b], Kimihito Sugiyama [c], Mamoru Ono [c], Moriharu Nagase [c]

[a] *Medical Care Section, Urawa Juvenile Classification Home, Ministry of Justice, Takasago 3-16-34-31, Urawa 336, Japan*
[b] *Department of Psychiatry, Namioka Town Hospital, Namioka, Aomori 036, Japan*
[c] *Research Institute of Showa Yakuhin Kako Co., Ltd., Shimonoge 3-13, Takatuku, Kawasaki 213, Japan*

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Abstract

The effects of repeated methamphetamine (4.0 mg/kg) plus scopolamine (0.5 mg/kg) treatment on behavioral sensitization and drug conditioning in rats were compared with the effects of repeated methamphetamine treatment. Behavioral sensitization induced by repeated methamphetamine plus scopolamine treatment was more vigorous than that induced by repeated methamphetamine treatment. Repeated methamphetamine plus scopolamine treatment produced sensitized responses, not only to methamphetamine plus scopolamine and methamphetamine but also, to a lesser extent, to scopolamine. Methamphetamine plus scopolamine-sensitized rats but not methamphetamine-sensitized rats exhibited conditioned responses to a low-frequency tone (300 Hz, 100 dB) associated with the drug state, suggesting that robust methamphetamine plus scopolamine-induced behavioral sensitization may lead to enhanced conditioning. It is plausible that robust behavioral sensitization might operate via a reciprocal balance between the dopaminergic and cholinergic systems in favor of dopaminergic dominance. Conditioning to the drug-associated tone may be mediated via a reciprocal balance between the two transmitter systems.

Keywords: Methamphetamine; Scopolamine; Cholinergic system; Behavioral sensitization; Drug conditioning; Low-frequency tone

1. Introduction

Repeated administration of amphetamine or methamphetamine induces a progressive and enduring enhancement of stereotyped behavior, a phenomenon known as behavioral sensitization (Robinson and Becker, 1986). Most of the proposed mechanisms focus on functional changes in the dopamine system; however, several studies have considered the role of other neuroeffector systems in the phenomenon (Kokkinidis and Anisman, 1980; Karler et al., 1990). It was reported from studies of the neural correlate of stereotyped behavior that a reciprocal balance between the dopaminergic and cholinergic inhibitory mechanisms in the brain was involved in its control (Carlton, 1961; Arnfred and Randrup, 1968; Naylor and Costall, 1971; Costall and Naylor, 1972; Kokkinidis and Anisman, 1980). Although previous studies revealed that anticholinergics, such as scopolamine hydrobromide (Arnfred and Randrup, 1968; Kokkinidis and Anisman, 1980), atropine sulphate (Costall and Naylor, 1972) and orphenadrine (Arnfred and Randrup, 1968; Naylor and Costall, 1971) enhanced amphetamine-induced stereotyped behavior, there has been a relative paucity of data concerning the influence of a reciprocal balance between the two transmitter systems on behavioral sensitization. Recently, we reported that repeated administration of methamphetamine (4.0 mg/kg/day) in combination with scopolamine (0.5 mg/kg/day) progressively enhanced methamphetamine-induced stereotyped behavior and behavioral sensitization to challenge injections of methamphetamine and metham-

☆ Name of laboratory: Department of Neuropsychiatry, School of Medicine, Hirosaki University, Zaifu-cho 34, Hirosaki 036, Japan.

* Corresponding author. Tel. +81-48-862-7520, fax +81-836-1372.

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phetamine plus scopolamine (Yui et al., 1988; Yui and Miura, 1991). Thus, the first purpose of the present study was to further determine the way in which the effects of potentially different drugs induce behavioral sensitization.

