

BEHAVIORAL SENSITIVITY TO AMPHETAMINE OR SCOPOLAMINE AFTER ACUTE ADMINISTRATION OF NICOTINE IN THE RAT

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Abstract

Objectives: Earlier experiments have revealed that a single pretreatment of the rat with chlorphenvinphos (CVP) at a subtoxic dose (1.0 mg/kg, about 1/10 of DL50) makes the animal hyposensitive to the locomotor stimulating effect of amphetamine (AMPH) or scopolamine (SCOP) given three weeks postexposure. Such a hyposensitivity did not develop after a single or multiple (at short intervals) dosing with oxotremorine (OXO), a direct muscarinic agonist, which suggests that it was not mediated by muscarinic receptors. The purpose of the present experiment was to find out whether activation of nicotinic receptors could induce behavioral hyposensitivity to AMPH. **Materials and Methods:** Male adult Wistar rats were pretreated once with 0.00, 0.5 or 1.0 mg/kg of nicotine (NIC), a nicotinic agonist, and challenged 15 days later with 1.0 mg/kg of AMPH or 0.75 mg/kg of SCOP. The pre- and postdrug open-field behavior of the rats was measured using a computerized set of activity meters. **Results:** Pretreatment with NIC, like pretreatment with OXO, did not make the animals hyposensitive to AMPH or SCOP. In a preliminary experiment we have also found that rats do not develop hyposensitivity to AMPH and SCOP after pretreatment with physostigmine, a reversible anticholinesterase and a direct nicotinic and possibly muscarinic agonist. **Conclusions:** The results suggest that a transient overstimulation of the cholinergic system cannot be the cause or a sufficient condition for the development of the CVP-induced diminution of sensitivity to AMPH and SCOP.

Key words:

Nicotine, Behavioral sensitization, Amphetamine, Scopolamine, Open field, Rat

INTRODUCTION

Organophosphorous (OP) compounds, irreversible inhibitors of acetylcholinesterase (AChE), are in broad use (pesticides, medicines, lubricants) and OP exposures are not uncommon [1,2]. The acute effects of OP intoxication are well recognized. Less is known about the nature of the long-term consequences of OP exposure, especially those involving the central nervous system; complaints suggesting disturbances in emotional (emotional lability, anxiety, depressive states) and cognitive (impaired memory) func-

tions are frequently reported by patients with the history of OP exposure [3–8]. In some earlier studies [9,10] we found that pretreatment of the rat with chlorphenvinphos (CVP), an OP pesticide, at a single i.p. dose (1.0 mg/kg 1/10 DL50), made the animal hyposensitive to the behavioral effect of amphetamine (AMPH), an indirect dopaminergic agonist, or scopolamine (SCOP), a direct cholinergic (muscarinic) antagonist. This effect was detected three weeks after the CVP exposure. These observations deserve attention because of at least two reasons. First, the pesticide dose was relatively low; at the 1.0 mg/kg CVP

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dose, the AChE activity in blood and in the brain is inhibited by about 50%, and a decrease in spontaneous locomotor activity is the only overt symptom of intoxication. Such low-level exposures are rather frequent among agricultural workers using OPs [2]. Second, the three-week exposure-test interval was sufficient for restitution of AChE activity in blood and in the brain [11], suggesting that the observed changes were protracted and possibly persistent.

A diminished sensitivity to dopaminergic agonists and to cholinergic antagonists is known to characterize rats with inborn cholinergic supersensitivity. It is worth noting that these rats were proposed as an animal model of human depression [12]. Thus, our observations suggested that a similar trait might develop as a result of exposure to CVP. The most likely trigger of this state could be the CVP-induced episode of cholinergic hyperexcitation due to AChE inhibition. Recently, we have undertaken investigations aimed at establishing which class of cholinergic receptors, muscarinic or cholinergic, could be responsible for the exposure-induced long-term decrease in behavioral sensitivity to AMPH and SCOP. To date we have found that neither single, nor repeated i.p. administration of oxotremorine (OX), a direct muscarinic agonist, induces hyposensitivity to AMPH. To the contrary, the OX pretreated rats developed an increased sensitivity to the psychostimulant [13,14] and to SCOP [15]. The purpose of the present experiment was to find out whether and if so, what changes may develop in the rat sensitivity to AMPH and SCOP after a single exposure to nicotine (NIC), a nonselective agonist of cholinergic nicotinic receptors. At first glance, doing so may look unreasonable. NIC is an addictive substance. In the rat, treatment with NIC may increase locomotor activity and its repeated administration is known to sensitize the animal to the locomotor stimulating effect of AMPH [16]. The NIC locomotor stimulating effect, however, develops in the course of repeated injections. First injection, like injection of CVP, suppresses spontaneous locomotion and elicits signs of discomfort [17]. Thus, it cannot be ruled out that alterations in behavioral sensitivity induced by an acute (single) treatment with NIC will resemble those produced by CVP. A preliminary test of the effect of physostigmine, a reversible carbamate anti-

cholinesterase, but also a direct nicotinic [18] and possibly muscarinic [19] agonist, was also performed.

