Chinese Journal of Physiology 49(1): 14-21, 2006

A Single Intrategmental Amphetamine Exposure Induces Transient Behavioral Sensitization in the Rats

Hsiao-Hua Wu[1], Chang-Ru Lee[1], Shi-Kwang Lin[1,2], Pen-Ho Yeh[1], and Wynn H.T. Pan[1*]

[1]*Institute of Pharmacology, School of Medicine*
National Yang-Ming University
Taipei, Taiwan, R.O.C.
[2]*Department of Geneal Psychiatry*
Taipei City Psychiatric Center
Taipei, Taiwan, R.O.C.

Abstract

Repeated treatment with psychostimulant drugs induces enduring behavioral sensitization and neuroadaptations which may play an important role in the development of drug addiction. However, different number and time course in drug administration and various lengths of drug withdrawal were employed in the literature, and there were inconsistent findings in the profile of extracellular dopamine level related to behavioral sensitization. Therefore, the effects of the number of drug exposure and the length of drug withdrawal period on the sensitized behavioral response were investigated in this study. Various lengths of amphetamine (AMPH) withdrawal (1, 3 and 5 days) after a single local administration of AMPH to bilateral ventral tegmental area (VTA) were used to observe the locomotor activity response. Besides, different amounts of administration of intra-VTA AMPH were given (1, 2 and 3 times of injection) to monitor the profile of travel distance and stereotypic movements of rats after 7 days of drug withdrawal. An early and short-lived behavioral sensitization to the single intra-VTA AMPH administration was induced. In the repeated treatment group, more drug exposures were associated with escalating and robust levels of travel distance after 7 days of drug withdrawal. The authors speculated that the transient and, a later augmented locomotor activity response might represent respective phases in the development of behavioral sensitization, which in turn contributed to the formation of more lasting behavioral and neuroplastic changes associated with drug addiction.

Key Words: behavioral sensitization, amphetamine, ventral tegmental area, withdrawal

Introduction

Systematically repeated administration of amphetamine (AMPH)-like stimulants produces an enduring enhancement of hyperactivity in rodents, a phenomenon known as behavioral sensitization (9, 20). Many efforts have been devoted to exploring the underlying mechanism of behavioral sensitization, with two major concerns: to locate the site of the action essential to the central nervous system in developing this sensitization, and to identify the changes occur in the nervous systems while there is sensitization.

In the first approach, the action site of AMPH to produce behavioral sensitization was extensively studied (developemental stage). Repeated intrategmental (intra-VTA) injections of AMPH, but not intra-accumbens, produces a sensitized locomotor response to a subsequent systemic or intra-accumbens injection of AMPH (3, 10, 15, 18, 24). These results suggest that an action on VTA is necessary for the development of behavioral sensitization.

Many laboratories have demonstrated that be-

Corresponding author: Dr. Wynn H.T. Pan, Institute of Pharmacology, School of Medicine, National Yang-Ming University, 155, Li-Nong St., Sec. 2, Beitou District, Taipei 112, Taiwan, R.O.C. Fax: 886-2-28264372, E-mail: wynn@ym.edu.tw
Received: February 2, 2005; Revised: May 23, 2005; Accepted: May 26, 2005.

havioral sensitization by systematically repeated administration of AMPH is associated with an enhanced extracellular dopamine (DA) increase in the nucleus accumbens (21, 26) and in the striatum (1, 11, 14, 19), but not in the medial prefrontal cortex (4). On the other hand, some other scientists have also demonstrated that a diminished extracellular DA increase occurs in the terminal fields after repeated administration of AMPH (5, 22). These results suggest that there is discordance between the DA and behavioral responses. Therefore, we hypothesized that there might be two different phases, no matter dopamine-dependent or not, in the progressive development of sensitization, which are related to not only the length of the drug withdrawal period but also the number of drug treatment (7, 8, 22).

The temporal development of behavioral sensitization awaits further study. To further investigate the development of behavioral sensitization, we simplfied the complex problem by using the easy ways in our studies: mentioning on the VTA, rather than on the system and focusing on the number of pretreatments or the length of the withdrawal period.

Materials and Methods

Subjects

Male Sprague-Dawley rats weighing 250-300 g on arrival were supplied by the Animal Center of National Yang-Ming University, Taipei, Taiwan, R.O.C. They were housed in a 12-hour light/dark cycle room with free access to food and water.

Apparatus

Total travel distance and stereotypic movements were measured by a video path analyzer (Model E61-21, Coulbourn Instruments). This analyzer was equipped with a cage (black background, 50 cm long x 50 cm wide x 50 cm high), a camera (Model TC650EA Series, Burle Technologies, Inc.), a television screen, a computer, and a printer. A detailed description of the principles of this video path analyzer can be found elsewhere (12).