It is well documented that repeated administration of amphetamine or methamphetamine can cause the subject to become conditioned to the experimental apparatus associated with the drug state (Tilson and Rech, 1973; Schiff, 1982; Beninger and Hahn, 1983). Earlier human studies have suggested that the high relapse rate seen in medically cured patients with amphetamine or methamphetamine psychosis may largely be a result of conditioning phenomena (Schiff, 1982) or excessive incentive learning associated with chronic amphetamine or methamphetamine abuse (Utena, 1974; Schiørring, 1982; Beninger and Hahn, 1983). The conditioned component of the behavior was suggested by Segal and Mandell (1974) to account for the increased responsiveness produced by repeated amphetamine treatment. In addition, it has been hypothesized that one of the general actions of amphetamine-like drugs is to cause an increased repetition of response, with response selection being partly dependent on environmental contingencies (Robbins, 1976). Actually, a previous animal study showed that under the influence of dopaminergic hyperactivity, the ability of drug-associated environmental stimuli to elicit behavioral responses was enhanced (Beninger and Hahn, 1983). It has also been suggested that psychological stress can act as a precipitant of methamphetamine psychosis following the development of methamphetamine-induced dopaminergic hypersensitivity (Sato et al., 1983). Recently, we reported that repeated methamphetamine plus scopolamine treatment enhanced methamphetamine plus scopolamine-induced vigorous stereotyped behavior in reaction to a low-frequency tone (300 Hz, 100 dB), following the development of behavioral sensitization (Yui et al., 1994). Accordingly, methamphetamine- or methamphetamine plus scopolamine-induced sensitization might facilitate the behavioral response to environmental stimuli. Taking into account the above-mentioned findings (Utena, 1974; Robbins, 1976; Schiørring, 1982; Schiff, 1982; Beninger and Hahn, 1983; Sato et al., 1983; Yui et al., 1994), conditioning to a tone associated with a drug state, using methamphetamine- or methamphetamine plus scopolamine-sensitized rats, might be a useful measure of conditioned responses to environmental stimuli. The second purpose of the present study was to determine conditioned responses including stereotypy in methamphetamine plus scopolamine-sensitized rats, especially to determine the ability of the co-administration of methamphetamine and scopolamine to augument the effects of methamphetamine on conditioning to a drug-associated tone.

2. Materials and methods

2.1. Animals

Wistar rats (Nihon Kurea Laboratory, Japan), weighing 250–350 g at the beginning of the study, were used. They were housed individually in an air-conditioned room (temperature: 25° C, relative humidity: 55%) under a reverse 12-h dark/light cycle (lights off: 07.00–19.00 h). Access to standard laboratory food and water was unrestricted. Testing was conducted during the dark cycle.

2.2. Behavioral sensitization: Experiment 1

The first experiment was designed to compare a progressive and enduring enhancement of stereotypy in order to determine whether behavioral sensitization would operate under a dopaminergic-cholinergic imbalance.

Drug treatment regimen

The 44 rats were randomly allocated to four groups. Each of the experimental groups received intraperitoneal injections of either methamphetamine (4.0 mg/kg, Philopon, Dainippon Co., Japan) in combination with scopolamine (0.5 mg/kg, Hysco, Kyorin Co., Japan) ($n = 20$), methamphetamine only (4.0 mg/kg) ($n = 8$), scopolamine only (0.5 mg/kg) ($n = 8$), or physiological saline in equivalent volumes to those of methamphetamine plus scopolamine ($n = 8$), once a day for 14 consecutive days (chronic administration session). When two drugs were administered simultaneously, they were injected in separate areas of the animal. To test the effects of the co-administration of methamphetamine and scopolamine on behavioral sensitization, and to determine the preserved levels of behavioral sensitization, methamphetamine plus scopolamine-treated rats were divided randomly into three subgroups at 7 days after the cessation of chronic treatment. Each subgroup received challenge injections of the same doses of methamphetamine plus scopolamine ($n = 7$), methamphetamine ($n = 7$) or scopolamine ($n = 6$) that had been given during the chronic administration session, followed by four subsequent challenge injections of the respective drugs at 7-day intervals (challenge session). The other three groups, chronically treated with methamphetamine, scopolamine or saline received five weekly challenge injections of their respective treatments.