MATERIALS AND METHODS

Animals

Male Wistar rats, outbreeds, from the breeding colony of the Nofer Institute of Occupational Medicine were used in the experiment. The rats were about 4 months old and weighed 310–350 g at the experiment onset. For two weeks prior to the start of the experiment and during the experiment, they were housed in single rat cages at 22°C, with a light/dark cycle of 12/12 h (light on at 06.00). Standard rat food pellets (Murigran) and tap water were accessible *ad libitum*.

Chemicals

The following chemicals were used for the experiment: nicotine (nicotine hydrogen tartrate – NIC), physostigmine (eserine – PHYS), amphetamine (d-amphetamine sulfate, AMPH), scopolamine (scopolamine hydrobromide – SCOP), and physiological saline (0.9% natrium chloratum – SAL). NIC, AMPH, and SCOP were purchased from SIGMA, PHYS from Fluka, and SAL from POLFA. Before use, NIC, PHYS, AMPH and SCOP salts were dissolved in bidistilled water to appropriate concentrations. The NIC solution was administered subcutaneously. The PHYS, AMPH, and SCOP solutions were administered intraperitoneally. The doses refer to the salt form of the drugs.

Exposure to cholinomimetics

The rats were injected with NIC or PHYS in the animal room. Immediately after the injection the animal was placed again in its home cage where it remained undisturbed, except for routine bedding and cage changes, until the test with pharmacological challenge.

Apparatus and the basic test procedure

The rat motoric activity was assessed with the use of a computerized 4-unit set of activity cages (63 • 63 • 40 cm) purchased from PROFEX Ltd, Białystok, Poland. The set was

located in a room, neighboring the animal rooms, and illuminated with white luminescence bulbs located at the ceiling. The ambient temperature and humidity inside the test room were the same as in the animal rooms. Each activity cage was equipped with 2 tiers of infrared motion sensors and a calculating system which transformed the beam interruptions into location of the animal within the cage 5 times/s. A state with no beam interruptions for at least 1 s was classified as "rest". For measurement of the motoric activity, the rat was transferred (in its home cage) from the animal room to the test room and placed in the activity cage for a predetermined period of time. Horizontal shifts of the rat's body equal or longer than 4 cm were regarded as ambulatory movements, and those shorter than 4 cm as non-ambulatory (short-distance) movements. Interruption of at least one beam of the upper tier of sensors was counted as a rearing episode.

The measurements were performed between 07:00 and 15:00. At first, the rats were adapted to activity cages 1 h/day for two successive days. Then three measurement sessions, denoted as 0, 1 and 2, were performed. Each measurement session consisted of two 50 min measurements, predrug and postdrug. After the predrug measurement, the rat was transferred to its home cage standing on the rack nearby and the activity cage was thoroughly cleaned. Then the rat was injected and placed again in the activity cage for the postdrug measurement. The interval between predrug and postdrug measurements was 8–10 min.

The sequence in the experimental protocol was as follows: adaptation – measurement session 0 – exposure to a cholinomimetic – measurement session 1 – measurement session 2.

The measurement session 0 was performed 2–3 days after the last adaptation stay in the activity cage and 2–3 days before the exposure to NIC or PHYS. Sessions 1 and 2 were performed on postexposure days 15 and 17, respectively. In sessions 0 and 2, after completion of the predrug measurement, the rats were challenged with 1 ml/kg of SAL. In session 1, they were challenged with 1.0 mg/kg of AMPH or 0.75 mg/kg of SCOP.

Challenge doses of AMPH or SCOP were the same as those used in our previous studies [9,10].

Statistical analysis

The following indices of the rat behavior in activity cages were analyzed: the number of ambulatory (long-distance) movements (AM), the distance covered during ambulation (DIS), the number of nonambulatory short-distance movements (NAM), and the number of rearings (R). A two way ANOVA (groups • sessions) was employed for statistical evaluation of the data. In cases of a significant interaction effect, it was followed by one-way ANOVA and Tukey's test for pairwise comparisons [20]. Differences were considered significant when the probability of the null hypothesis was 5% or less.

Experiment 1. Behavioral responsiveness to amphetamine or scopolamine after a single exposure to nicotine

Experimental groups and procedure. The experiment was composed of two parts: the amphetamine part and the scopolamine part. For each part three groups of rats ($n = 8$ in each group) were used: the control (C) group and two groups of rats injected with nicotine at a dose of 0.5 mg/kg (group NIC-0.5) or 1.0 mg/kg (group NIC-1.0). The solutions, NIC in the nicotine groups and SAL in the C group, were administered subcutaneously. The administration route and NIC doses were established in a pilot experiment. Subcutaneous administration at a dose of 1.0 mg/kg produced, with latency of several minutes, symptoms suggestive of fear: a decrease in spontaneous locomotion, frequent urination and defecation, subsiding within 25–30 min. Symptoms produced by the 0.5 mg/kg dose were similar, but less pronounced. The tests with AMPH or SCOP (session 1), and SAL (session 2) challenges were performed on days 15 and 17, respectively, after NIC exposure.