Drugs

d-AMPH sulfate dissolved in sterile 0.9% saline was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Pentobarbital sodium was kindly supplied by the Veterans General Hospital, Taipei.

Surgery

The rats were anesthetized with 50 mg/kg i.p. sodium pentobarbital and placed on a stereotaxic apparatus (Kopf models 1430 & 1460). Two guided cannulae were then bilaterally implanted (22 g, Plastic Products) into the VTA (A/P -3.6, M/L ±0.6 from bregma, and D/V -8.9 from skull) and positioned 1 mm above the final injection site (26). An incisor bar was then placed 5.0 mm above the interaural line. Both guide cannulae were angled at 16° to the vertical, and then secured in place by skull screws and dental cement. Penicillin (60,000 I.U.) was administered i.m. immediately after surgery. Next, 28 gauge Plastic Products obturators were inserted into the VTA guide cannulae. Finally, the rats were returned to their home cages for a 5 to 7-day recovery period.

Microinjection Procedures

Simultaneous bilateral intracranial micro-injections (d-AMPH sulfate 2.5 μg/side) were made in the conscious rats. Saline solution was administered in the same volume to serve as a control. All the microinjections were given in a volume of 0.5 μl/side over 45s with a 28 gauge injector cannula (Plastic One Products) inserted to a 1 mm depth below the guide cannulae tip. Bilateral injection cannulae were connected by PE-20 tubing (Clay Adams, Parsippany, NJ) to 1 μl syringes (Hamilton, Reno, NV). Seventy-five seconds after injection, the injector cannulae were replaced by obturators. The rats were returned to their home cages immediately after the microinjection procedure.

Kalivas and Weber (1988) find that the larger the dosage of intra-VTA injections, the easier the sensitization would be produced (1.5 μg or more) (10). Therefore, we chose 2.5 μg as the dosage for intra-VTA injection. In addition, because an enhanced dopamine increase can be observed after a withdrawal period no shorter than 7 days (16), we set the withdrawal period to be 7 days in this study.

Experiment I

The rats first received one bilateral intra-VTA injection of either AMPH (2.5 μg / 0.5 μl/side) or saline (0.5 μl). Then, on the first, third, and fifth day after the injection, each rat's behavior was monitored.

Experiment II

The rats underwent a 7-day withdrawal period after receiving one, two, or three bilateral intrategmental injections of either AMPH (2.5 μg /0.5 μl/side) or saline (0.5 μl). If more than one injection were given, it would be seperated for two days. On the test day, all rats received a challenge administration of AMPH (1.0 mg/kg, i.p.), and their behavioral activities were monitored by the video path analyzer.