Behavioral measurement

Rats were placed individually in each observation cage made of transparent plastic with the same dimensions as the home cage ($33 \times 24 \times 17$ cm high). Red opaque barriers were used to prevent each rat from

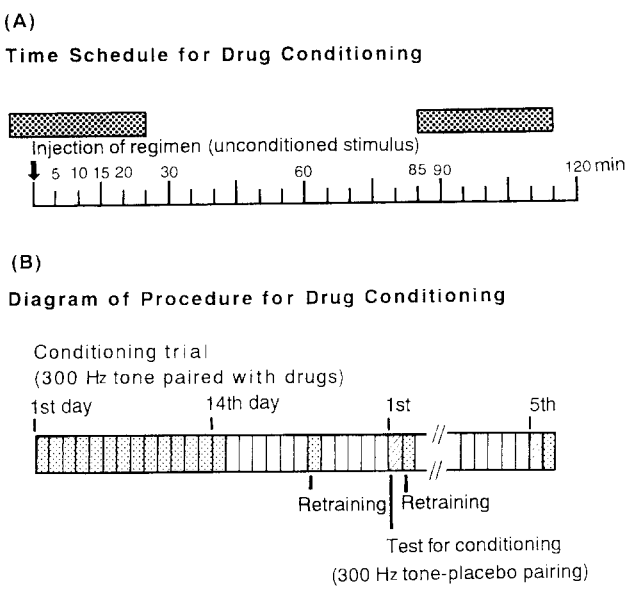

Fig. 1. Procedure for drug conditioning. Rats received 14 conditioning trials consisting of a daily session of tone (300 Hz, 100 dB) as a conditioned stimulus, paired with injections of methamphetamine (4.0 mg/kg) plus scopolamine (0.5 mg/kg), methamphetamine (4.0 mg/kg), scopolamine (0.5 mg/kg), or saline. The tone was presented for 30 min beginning 5 min before and 85 min after the injections (A, upper panel). Retraining identified with the training trials was conducted 5 times at 7-day intervals. Test sessions occurred 6 days after every retraining (B, lower panel).

being influenced by the behavior of its neighbors. Following a 3-h session of habituation to the observation cage for 3 days, each animal was videotaped and rated for 5 min at 5, 10, 15, 20, 30, 45, 60, and 120 min after injections by two trained raters, blinded to the treatment groups (Fig. 1A). The evaluation of behavioral responses was based on the following five-point rating scale adapted from previously established systems (Costall and Naylor, 1972; Sahakian et al., 1975; Eichler et al., 1980; Kolta et al., 1990; Paulson et al., 1991; Dall'olio et al., 1994): 0 = animal is asleep or stationary; 1 = mild, discontinuous sniffing associated with continuous locomotor activity; 2 = burst of sniffing with hyperactivity; 3 = continuous sniffing with very brief locomotor activity or rearing; 4 = continuous sniffing without rearing and locomotion; 5 = continuous (focussed) sniffing intermingled with gnawing and licking in one place.

Data analysis

The cumulative behavioral score collected over a 120-min period on each test day was calculated as the average of eight observations for each rat in each respective groups, with a maximum possible score of 5.0, during both the chronic administration session and the challenge session. To assess the time course of behavioral responses elicited by the first challenge injections of the regimen in comparison with the time course of those induced by the first injections of the chronic administration session, the time point value at each observation on each test day was calculated as the average of 6-8 rats in each respective group except for values from the chronic methamphetamine plus scopolamine treatment group which was the average of 20 rats. Because stereotyped behavior rankings are on an ordinal scale, behavioral data were analyzed using the non-parametric Kruskal-Wallis test. When significance was found, the Mann-Whitney *U*-test was used to test differences between a single pair from any group (Schiff, 1982; Camp and Robinson, 1988; Paulson et al., 1991). All comparisons were based on two-tailed probabilities.

Dose-response

With increased doses of amphetamine in the dose range of 3.0–15.0 mg/kg (Ellinwood and Balster, 1974; Fray et al., 1980), or with the addition of 3.0 mg/kg scopolamine to 10.0 mg/kg amphetamine (Arnfred and Randrup, 1968), stereotypy was reported to be intermingled with gnawing and licking. Our preliminary study showed that methamphetamine in the dose range of 2.0–10.0 mg/kg, either alone or in combination with 0.5 mg/kg scopolamine produced a dose-dependent, focussed stereotypy intermingled with gnawing and licking.