RESULTS

Response to the amphetamine challenge

Ambulation distance. The mean predrug DIS value varied from 31.23 to 34.96 m in session 0 and decreased in successive sessions (Fig. 1A). In sessions 0 and 2 (with SAL), the postdrug DIS counts were by 10 to 50% smaller compared



to the predrug ones, but in session 1 (with AMPH) they were about three times higher. The groups did not differ with respect to the direct DIS values in session 1 (Fig. 1B). However, there were significant differences in compari-

sons with indirect values; the effect of the psychostimulant was significantly stronger in the NIC-1.0 group than in the remaining groups (Fig. 1C). This was due to a very low predrug DIS value in this group.

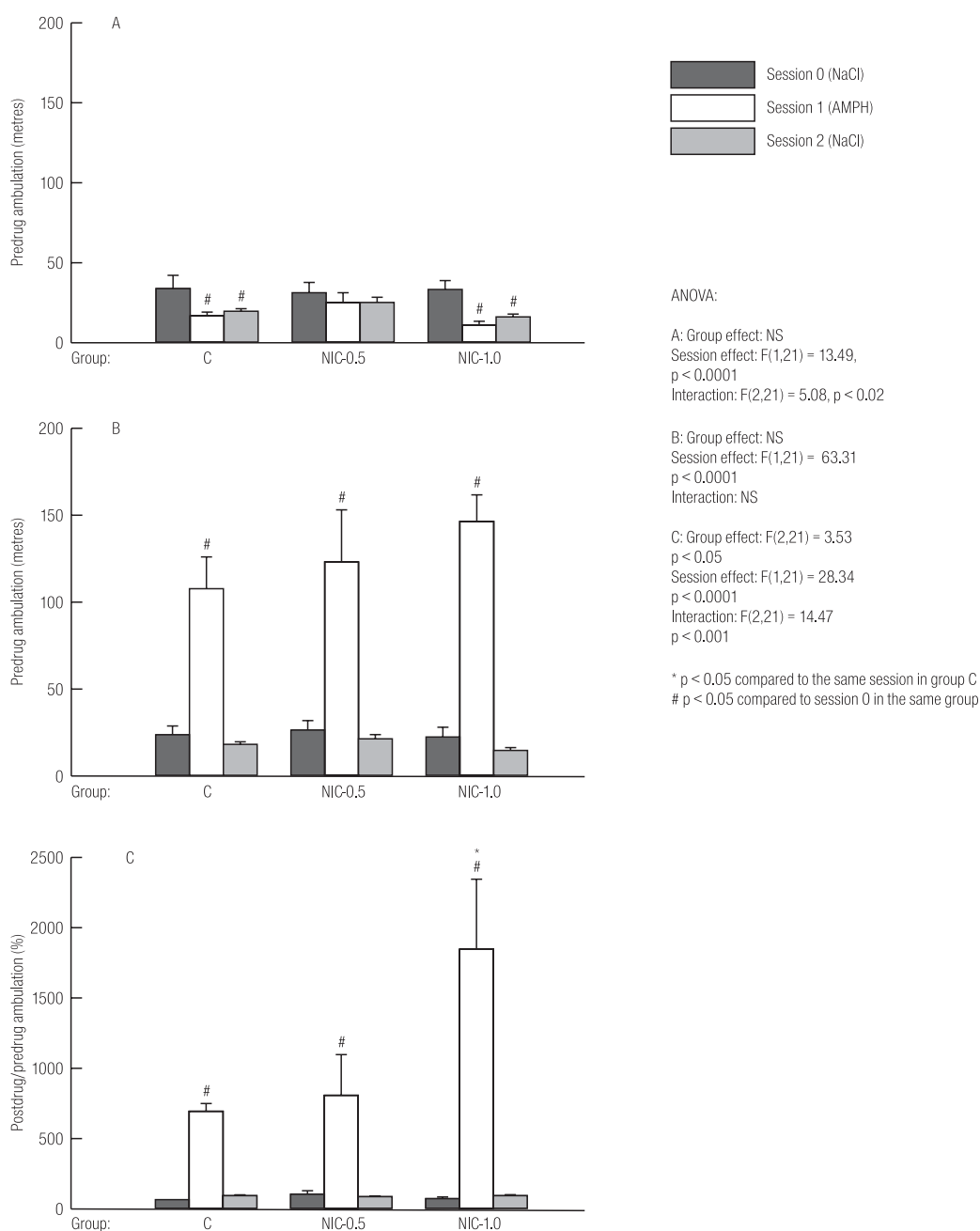


Fig. 1. The behavioral response to amphetamine challenge after treatment with nicotine. Ambulation distance. Nicotine was administered s.c. once at a dose of 0.0 mg/kg (group C), 0.5 mg/kg (group NIC-0.5), or 1.0 mg/kg (group NIC-1.0). Behavior was monitored for 50 min preceding and 50 min following i.p. injection of a test substance: 1.0 ml/kg of physiological saline in session 0 (before the treatment), 1.0 mg/kg of amphetamine in session 1 (on day 14 after the treatment), and 1.0 ml/kg of physiological saline in session 2 (on day 15 after the treatment). A – total distance covered in the open-field during the 50-min measurement preceding the injection of the test substance. B – distance covered during the 50-min measurement following the injection. C – relative postinjection distance in % $[(B/A) \cdot 100]$. The bars represent means and SEM.