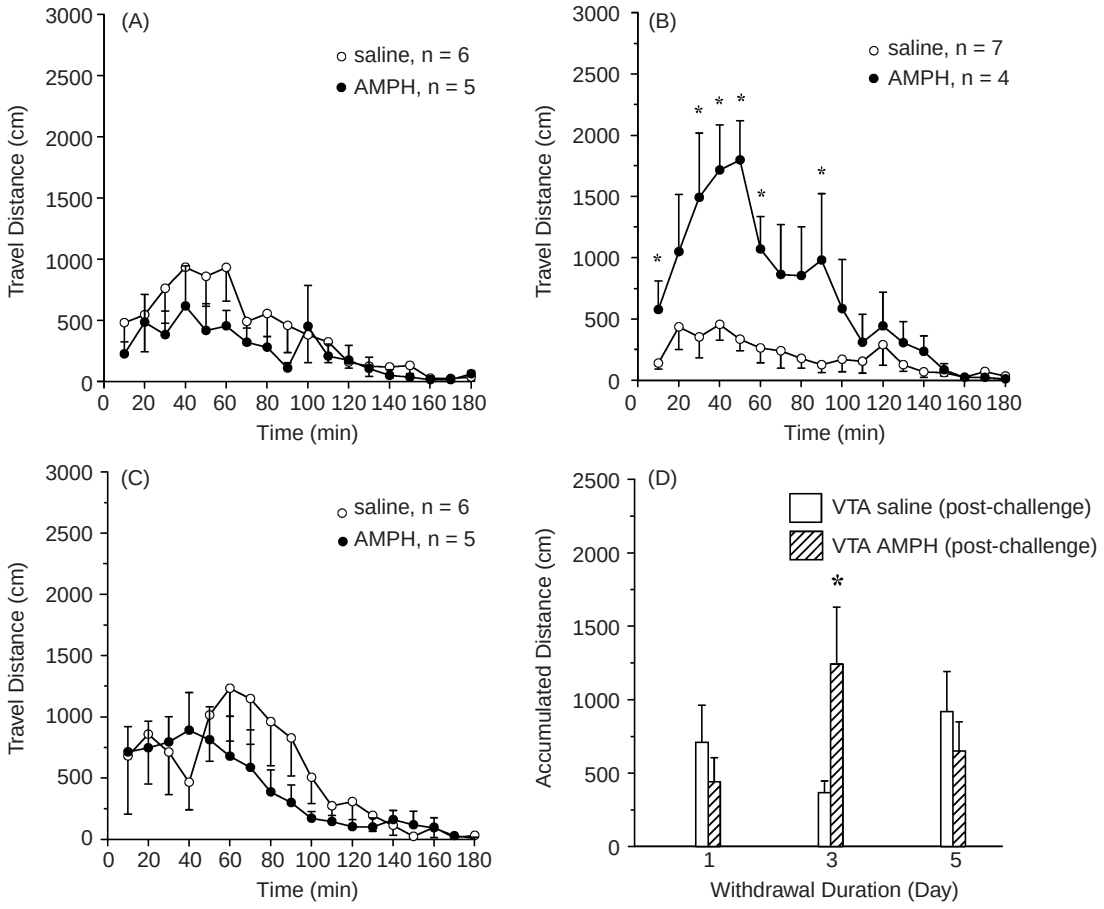

Fig. 1. Summary of the effects of single intra-VTA injection with AMPH on travel distance in rats post the challenge injection of AMPH (1.0 mg/kg, i.p.) given 1, 3, or 5 days of the withdrawal period. Panel (A), (B), and (C) depict the profile of travel distance in the time course post a challenge injection on withdrawal days 1, 3, and 5, respectively. Panel (D) shows the total travel distance for the 180-min test period. Values are presented as mean ± S.E.M. The asterisk (*) indicates statistically significant difference between AMPH-pretreated and saline-pretreated animals ($P < 0.05$).

Behavioral Test Procedure

All experiments were conducted from 9.00 a.m. to 6.00 p.m. Each rat was placed into a behaviroal box hooked-up with the video path analyzer and was allowed a habituation period for at least two hr prior to behavioral monitoring. After that, intraperitoneal injections of AMPH were given to rats. Finally, the travel distance and the number of the stereotypic movements of the rats were monitored for 18 successive sessions (10 min for each session). Stereotypic movements were defined and measured *via* the software of the video path analyzer as decribed in our previous study (12).

Histology

After completion of testing, in order to assess the site of cannulae placements, subjects were sacrificed by a large dose of sodium pentobarbital. Next, the brain was removed and fixed in a saline solution composed of 30% sucrose and 10% formaldehyde. After fixation, 40 μm coronal sections were cut on a freezing microtome and each section was mounted on a glass slide, and stained with 0.5% Cresyl violet for histological verification of the path of the cannulae tips.

Statistical Analysis

Values are presented as mean ± SEM. The temporal changes of travel distance and stereotypic movements were evaluated by a two-way ANOVA with repeated measures. This was followed by a post-hoc Scheffe multiple range test to assess the difference between individual means. The total travel distance and the total stereotypical movements in the entire 180-min time span were evaluated with unpaired Student's *t*-test to assess the difference between individual means. $P < 0.05$ was taken to indicate statistical significance.