2.3. Conditioned responses: Experiment 2

The second experiment assessed the ability of methamphetamine combined with scopolamine to produce conditioned responses using a low-frequency tone (300 Hz) as conditioned stimulus, in order to determine whether co-administered scopolamine augments the effects of methamphetamine on conditioning. To properly carry out this procedure, the animals were trained repeatedly with paired presentations of methamphetamine or methamphetamine plus scopolamine treatment, as an unconditioned stimulus, and a tone (300 Hz, 100 dB), as conditioned stimulus. To test for conditioned effects, the tone was paired with placebo injections. This allowed us to assess that the ability of the tone to enhance the conditioned responses was identified with environment-specific conditioning (Schiff, 1982; Beninger and Hahn, 1983) including conditioned stereotypy. The methods were similar to those in experiment 1 with the following three exceptions (*Conditioning*, *Reconditioning*, *Conditioned effects post-test*):

Conditioning

The 32 rats were randomly assigned to four groups ($n = 8$). As depicted in Fig. 1A, each of the four groups received 14 conditioning trials consisting of a daily

session with tone (300 Hz, 100 dB) as a conditioned stimulus, paired with intraperitoneal injections of methamphetamine (4.0 mg/kg) plus scopolamine (0.5 mg/kg), methamphetamine (4.0 mg/kg), scopolamine (0.5 mg/kg), or physiological saline in equivalent volumes to those of methamphetamine plus scopolamine, as an unconditioned stimulus in each observation cage. Results from previous studies suggested that rats which exhibit stereotyped behavior, i.e., a relatively invariant pattern, regular repetition, and apparent uselessness (Randrup and Munkvad, 1974), might lose their appropriate responses to environmental factors (Randrup and Munkvad, 1974). Thus, methamphetamine plus scopolamine-treated rats and methamphetamine-treated rats might be disturbed in their normal responses to a tone during a resultant phase of stereotypy. A sequence of behavior induced by amphetamine in the dose range of 5.0–10.0 mg/kg includes a 'pre-phase' of hyperactivity (approximately 10–20 min after injection), a 'stereotypy phase' (approximately 60 min after injection), and an 'after-phase' of enhanced motor activity (approximately 90 min after injection) (Segal and Mandell, 1974; Kokkinidis and Anisman, 1980). Taking into account the above-mentioned findings, a tone acting as a conditioned stimulus must be presented both during the 'pre-phase' and the 'after-phase'. Thus, we presented a tone for 30 min, beginning 5 min before injection and again 85 min after injection (Fig. 1A). The 300 Hz tone was generated by a CR audiogenerator (Model AG202A, Trio Co., Japan) situated at the left front of the observation cage, and amplified through a 100-W power amplifier. Background noise (40 dB) generated by an airconditioning system was constant in the rearing and experimental rooms. The 300-Hz tone is considered to have some property to alarm because drug-free rats exhibited startle responses at the start of presentation.

Reconditioning

In order to assess the conditioned responses to a tone-conditioned stimulus, two groups of rats underwent reconditioning trials. Group one consisted of methamphetamine plus scopolamine- ($n = 8$) and methamphetamine-sensitized rats ($n = 8$) which exhibited behavioral sensitization to challenge injections of their respective drugs. Group two consisted of scopolamine- ($n = 8$) and saline- ($n = 8$) pretreated rats. The rats received reconditioning trials with an original conditioned stimulus (tone)-unconditioned stimulus (drug) protocol, 7 days after the discontinuation of their 14 training trials. To determine the establishment of conditioned responses, subsequent retraining followed by testing was repeated 4 times, at 7-day intervals (Fig. 1B).

Conditioned effects post-test

In order to test conditioned responses in the methamphetamine plus scopolamine- and methamphetamine-sensitized rats and to avoid the possible residual drug effects of the methamphetamine metabolite (*p*-hydroxy-norepinephrine), which has been reported to be undetectable in the brain of rats 6 days after injection, on the behavioral augmentation (Browne and Segal, 1977), or unexcreted methamphetamine and scopolamine, we waited 6 days after each retraining session before testing. A placebo (physiological saline) was substituted for the respective drugs during each session. Using only the tone, the behavioral effects were measured to determine the degree of conditioning. Because conditioned responses which consisted of mild discontinuous sniffing associated with continuous exploration ('1' on the rating scale in the present experiment) were not clearly distinct from the normal pattern of sniffing associated with normal exploration, testing was repeated 5 times at weekly intervals in order to determine conditioning (Fig. 1B). Each rat was rated for 5 min by two trained raters, blinded to the treatment groups at 5, 10, 15, 20 and 30 min after injections according to the scoring system used in experiment 1.

