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Immunohistochemical and Behaviour Pharmacological Analysis of Rats Inoculated Intranasally with Vesicular Stomatitis Virus

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ABSTRACT

A temperature-sensitive mutant of vesicular stomatitis virus was inoculated intranasally into infant Sprague Dawley rats aged 9 to 17 days. Rats receiving the virus at 9 days of age had an extensive spread of infection throughout the brain and the animals died after a few days. Rats inoculated at day 11 postnatally survived and the infection was limited to the olfactory pathways, hypothalamus, diagonal bands and the anterior raphe nuclei. Stereological measurements showed that the volume of infected neurons constituted 67 ± 10% of the total neuronal volume in the dorsal raphe nucleus. Double-labelling experiments revealed that both 5-hydroxytryptamine- and substance P-immunoreactive neurons contained the virus antigen. The motor stimulant effect of amphetamine was studied at 3 months post infection. The increase in amphetamine-induced frequency and duration of rearing was significantly attenuated in infected rats and the amphetamine-induced locomotion was slightly reduced.

KEY WORDS: Virus infection 5-Hydroxytryptamine Immunohistochemistry Stereology Amphetamine Locomotion Rearing

INTRODUCTION

Certain neurotropic viruses can invade the brain along the olfactory route after an infection of the olfactory mucosa (for review see Johnson, 1982; Tomlinson and Esiri, 1983; Morales *et al.*, 1988; Barthold, 1988; Perlman *et al.*, 1989; Shankar *et al.*, 1992). Neurons in the diagonal bands, locus coeruleus and anterior raphe nuclei, which form part of the reticular core, project directly to the olfactory bulbs and can, via retrograde axonal transport, be labelled by tracers injected into the olfactory bulb (Shipley and Adamek, 1984) or even instilled into the nasal cavity (Shipley, 1985). These groups of neurons were also labelled by vesicular stomatitis virus (VSV) antigen following intranasal infection with the virus in mice (Lundh *et al.*, 1987). By using a temperature-sensitive mutant (G41) of VSV and the fact that olfactory spread of VSV is age-dependent (Sabin and Olitsky, 1937) a non-lethal infection involving these neurons was obtained in suckling mice (Lundh *et al.*, 1988). In order to perform correlative neurochemical and behavioural studies, infant rats were then infected and we have previously shown that intranasal instillation of the VSV mutant G41 results in a marked depletion of serotonin in the hippocampus and cerebral cortex with no substantial effects on dopamine, noradrenaline, choline acetyltransferase and glutamate decarboxylase (Mohammed *et al.*, 1990, 1991). Behavioural changes induced by the infection included increased motor activity. Both the serotonergic raphe neurons and the dopaminergic nigro-cortical neurons have been implicated in arousal and motor activation (Fibiger and Campbell, 1971; Lucot and Seiden, 1986; Mabry and Campbell, 1974). As a first step towards studying the behavioural pharmacology of VSV-induced hyperactivity, we examined the effect of amphetamine. This drug has been widely used as a tool to release monoaminergic transmitters from their axon terminals in studies on the effects of monoamines on motor activity (for review see Moore, 1974). Here we present a detailed account of the distribution of VSV antigen in the rat brain and report that VSV antigen is present in a majority of the anterior raphe neurons and that amphetamine-induced increase in duration and frequency of rearing was attenuated by VSV infection.

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MATERIALS AND METHODS

Virus infection

A temperature-sensitive mutant of VSV, designated G41, was used in all experiments. The characteristics of this mutant have been described previously (Rabinowitz *et al.*, 1976). The VSV strain was generously provided by Dr Mauro Dal Canto, Department of Pathology, Northwestern University, Illinois, USA. The mutants were grown in BHK_{21} cells and plaque titrated as described earlier (Lundh *et al.*, 1987, 1988). Ten μl of virus suspension were instilled twice into each nostril of suckling Sprague–Dawley rats (ALAB, Sollentuna, Sweden) ($n = 197$) using a micropipette, exposing each animal to about $3–6 \times 10^6$ plaque-forming units of the virus. Controls ($n = 77$) received an equal amount of the virus vehicle (Eagle's Minimal Essential Medium, Flow Laboratories, Scotland). The animals were kept under standard laboratory conditions, employing a 12/12 h light cycle.

In order to determine age-dependency of virus spread through the olfactory system, one to four litters, aged 9, 10, 11, 12, 13, 15 and 17 days, were used (in total 140 infected and 40 uninfected rats). The size of the litters was standardized immediately after birth to ten pups per litter, each consisting of five male and five female pups. The rats were weighed once a day during the whole observation period.