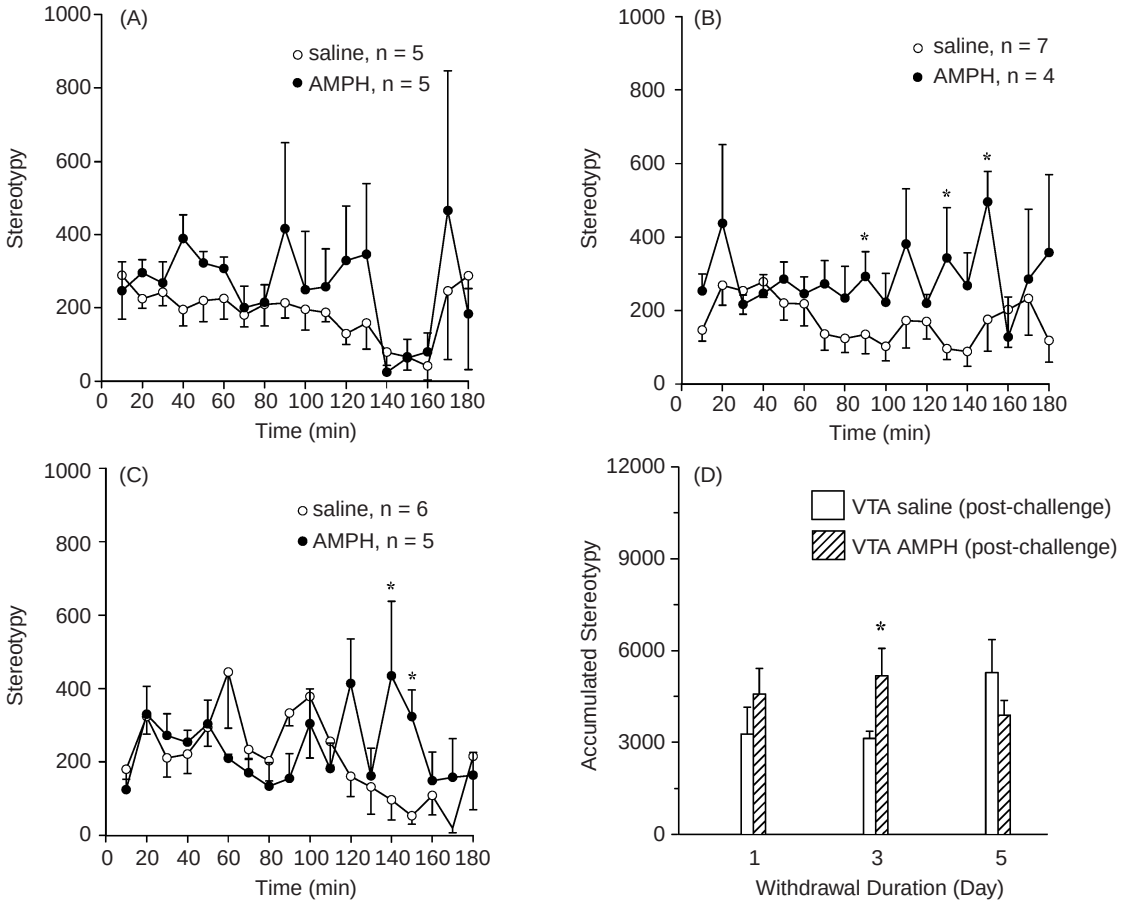

Fig. 2. Summary of the effects of 1 intra-VTA injection with saline or AMPH on stereotypic movements post a challenge injection of AMPH (1.0 mg/kg, i.p.) given 1, 3, or 5 days of the withdrawal period. Panel (A), (B), and (C) depict the profile of stereotypic movements in the time course post a challenge injection on withdrawal days 1, 3, and 5, respectively. Panel (D) shows the total number of stereotypic movements for each 180-min test period post a challenge injection. Values are presented as mean ± S.E.M. The asterisk (*) indicates statistically significant difference between AMPH-pretreated and saline-pretreated animals ($P < 0.05$).

Results

Behavioral Effects of a Single Intrategmental AMPH Injection after a 1, 3 or 5-day Withdrawal Period

Figures 1A, 1B, and 1C show the profile of travel distance during the 180 min recording period for both the experimental and control groups. Figure 1A is the group with a 1-day withdrawal period, Fig. 1B is the group with a 3-day withdrawal period and Fig. 1C is the group with a 5-day withdrawal period after a single intrategmental AMPH injection. In compared with the saline-injected control group, only the 3-day withdrawal group showed a significant increase in travel distance activity. In comparing the total travel distance during the whole 180 min period between the saline-control and treatment group (Fig. 1D), only the 3-day withdrawal group exhibited a significant difference (3672±792 *vs*. 12417±3879).

Figures 2A, 2B, and 2C show the changes in stereotypic movements *vs*. time lapse during the 180 min recording period for both control and treatment groups. Figure 2A is the group with a 1-day withdrawal period, while Fig. 2B is the group with a 3-day withdrawal period and Fig. 2C is the group with a 5-day withdrawal period after a single intrategmental AMPH injection. Comparing the total accumulated stereotypic movements during the entire 180 min period between the saline-control and treatment groups (Fig. 2D), again, only the 3-day withdrawal group exhibited a significant difference (3133±236 *vs*. 5176±898).

Behavioral Effects of Multiple Intrategmental AMPH Injections after a 7-day Withdrawal Period

Figures 3A, 3B and 3C show the changes in travel distance *vs*. time during the 180 min recording period for both the control and treatment groups.