Data analysis

To determine the conditioned responses for each rat, in the respective group on each test day, the cumulative activity over a 30-min period and the time point value at each observation were collected over 5 test days. The values were calculated as the average of 5 test days for each rat in the eight-rat groups, respectively. Data were analyzed with the same statistical methods as used in experiment 1.

3. Results

3.1. Behavioral sensitization to methamphetamine plus scopolamine and methamphetamine alone

Chronic treatment with methamphetamine plus scopolamine induced a significantly progressive augmentation in behavioral responses throughout most of the chronic administration session when compared to chronic methamphetamine treatment ($U = 10.00$–42.50, $Z = 1.92$–3.58, $P < 0.01$ or $P < 0.05$).

The first challenge injections of methamphetamine plus scopolamine given to the methamphetamine plus scopolamine-pretreated rats induced a significant augmentation of stereotyped behavior intermingled with gnawing and licking when compared to the first injections of methamphetamine plus scopolamine at any time (group mean, $U = 137.50$, $Zc = 3.77$, $P < 0.01$;

time point values, $U = 138.50–127.50$, $Zc = 4.20–3.32$, $P < 0.01$). The first challenge injection of methamphetamine given to the methamphetamine plus scopolamine-pretreated rats produced a significant enhancement in stereotyped behavior when compared to the first injections of both the methamphetamine group (group mean, $U = 48.00$, $Z = 3.11$, $P < 0.01$; time point values, $U = 48.00$, $Z = 2.96–3.53$, $P < 0.01$) and the methamphetamine plus scopolamine group (group mean, $U = 116.00$, $Zc = 3.40$, $P < 0.01$; time point values, $U = 108.00–120.00$, $Zc = 3.11–4.17$, $P < 0.01$) throughout most of the session. The scopolamine challenge given to the methamphetamine plus scopolamine-pretreated rats elicited a significantly more augmented behavioral response than did the first injection of scopolamine ($U = 46.50$, $Z = 2.92$, $P < 0.01$) at 20 ($U = 41.00$, $Z = 2.55$, $P < 0.01$) and 45–120 min ($U = 38.00–48.00$, $Z = 2.08–3.53$, $P < 0.01$ or $P < 0.05$) (Table 1).

The weekly challenge injections of methamphetamine plus scopolamine, and methamphetamine only to the methamphetamine plus scopolamine-pretreated rats induced about the same degree of intense (focussed) stereotypy (statistically non-significant) across 5 challenge test days. However, the weekly challenge injections of methamphetamine to the methamphetamine plus scopolamine-pretreated rats induced a significant augmentation of stereotyped behavior when compared to the methamphetamine-pretreated rats throughout most of the challenge session ($U = 13.00–1.50$, $Z = 1.45–2.95$, $P < 0.01$ or $P < 0.05$). Interestingly, the weekly challenge injections of scopolamine to the methamphetamine plus scopolamine-pretreated rats induced a significant enhancement of behavioral responses when compared to the scopolamine-pretreated rats over the 5 challenge test days ($U = 0.00–0.50$, $Z = 3.07–3.23$, $P < 0.01$) (Table 1). Thus, methamphetamine plus scopolamine-induced stereotypy was consistently reproduced by the challenge injections of both methamphetamine plus scopolamine and methamphetamine only. Additionally, methamphetamine plus scopolamine-induced stereotyped behavioral responses were, to a lesser extent, elicited by scopolamine challenges.