Ambulation number

Analysis of the AM number produced similar results as that of the DIS values (data not shown).

Rearings (vertical activity)

The predrug R values like the predrug DIS values decreased in successive sessions. The postdrug R measurements leave no doubt as to a stimulating effect of AMPH on this form

of the rat's behavior (significant effect of the session factor). Pretreatment with NIC had no effect on the response to the AMPH challenge, which is suggested by the insignificant group • session interaction (data not shown).

Short-distance nonambulatory movements

Analysis of the NAM values, both direct and indirect ones, revealed no significant differences (data not shown).

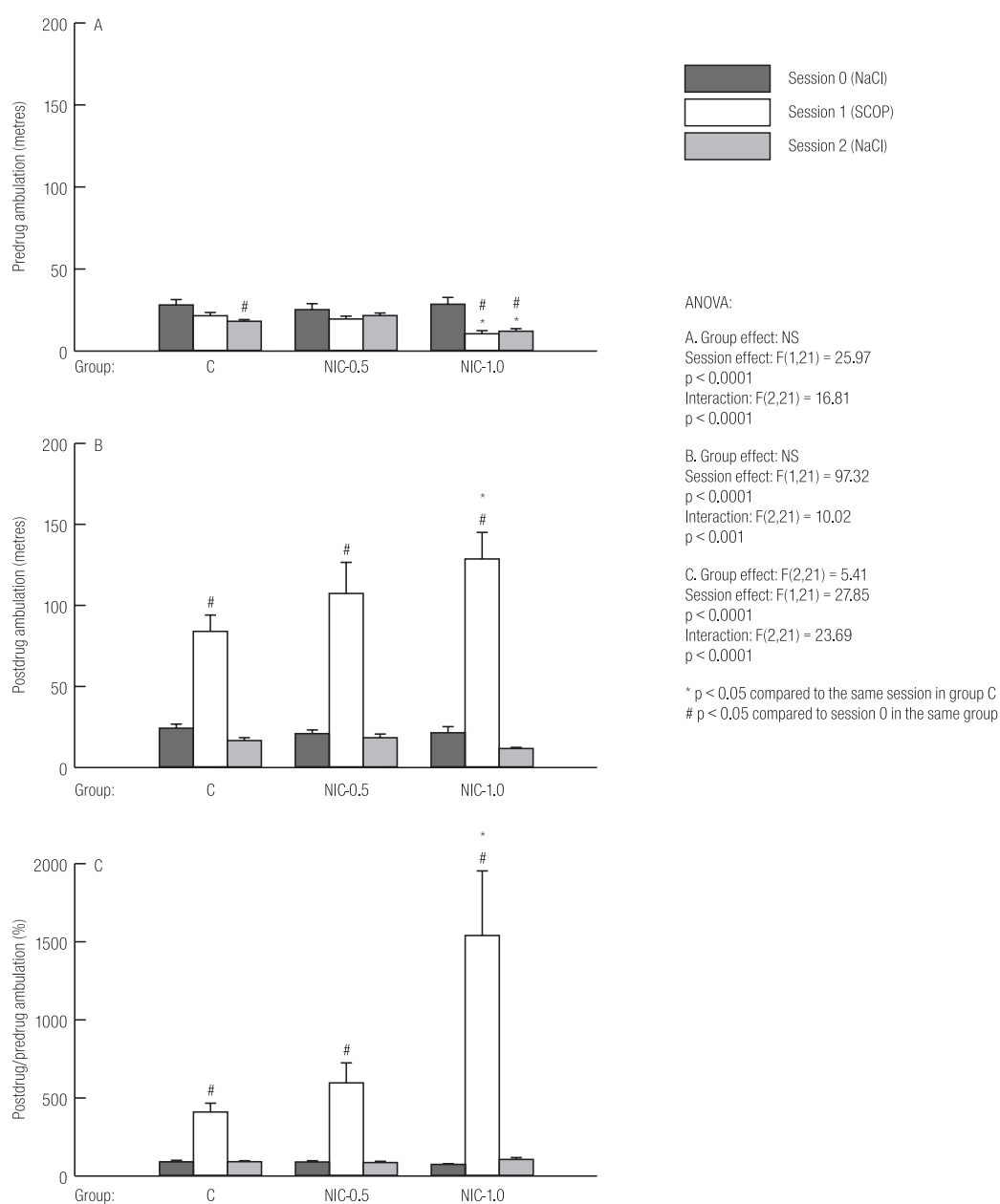


Fig. 2. The behavioral response to scopolamine challenge (0.75 mg/kg, i.p.) after treatment with nicotine. Ambulation distance. Remaining descriptions are as in Fig. 1.