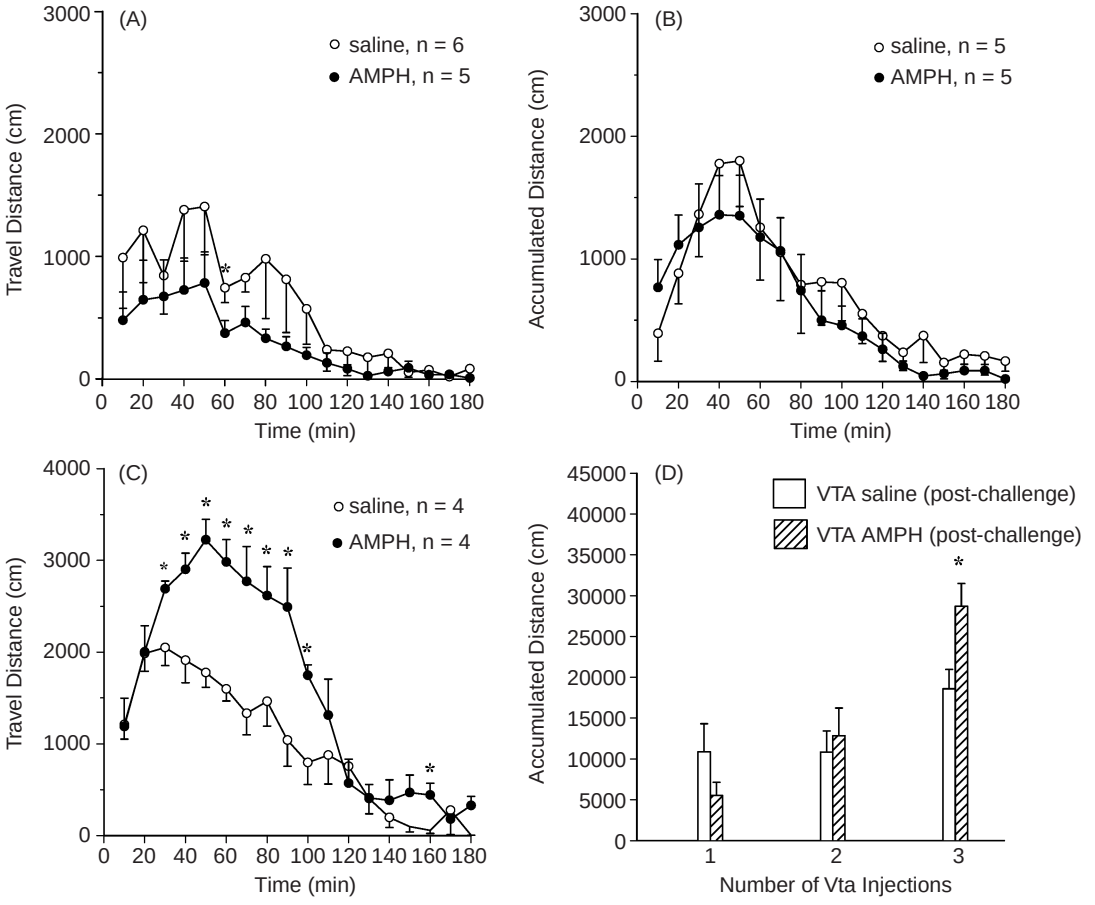

Fig. 3. Summary of the effects of various numbers of intra-VTA injections with AMPH on travel distance in rats post a challenge injection of AMPH (1.0 mg/kg, i.p.) after a 7-day withdrawal period. Panel (A), (B), and (C) depict the profile of travel distance in the time course post a challenge injection on the 7th withdrawal day with 1, 2, and 3, times of intra-VTA injection, respectively. Panel (D) shows the total travel distance for the 180-min test period. Values are presented as mean ± S.E.M. The asterisk (*) indicates statistically significant difference between AMPH-pretreated and saline-pretreated animals ($P < 0.05$).

Figure 3A is the group of a single intrategmetnal AMPH injection, while Figure 3B is the group of a double intrategmetnal AMPH injection, and Figure 3C is the group of a triple intrategmental injection after a 7-day withdrawal period. Compared with the saline-injected controls, only the triple intrategmental AMPH injection group showed a significant increase in travel distance. Meanwhile, comparing the total distance during the entire 180 min period between the saline-control and the treatment groups (Fig. 3D), only the triple intrategmental injection group exhibited a significant difference (18591±2380 *vs*. 28708±2783).

Figures 4A, 4B, and 4C show the changes in stereotypical movements *vs*. time lapse during the 180 min recording period for both the control and treatment groups. Figure 4A is the single intrategmental AMPH injection group, Figure 4B is the double intrategmental AMPH injection group, and Figure 4C is the triple intrategmental injection group after a 7-day withdrawal period. Comparing the total accumulated stereotypic movements during the entire 180 min period between the saline-control and the treatment groups (Figure 4D), again, only the triple intrategmental AMPH injection group exhibited a significant difference (3829±217 *vs*. 6929±1103).