3.2. Conditioned responses in methamphetamine plus scopolamine and methamphetamine-sensitized rats

Methamphetamine plus scopolamine-sensitized rats exhibited significantly enhanced behavioral responses, consisting of mild discontinuous sniffing associated with continuous exploration, when compared to methamphetamine-sensitized rats ($U = 7.50$, $Z = 2.61$, $P < 0.05$), scopolamine-pretreated rats ($U = 7.50$, $Z = 2.60$, $P < 0.05$), and saline-treated controls ($U = 5.50$, $Z = 2.63$, $P < 0.01$). Time-course analysis revealed that methamphetamine plus scopolamine-sensitized rats showed significantly more augmented behavioral responses than did the methamphetamine- and scopolamine-pretreated rats at 10 min (methamphetamine-pretreated rats, $U = 4.00$, $Z = 3.27$, $P < 0.01$; scopolamine-pretreated rats, $U = 6.50$, $Z = 2.91$, $P < 0.01$) and at 15 min (methamphetamine-pretreated rats, $U = 12.00$, $Z = 2.44$, $P < 0.05$; scopolamine-pretreated rats, $U = 12.00$, $Z = 2.44$, $P < 0.05$), and more than the saline-pretreated controls over the first 20 min ($U = 6.00–8.00$, $Z = 2.40–2.86$, $P < 0.01$ or $P < 0.05$). Methamphetamine-sensitized rats failed to demonstrate a significant acquisition of conditioned re-

Table 1
Stereotyped behavioral response to challenge injections of regimen in comparison with the first injections of the drug regimen

	Methamphetamine + scopolamine ($n = 20$)			Methamphetamine ($n = 8$)	Scopolamine ($n = 8$)	Saline ($n = 8$)
Chronic session						
1st	2.34 ± 0.11			1.58 ± 0.09	0.38 ± 0.09	0.09 ± 0.05
	Methamphetamine + scopolamine ($n = 7$)	Methamphetamine ($n = 7$)	Scopolamine ($n = 6$)	Methamphetamine ($n = 8$)	Scopolamine ($n = 8$)	Saline ($n = 8$)
Challenge session						
1st	4.20 ± 0.21 [a]	3.75 ± 0.09 [a,b,d]	0.98 ± 0.09 [c,f]	3.41 ± 0.15 [b]	0.23 ± 0.08	0.06 ± 0.03
2nd	4.29 ± 0.14	4.00 ± 0.11 [d]	1.58 ± 0.26 [f]	3.34 ± 0.19	0.08 ± 0.04	0.03 ± 0.02
3rd	4.34 ± 0.13	4.04 ± 0.15 [e]	1.10 ± 0.16 [f]	2.97 ± 0.30	0.08 ± 0.06	0.03 ± 0.02
4th	4.39 ± 0.10	3.77 ± 0.13	1.35 ± 0.17 [f]	3.56 ± 0.13	0.09 ± 0.05	0.02 ± 0.02
5th	4.16 ± 0.26	3.88 ± 0.13 [d]	1.73 ± 0.18 [f]	3.33 ± 0.22	0.19 ± 0.08	0.02 ± 0.02

Each value represents the mean ± S.E.M. Rats pretreated with methamphetamine (4.0 mg/kg) plus scopolamine (0.5 mg/kg) were divided into three subgroups, and each subgroup received challenge injections equal to the initial doses of methamphetamine plus scopolamine, methamphetamine, or scopolamine that were given during chronic treatment. Statistical analysis was done with the Mann-Whitney U-test between applied to any single pair from any group. [a] $P < 0.01$ vs. the first injections of methamphetamine plus scopolamine; [b] $P < 0.01$ vs. the first injection of methamphetamine; [c] $P < 0.01$ vs. the first injection of scopolamine; [d] $P < 0.05$, [e] $P < 0.01$ vs. methamphetamine challenge to methamphetamine-pretreated rats; [f] $P < 0.01$ vs. scopolamine challenge to scopolamine-pretreated rats.

sponses. Scopolamine-pretreated rats showed no significant difference from saline-treated controls.

4. Discussion

4.1. Effects of methamphetamine plus scopolamine on behavioral sensitization

The results of Costall and Naylor (1972) had indicated that the co-administration of amphetamine and anticholinergic agents such as atropine and orphenadrine may induce exhaustion of the latent compensatory effects of the cholinergic system following the elicitation of stereotypy. Then, the inhibition of the cholinergic system would further enhance the effects of dopaminergic stimulation and thus increase the intensity of stereotypy. According to this possibility, treatment with methamphetamine alone can not induce a dopaminergic-cholinergic imbalance. Accordingly, in order to evaluate the influence of the dopaminergic-cholinergic imbalance on behavioral sensitization, we carried out challenge injections of methamphetamine plus scopolamine, methamphetamine only and scopolamine only in the methamphetamine plus scopolamine-pretreated rats.