Effect of NIC pretreatment on the response to SCOP challenge

Ambulation distance and ambulation number

In the SCOP experiment, the effects of the cholinolytic on the locomotor activity resembled those produced by AMPH. In sessions 1 and 2, the predrug DIS values were

significantly lower in group NIC-1.0 than in group C (Fig. 2A), indicating a persistent suppression of this form of behavior by NIC. In all groups, administration of SCOP in session 1 resulted in an increased locomotion. In group NIC-1.0, however, both direct and indirect ambulation values (DIS and NAM) were significantly higher than in

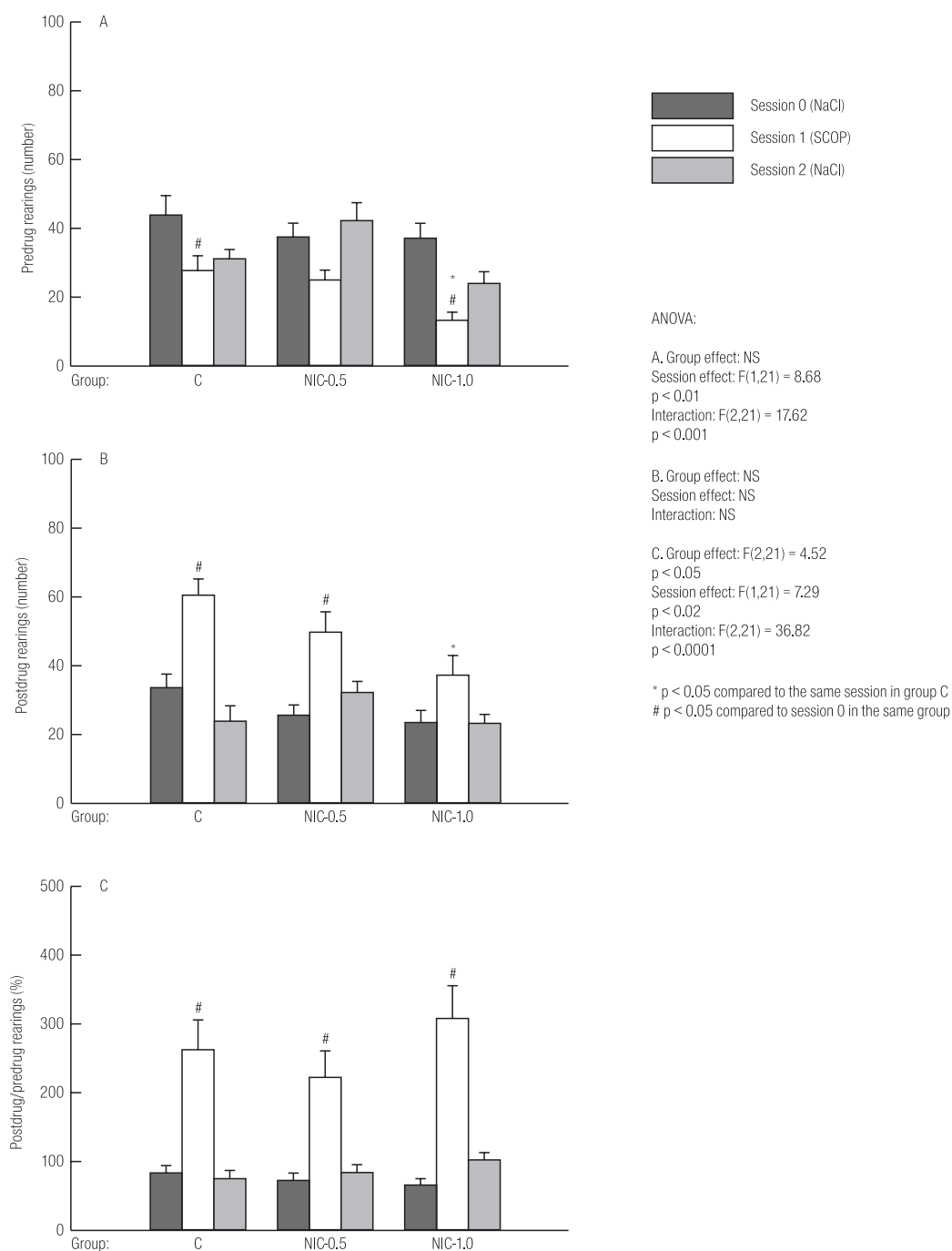


Fig. 3. The behavioral response to scopolamine challenge (0.75 mg/kg, i.p.) after treatment with nicotine. Rearing. Remaining descriptions are as in Fig. 1.

group C (Fig. 2 B and C). This suggests an increased behavioral sensitivity to SCOP after exposure to 1.0 mg/kg of NIC (The NAM data are not shown).