Discussion

This study demonstrated that both single and well as repeated intra-VTA injection of AMPH induced behavioral sensitization. The results clearly indicated that a single intra-VTA injection with AMPH was sufficient in eliciting enhanced locomotor responses. Noteworthily, after single intrategmental injection, the increased motor activity appeared transient. Our data showed that the amplitude of sensitized behavioral manifestation tended to escalate as the exposure of VTA to AMPH increased, especially for the locomotor travel distance. Together, the above results provided further support to the

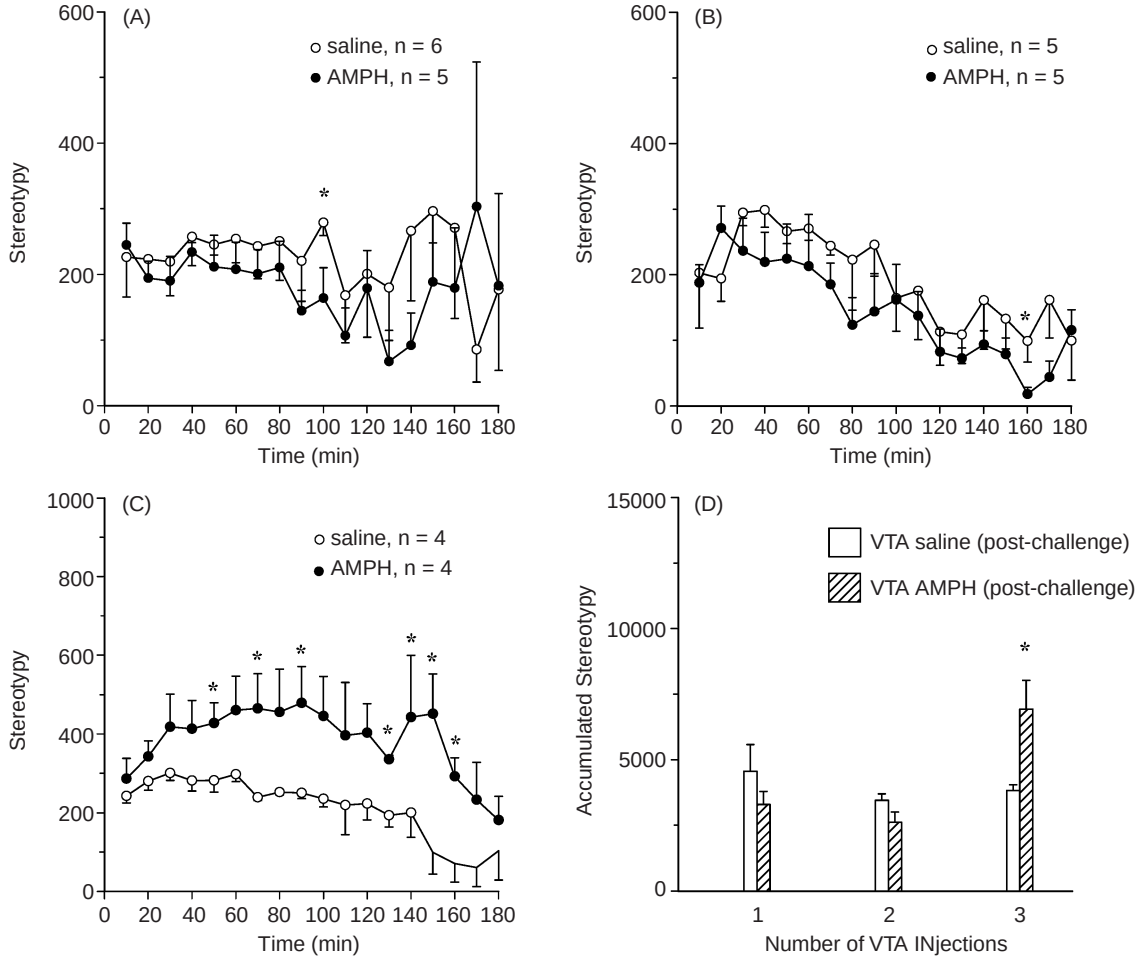

Fig. 4. Summary of the effects of various numbers of intra-VTA injections with AMPH on stereotypic movements in rats post a challenge injection of AMPH (1.0 mg/kg, i.p.) after a 7-day withdrawal period. Panel (A), (B), and (C) show the time course of the number of stereotypic movements post a challenge injection. Panel (D) shows the total number of stereotypic movements for each 180-min test period post a challenge injection. Values are presented as mean ± S.E.M. The asterisk (*) indicates statistically significant difference between AMPH-pretreated and saline-pretreated animals ($P < 0.05$).

notion that VTA is the action site responsible for initiating the sensitized locomotor responses, which may be augmented with more exposure of the VTA to the challenge psychostimulant.