The finding that repeated methamphetamine plus scopolamine treatment augmented stereotyped behavior is consistent with previous reports (Carlton, 1961; Arnfred and Randrup, 1968; Naylor and Costall, 1971; Costall and Naylor, 1972). In addition, the present findings indicate that repeated treatment with methamphetamine plus scopolamine induced a significant augmentation of behavioral sensitization compared to repeated methamphetamine treatment. The challenge injection of methamphetamine to the methamphetamine plus scopolamine-pretreated rats, administered 7 days after the last pretreatment doses, induced a significantly more augmented stereotypy than the first injections of methamphetamine and also the first injections of methamphetamine plus scopolamine. There was no significant difference in stereotypy between methamphetamine plus scopolamine and methamphetamine challenges in the methamphetamine plus scopolamine-pretreated rats. However, challenge injections of methamphetamine to the methamphetamine plus scopolamine-pretreated rats consistently produced significant sensitization effects when compared to methamphetamine challenges to the methamphetamine-pretreated rats. These findings indicate the predominance of the dopaminergic system over behavioral sensitization. Of particular interest in the present study was that the scopolamine challenges to the methamphetamine plus scopolamine-pretreated rats consistently induced more significantly enhanced behavioral responses than in the scopolamine-pretreated rats. Thus, it is important to note that intense stereotyped behavior induced by repeated methamphetamine plus scopolamine treatment was elicited not only by every challenge injection of methamphetamine plus scopolamine and methamphetamine, but also, to a lesser extent, by scopolamine challenges as well. In general, it is suggested that behavioral sensitization may be mediated via the reciprocal balance between the dopaminergic and cholinergic inhibitory systems in favor of dopaminergic dominance.

4.2. Conditioned responses to the tone as a conditioned stimulus

In the classical conditioning to drug effects, a drug (unconditioned stimulus) is administered for a number of trials in association with environmental stimuli (conditioned stimulus) such as placement in a novel observation chamber, or with complex conditioned stimuli such as tone-injection-novel cage placement stimuli (Schiff, 1982). After a number of pairings of an unconditioned stimulus such as amphetamine or methamphetamine and a conditioned stimulus such as placement in a novel observation chamber (Tilson and Rech, 1973; Herz and Beninger, 1987; Schiff, 1982; Beninger and Hahn, 1983), a placebo is substituted for the drug. Then, with the conditioned stimulus alone, measurement of the behavioral effects determines the degree of conditioning obtained. These components were involved in the present experiment. An earlier study suggested that a general action of stimulant drugs can induce conditioning of stereotypy to drug-associated environmental contingencies (Robbins, 1976). Therefore, we used the 0–5 point rating scale, which can quantitatively characterize the gamut of methamphetamine- and methamphetamine plus scopolamine-induced behaviors, in order to evaluate conditioned responses, including stereotypy.

The present results revealed that methamphetamine plus scopolamine-sensitized rats exhibited significantly more augmented behavioral responses to the tone-placebo pairing than did the methamphetamine-sensitized, scopolamine- and saline-pretreated rats. This finding indicates that there was conditioning to the methamphetamine plus scopolamine-associated low-frequency tone in the methamphetamine plus scopolamine-sensitized rats. However, methamphetamine-sensitized rats failed to demonstrate a significant acquisition of conditioned responses. Previous studies have demonstrated that rats with a history of receiving amphetamine in the dose range of 0.5–1.8 (Tilson and Rech, 1973) or 0.5–5.0 mg/kg (Herz and Beninger, 1987), or at the dose of 2.5 mg/kg (Beninger and Hahn, 1983) in a specific environment showed conditioned locomotor activity. Furthermore, it was reported that ten training trials consisting of a daily session of