Rearings (vertical activity)

In all groups, the predrug R values were significantly lower in session 1 than in session 0 and this decrease was

particularly well evident in group NIC-1.0 (Fig. 3A). This suggests an enduring suppressive effect of the NIC exposure on the exploratory activity. Administration of SCOP in session 1 resulted in an increase in the vertical activity in all groups, but the postdrug R values were significantly increased only in groups C and NIC-0.5. Moreover, in session 1, the direct R value was significantly lower in group

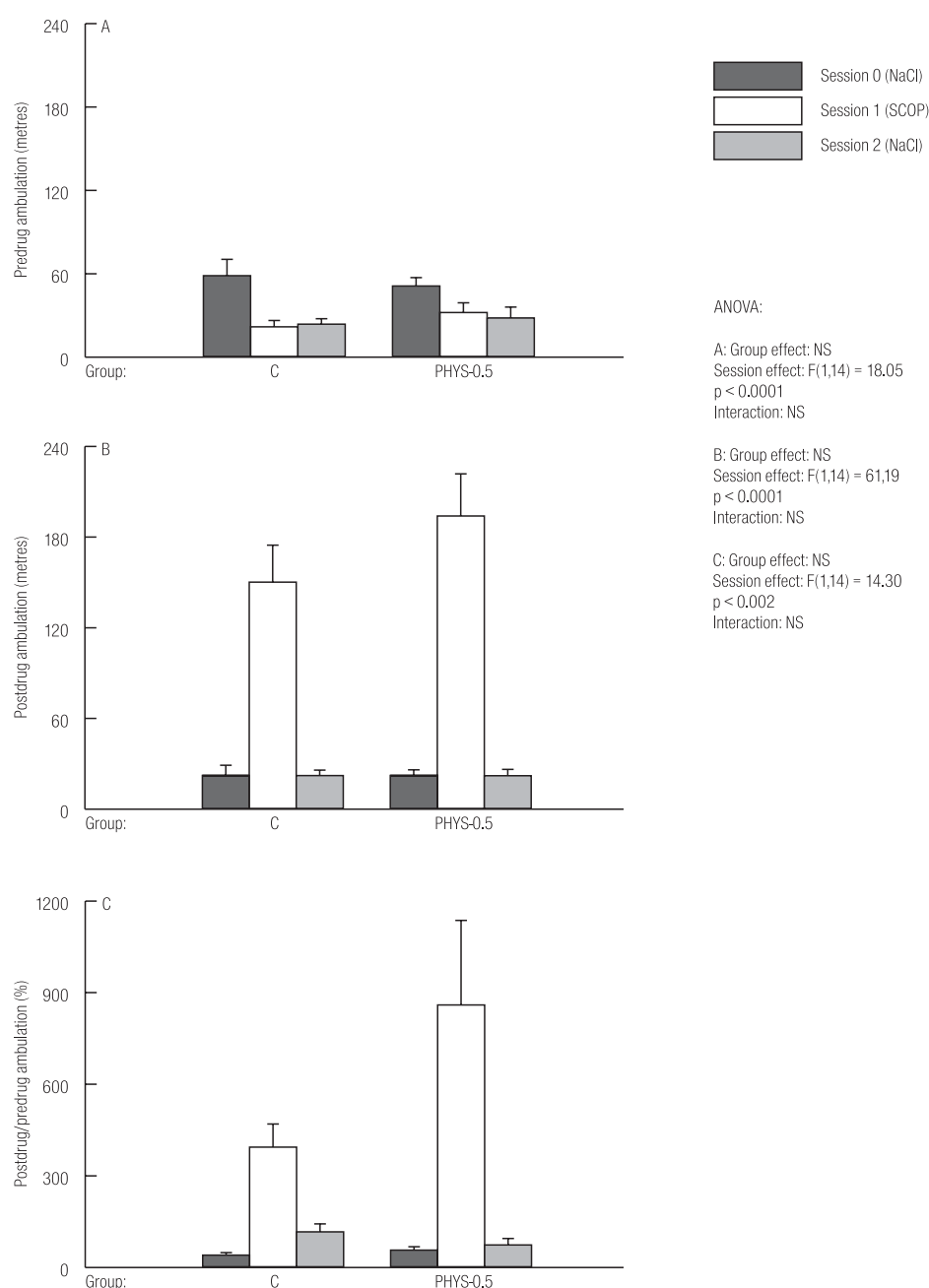


Fig. 4. The behavioral response to amphetamine challenge after a single i.p. treatment with physostigmine at a dose of 0.5 mg/kg (Group PHYS-0.5) or 1.0 ml/kg of physiological saline (group C). Ambulation distance. Remaining descriptions are as in Fig. 1.