Behavioral sensitization has been reported to be induced by a single exposure to systemic psychostimulant administration, such as amphetamine or cocaine (17, 19, 23). The enhanced locomotor activities resulted from one single exposure to systemic AMPH administration were evident at 7 days (19) and even more robust at 3 weeks than a 1-week or a 3-day withdrawal period upon challenge dose (23). Our data illustrated that the sensitized locomotor response caused by one single intra-VTA injection of AMPH was evident only at a 3-day, but not at a 1, 5 or 7-day withdrawal period (Fig. 1D & 3D). Thus, these results suggested that transient behavioral sensitization can be elicited *via* just one single local, intra-VTA, exposure to AMPH. We argued that systemic administration of a psychostimulant "showered" the whole mesocorticolimbic pathway, including the area of dopaminergic cell body (VTA) and the dopaminergic terminals (e.g., medial prefrontal cortex and nucleus accumbens). However, the response of dopaminergic neurotransmission at mPFC is not the same in correspondance to systemic (decreased) (23) *vs*. intra-VTA (increased) (13) exposure to AMPH. Besides, prefrontal cortex treatments of AMPH attenuate the sensitized locomotor effects of systemic AMPH that developed over days (2). We speculated that the earlier appearance of sensitized locomotor activity *via* intra-VTA injection (this study), as compared to those produced *via* systemic administration of AMPH (19, 23), might be due to the lack of an initial opposing effect of AMPH on mPFC, which in turn boots up the behavioral sensitization. The data profile of

sensitized stereotypy to one single intra-VTA AMPH injection in our study was compatible with this pattern.

Another major finding of this study was that repeated intra-VTA administration of AMPH escalated the amplitude of sensitized locomotor activity. Our results showed that both the peak amplitude and accumulated amount of travel distance in the repeated treatment group turned out to be more robust than those in the single treament group, although the change of magnitude of stereotypy are not as sensitive as the travel distance. This implied that the perikarya in VTA (most likely dopaminergic) might be sensitized and hence the VTA might play a significant role in the development of behavioral sensitization after multiple exposure to AMPH.

We further argued that there might be respective phases in the progressive development of behavioral sensitization, i.e., the one initially developed and the other developed in a later phase. Neurochemical data derived from repeated systemic AMPH administration (6) also reveal different profiles of extracellular DA levels in different phases of the development of sensitization. Imperato *et al*. reported that during the repeated systemic AMPH treatments, an increase of DA efflux (day 1 & 3) in the ventral striatum was first observed, then latter but persistent reduced levels of extracellular DA were found (day 5 & thereafter). The various phases in the time course of development of behaviroal sensitization may partly explain the inconsistent findings that there were different profiles (i.e., decreased *vs*. increased) of extracellular DA level during behavioral sensitization to repeated AMPH treatments (5, 14, 21, 22).

The observation that behavioral sensitization could be induced by just one single exposure to AMPH, either *via* local (e.g., intra-VTA) or systemic route, arouses clinical and research attention. Although transient, the neuroadaptations occurring in this initial stage may undermine subsequent sustained neuroplastic changes, which then lead to enduring sensitized neurobiological and behavioral responses enhancing the development of addiction to psychostimulants.

Acknowledgements

This study was supported in part by research grants NSC 92-2314-B-109-002 to SK Lin from the National Science Council, Taipei, Taiwan, R.O.C.