amphetamine at the doses of 0.8, 2.6 and 4.7 mg/kg, paired with a tone (Sonalert signal, 1 min) and placement in a novel observation cage induced conditioned sniffing, rearing and horizontal locomotor activity (Schiff, 1982). The present finding with methamphetamine-sensitized rats is contrary to the above-mentioned previous results (Ellinwood, 1971; Tilson and Rech, 1973; Herz and Beninger, 1987; Schiff, 1982; Beninger and Hahn, 1983). The source of this variation is not certain but could be either procedural differences in the measurement of behavioral responses or the dose-effect relations of drugs in the different species tested (rats vs. cats). For example, in previous studies of drug conditioning, locomotor activity and vertical activity (rearing or jumping) were quantified with microswitches or automatic counters (Tilson and Rech, 1973; Herz and Beninger, 1987; Schiff, 1982) which reflected not only entirely constant locomotion but non-specific general activity, and these were scored according to a rating scale ranging from asleep (1), through normal alert activity (4), up to stereotyped behavior (8) (Beninger and Hahn, 1983). The scoring system in the present experiment rates mild, discontinuous sniffing associated with continuous exploratory activity for more than 4 min as a score of (1). In the case of cats (Ellinwood, 1971), the conditioned stereotypy might be due to the toxic effects of higher doses (15.0–30.0 mg/kg) of methamphetamine in a species different from rats. A previous review indicated that rats were more sensitive to frequencies of the tone used in the above-mentioned previous studies than to the 300-Hz tone (Glough, 1982). Our previous study, however, showed that methamphetamine plus scopolamine-treated rats progressively had a significant augmentation in reaction of their stereotypy to the 300-Hz tone as compared with methamphetamine-treated rats (Yui et al., 1994). On the basis of the two findings, it is suggested that methamphetamine-sensitized rats may be less sensitive to the low-frequency tone than methamphetamine plus scopolamine-sensitized rats, and thus the methamphetamine-sensitized rats might fail to display conditioning.

The important point is that methamphetamine plus scopolamine-sensitized rats exhibited conditioned responses to the drug associated with a low-frequency tone. This indicates that co-administration of methamphetamine and scopolamine can augment the effects of methamphetamine in drug conditioning. In light of the experiment 1 finding that methamphetamine plus scopolamine induced more robust behavioral sensitization than methamphetamine, it is suggested that robust methamphetamine plus scopolamine-induced behavioral sensitization may lead to enhanced ability of conditioned responses to the tone.

A previous study based on the effects of scopolamine on conditioning had indicated the depressive effects of scopolamine on behavior suppressed by punishment (electric shock), conditioned emotional response (non-contingent shock) and extinction in rats (McKim, 1980). Administration of scopolamine has been reported to disrupt classical conditioning as evaluated from the rabbit nictitating membrane response (Harvey et al., 1983; Salivatierra and Berry, 1989) and to retard the rate of conditioned response acquisition to a tone conditioned stimulus by blocking the unconditioned and conditioned excitatory properties of tone stimuli (Harvey et al., 1983). Accordingly, it is concluded that scopolamine can disrupt conditioned responses to a tone associated with a drug state by impairing the transfer of experience from the training session to the test trial (Warburton and Groves, 1969). However, in the present study, scopolamine administered in combination with methamphetamine induced conditioning. This result is partially in agreement with results of a previous study of the conditioned effects of scopolamine combined with apomorphine (Carey, 1991). Scopolamine showed a functionally acquired conditioned stimulus property by eliciting an apomorphine response in rats with 6-hydroxydopamine substantia nigra lesions (Carey, 1991).

As mentioned above, vigorous methamphetamine plus scopolamine-induced behavioral sensitization may mediate conditioning. Therefore, taking into account previous findings that the establishment of conditioned responses to amphetamine-associated environments involves a dopaminergic action (Schiff, 1982; Beninger and Hahn, 1983; Herz and Beninger, 1987), the present results support the possibility that a reciprocal balance between the dopaminergic and cholinergic systems may be involved in drug conditioning in which the methamphetamine effects are enhanced by anticholinergics such as scopolamine.

In conclusion, the present findings indicate that behavioral sensitization may operate via the reciprocal balance between the dopaminergic and cholinergic inhibitory systems in favor of dopaminergic dominance. Conditioning to the methamphetamine plus scopolamine-associated tone might be mediated via the reciprocal balance between the two transmitter systems. It is important to note that robust methamphetamine plus scopolamine-induced behavioral sensitization may lead to enhanced conditioning to the methamphetamine plus scopolamine-associated low-frequency tone.

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