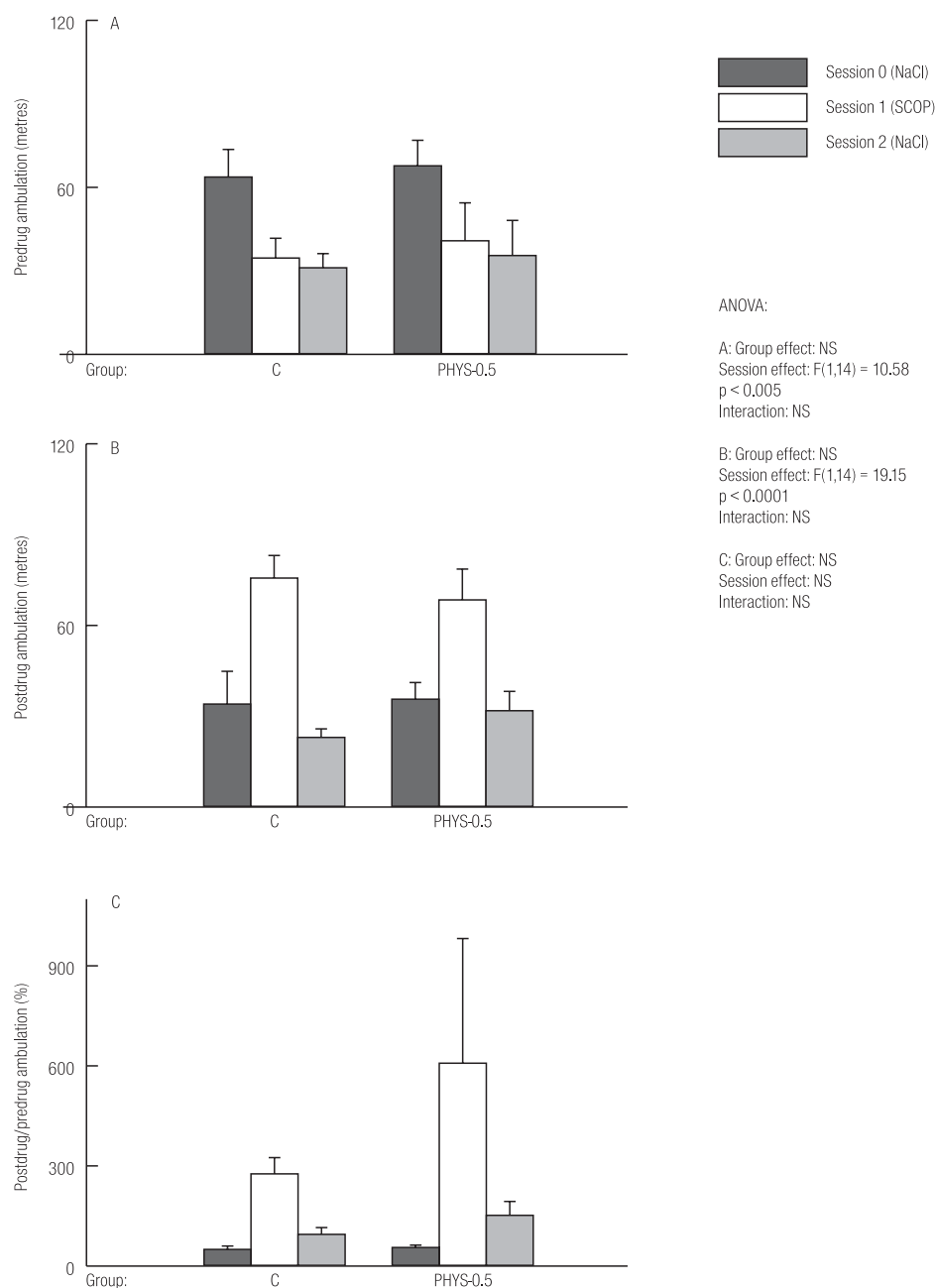


Fig. 5. The behavioral response to scopolamine challenge (0.75 mg/kg, i.p.) after a single i.p. treatment with physostigmine at a dose of 0.5 mg/kg (Group PHYS-0.5) or 1.0 ml/kg of physiological saline (group C). Ambulation distance. Remaining descriptions are as in Fig. 1.

NIC-1.0 than in group C (Fig. 3B). This indicates that in the case of SCOP, the drug-induced increase in locomotor activity went along with a decrease in exploratory (vertical) activity.

What concerns the indirect R values, differences between groups have been obliterated due to a very low predrug R value in group NIC-1.0 (Fig. 3C).

Nonambulatory, short distance movements

Analysis of this measurement revealed no reliable differences (data not shown).

Experiment 2. Response to amphetamine or scopolamine after pretreatment with physostigmine

Physostigmine is a reversible AChE inhibitor but also a direct agonist of nicotinic [18] and possibly muscarinic [19]

cholinergic receptors. Therefore, a change in response to the AMPH or SCOP challenge following PHYS administration may be considered as a consequence of a combined activation of nicotinic and muscarinic receptors. In experiment 2, the approach was the same as in experiment 1. Since we regarded the experiment with PHYS as a preliminary one, only one dose, 0.5 mg/kg, was tested. The acute behavioral symptoms induced by 0.5 mg/kg of PHYS (motor slowing, crouching, tremor, piloerection, salivation, frequent urination, and defecation) resembled in intensity those produced by 1.0 mg/kg of oxotremorine [13]. The rats were challenged with AMPH or SCOP on day 15 and with SAL on day 17 after the PHYS exposure. The results showed that in the PHYS pretreated rats the locomotor response to AMPH or SCOP was apparently stronger than in control rats (Figs. 4 and 5). Analysis of ambulation scores, however, revealed no significant differences between C group and the AMPH or SCOP challenged groups.