References

1. Akimoto, K., Hamamura, T., Kazahaya, Y., Akiyama, K. and Otsuki, S. Enhanced extracellular dopamine level may be the fundamental neuropharmacological basis of crossbehavioral sensitization between methamphetamine and cocaine—an *in vivo* dialysis study in freely moving rats. *Brain Res*. 507: 344-346, 1990.
2. Ben-Shahar, O. and Ettenberg, A. Amphetamine infusions into the prefrontal cortex attenuate the sensitization to amphetamine. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 22: 763-773, 1998.
3. Dougherty, G. G. and Ellinwood, E.H.Jr. Chronic D-amphetamine in nucleus accumbens: lack of tolerance or reverse tolerance of locomotor activity. *Life Sci*. 28: 2295-2298, 1981.
4. Hamamura, T. and Fibiger, H. C. Enhanced stress-induced dopamine release in the prefrontal cortex of amphetamine-sensitized rats. *Eur. J. Pharmacol*. 237: 65-71, 1993.
5. Hurd, Y.L., Lindefors, N., Brodin, E., Brene, S., Persson, H., Ungerstedt, U. and Horfelt, T. Amphetamine regulation of mesolimbic dopamine cholecystokinin neurotransmission. *Brain Res*. 578: 317-326, 1992.
6. Imperato, A., Obinu, M.C., Carta, G., Mascia, M.S., Casu, M.A. and Gessa, G.L. Reduction of dopamine release and synthesis by repeated amphetamine treatment: Role in behavioral sensitization. *Eur. J. Pharmacol*. 317: 231- 237, 1996.
7. Kalivas, P.W. and Duffy, P. Time course of extracellular dopamine and behavioral sensitization to cocaine I – Dopamine axon terminals. *J. Neurosci*. 13: 266-275, 1993a.
8. Kalivas, P.W. and Duffy, P. Time course of extracellular dopamine and behavioral sensitization to cocaine II – Dopamine perikarya. *J. Neurosci*. 13: 276-284, 1993b.
9. Kalivas, P.W. and Stewart, J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res. Rev*. 16: 223- 244, 1991.
10. Kalivas, P.W. and Weber, B. Amphetamine injection into the ventral mesencephalon sensitizes rats to peripheral amphetamine and cocaine. *J. Pharmacol. Exp. Ther*. 245: 1095-1102, 1988.
11. Kolta, M.G., Shreve, P., Souza, V.D. and Uretsky, N.J. Time course of the development of the enhanced behavioral and biochemical responses to amphetamine after pretreatment with amphetamine. *Neuropharmacology* 24: 823-829, 1985.
12. Pan, W.H.T., Lee, C.R. and Lim, L.H. A new video path analyzer to monitor travel distance, rearing, and stereotypic movement of rats. *J. Neurosci. Meth*. 70: 39-43, 1996a.
13. Pan, W.H.T., Sung, J.C. and Fuh, S.M.R. Locally application of amphetamine into the ventral tegmental area enhances dopamine release in the nucleus accumbens and the medial prefrontal cortex through noradrenergic neurotransmission. *J. Pharmacol. Exp. Ther*. 278: 725-731, 1996b.
14. Patrick, S.L., Thompson, T.L., Walker, J.M. and Patrick, R.L. Concomitant sensitization of amphetamine-induced behavioral stimulation and *in vivo* dopamine release from rat caudate nucleus. *Brain Res.* 538: 343-346, 1991.
15. Paulson, P.E. and Robinson, T.E. Sensitization to systemic amphetamine produces an enhanced locomotor response to a subsequent intra-accumbens amphetamine challenge in rats. *Psychopharmacology* 104: 140-141, 1991.
16. Paulson, P.E. and Robinson, T.E. Amphetamine-induced time-dependent sensitization of dopamine neurotransmisson in the dorsal and ventral striatum:a microdialysis study in behaving rats. *Synapse* 19: 56-65, 1995.
17. Peris, J. and Zahniser, N.R. One injection of cocaine produces a long-lasting increase in [3H]-dopamine release. *Pharmacol. Biochem. Behav*. 27: 533-535, 1987.
18. Perugini, M. and Vezina P. Amphetamine administered to the ventral tegmental area sensitizes rats to the locomotor effects of nucleus accumbens amphetamine. *J. Pharmacol. Exp. Ther.* 270: 690-696, 1994.
19. Robinson, T.E., Becker, J.B. and Presty, S.K. Long-term facilitation of amphetamine- induced rotational behavior and striatal dopamine release produced by a single exposure to

amphetamine: sex differences. *Brain Res.* 253: 231-241, 1982.
20. Robinson, T.E. and Becker, J.B. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res. Rev.* 11: 157-198, 1986.
21. Robinson, T.E., Jurson, P.A., Bennett, J.A. and Bentgen K.M. Persistent sensitization of dopamine neuro-transmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)-amphetamine: A microdialysis study in freely moving rats. *Brain Res.* 462: 211-222, 1988.
22. Segal, D.S. and Kuczenski, R. *In vivo* microdialysis reveals a diminished amphetamine- induced DA response corresponding to behavioral sensitization produced by repeated amphetamine pretreatment. *Brain Res.* 571: 330-337, 1992.
23. Vanderschuren, L.J.M.J., Schmidt, E.D., De Vries, T.J., Van Moorsel, C.A.P., Tilders, F.J.H. and Schoffelmeer, A.N.M. A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. *J. Neurosci.* 19: 9579-9586, 1999.
24. Vezina, P. Amphetamine injected into the ventral tegmental area sensitizes the nucleus accumbens dopaminergic response to systemic amphetamine: an *in vivo* microdialysis study in the rat. *Brain Res.* 605: 332-337, 1993.
26. Wolf, M.E., White, F.J., Nassar, R., Brooderson, R.J. and Khansa, M. R. Differential development of autoreceper subsensitivity and enhanced dopamine release during amphetamine sensitization. *J. Pharmacol. Exp. Ther.* 264: 249-255, 1993.