DISCUSSION AND CONCLUSIONS

The results of the present experiments show that pretreatment with NIC at a single dose, like pretreatment with OXO [13,14], induces hypersensitivity rather than hyposensitivity to the locomotor stimulating effect of AMPH or SCOP in the rat. This indicates that not only a repeated [16], but also a single dosing with NIC may sensitize the rat to AMPH. Like in the case of OXO, the effect is dose-dependent (only the highest dose was effective) being particularly well pronounced in comparisons of relative values. (It is related with the marked and persistent depression of spontaneous locomotor activity after administration of the cholinomimetic). Thus it appears that the effect of a single exposure to CVP, i.e. a decreased behavioral sensitivity to AMPH or SCOP, cannot be reproduced by exposure either to muscarinic or nicotinic agonist. The results of the preliminary experiment suggest that neither it can be reproduced by administering the rat with PHYS – an reversible AChE inhibitor. These observations by no means support the supposition that the decrease in behavioral sensitivity to AMPH and SCOP found after exposure

to CVP at a moderate dose was a consequence of a transient cholinergic hyperactivity. To the contrary, they suggest that an episode of cholinergic hyperactivity may result weeks later in an increased sensitivity to both AMPH and SCOP.

There is rather a common agreement that the AMPH-induced psychomotor activation results from excitation of the dopaminergic projection from the ventral tegmental area (VTA) to nucleus accumbens (NAcc) [21]. A number of reports makes it likely that the locomotor response to SCOP is mediated at least in part by the same system. The dopaminergic neurons in VTA and substantia nigra receive excitatory projection from mesopontine cholinergic neurons [22]. According to some authors, the SCOP-induced locomotion results from blocking muscarinic receptors (autoreceptors) on these neurons, which in turn, results in excitation of the dopaminergic VTA (and nigral) neurons [23,24]. The progressive and persisting augmentation of the locomotor response to AMPH (behavioral sensitization), which may be induced by a number of pharmacological and non-pharmacological agents, is being attributed to persistently enhanced responsiveness of the dopaminergic VTA neurons and/or neurons from other areas projecting to VTA [25]. According to the above, our previous and present results may indicate that a single exposure to OXO or NIC can suffice for the induction of such an enhancement. This is quite conceivable considering that cholinergic muscarinic and nicotinic excitation are both important determinants of the dopaminergic activity. Cholinergic terminals excite monosynaptically mesencephalic dopaminergic neurons (in VTA and s. nigra) through postsynaptic muscarinic receptors [22], and the dopamine release in the striatum is heavily dependent on nicotinic activation [26]. What is more, it has been recently shown that nicotinic activity is a *sine qua non* for induction of behavioral sensitization to NIC and other drugs [27].

Activation of the hypothalamo-pituitary-adrenal system (the HPA axis) might be another factor contributing to the OXO- or NIC-induced sensitization to AMPH. Both these drugs are known as powerful activators of the HPA axis [28–30]. Activation of the HPA axis results in a raise in plasma glucocorticoid level, and glucocorticoids are known



to exert a strong activatory influence on the dopaminergic system [31]. There are data indicating that the increase in the plasma corticosterone (CORT) level, the CORT response, is crucial for the induction of the behavioral sensitization to AMPH by a pharmacological or nonpharmacological “stressor”. It has been shown, for example, that blockade of the CORT response prevents sensitization development and that sensitization to AMPH may be induced by repeated CORT administration [32,33].

Whereas in the light of the above, the effect of exposure to OXO [14] and to NIC (present experiment), i.e. the increased response to AMPH (and SCOP) challenges, is understandable, the opposite effect of a single CVP exposure [9] becomes even more obscure. Considering that CVP, an AChE inhibitor, acts as an indirect muscarinic and nicotinic agonist, being also a powerful activator of the HPA axis [34], one may ask why the effect of CVP exposure on AMPH responsiveness does not resemble that produced by OXO or NIC. At least two likely causes may be pointed out. First, a possible difference in duration of direct effects on the cholinergic and HPA systems; those produced by CVP last longer. However, we have recently shown that even prolonged exposure to OXO (multiple injections at short intervals) does not make the rats hyposensitive to AMPH [14]. This makes the above difference rather an unlikely cause of the dissimilarity at least between the effect of CVP and OXO. Second, the effect of CVP on the sensitivity to AMPH and SCOP is not possibly mediated by the cholinergic system. This possibility is suggested by the results of our recent studies, in which the effects of a single exposure to CVP or another organophosphorus pesticide, chlorpyrifos (CPF), were compared. They have shown that exposure to CPF, contrary to CVP exposure, does not make the rat hyposensitive to AMPH or SCOP [35]. In fact, there are reports showing that the rat may become persistently hypersensitive to SCOP after CPF exposure [36]. This makes it likely that the CVP-induced hyposensitivity to AMPH and SCOP may not be directly related to AChE inhibition, and thus to transient cholinergic hyperactivity. This also suggests that cholinergic hyperactivity may not be the cause or a sufficient condition for the development of long-term alterations in the central nervous

system functional state reported in humans with history of OP exposure.

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