Histological and immunohistochemical analysis

Seventeen rats, inoculated with virus at 11 days of age, were perfused through the heart with 4% paraformaldehyde in Sörensens phosphate buffer (pH 7.2) at days 3, 5, 7, 9 and 11 post inoculation (p.i.). The brains were dissected, postfixed for 90 min, subsequently rinsed and kept in 10% sucrose for at least 24 h prior to cutting on a cryostat (Frigocut 2800E, Reichert-Jung, Germany). Four uninfected rats perfused at days 5 and 11 p.i. served as controls. Twelve μm thick sections, from more than 50 different levels of each brain, were stained with cresyl violet and mounted in Entellan (Merck, Germany). Viral antigen was demonstrated in adjacent sections by the peroxidase-antiperoxidase method (Sternberger, 1979) employing a sheep anti-VSV hyperimmune serum (kindly provided by Dr Jan Závada, Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia; for characterization and details of procedure see Lundh *et al.*, 1988). Sections were also processed for double-labelling with the sheep anti-VSV hyperimmune serum and antibodies raised in rabbits to 5-hydroxytryptamine (5HT) (Steinbusch, 1981; Steinbusch and Verhofstad, 1979) (generously provided by Dr Harry W. M. Steinbusch, Faculty of Medicine, Department of Pharmacology, Free University of Amsterdam, The Netherlands) or synthetic substance P (Brodin *et al.*, 1986). Incubation with the primary antisera at 4°C overnight was followed by a thorough rinse of the sections in 0.01 M-phosphate-buffered saline (PBS; pH 7.4). The sections were then incubated for 30 min at 37°C with tetramethylrhodamine isothiocyanate-labelled swine anti-rabbit antibodies (Dakopatts, Copenhagen, Denmark) and after thorough washing in PBS, with fluorescein isothiocyanate-labelled rabbit anti-sheep antibodies for 30 min. Finally, the slides were washed in PBS, mounted in PBS-buffered glycerine with 0.1% *p*-phenylene diamine (Johnson and de C. Nogueira Araujo, 1981) and examined in a Nikon Optiphot fluorescence microscope.

In order to improve visualization of substance P immunoreactivity in nerve cell bodies, three 11-day-old rats were infected and at day 7 p.i. injected stereotactically into the right lateral ventricle with 5 μl of colchicine (2 μg/μl; Sigma Chemical Company, St Louis, USA) dissolved in 0.9% saline. Two uninfected control rats were treated in the same way. Twenty-four hours later the rats were perfused and processed for immunohistochemistry using the double-labelling method described above.

Four animals, inoculated at 9 days of age, were perfused at day 5 p.i. and processed for demonstration of viral antigen as described above.

Stereology

In order to quantify the extent of virus infection in the raphe nuclei, the proportion of infected neurons in the dorsal raphe nucleus was determined by stereological measurements (see Weibel, 1979) in five infected rats and two controls, sampled at days 5 and 7 p.i. Firstly, the volume density (V_v) of the neurons in the dorsal raphe nucleus, i.e. their relative volume as compared to the total volume of the nucleus, was calculated in sections from six random levels of the dorsal raphe nucleus of each animal (infected and uninfected). This was done in order to evaluate if there was any cell loss in the raphe nuclei of the infected rats. Secondly, the volume density of the *infected* neurons, i.e. their relative volume as compared to the total volume of neurons, was determined. In addition, the absolute volume of the dorsal raphe nucleus was calculated. The volume of the infected neurons was calculated from micrographs of sections immunohistochemically labelled for virus antigen, whereas the total volume of nerve cells and the volume of the whole dorsal raphe nuclei was evaluated on adjacent cresyl violet-stained sections using a Reichert-Jung Visopan microscope.

Motor activity tests

Rats inoculated at 11 days of age were used for motor activity tests. This group consisted of 64 animals (26 males and 38 females), about half of which were infected. Motor activity was tested 3 months p.i., separately for males and females, using automated activity cages (Mohammed *et al.*, 1990). The

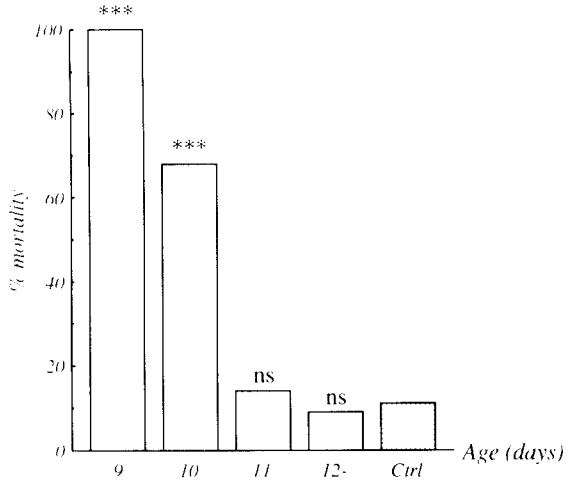

Fig. 1. After virus inoculation of 9-day-old rats, 100% of the animals died within 1 week, whereas 68% of the rats died when the inoculation was made at the age of 10 days. These figures differed significantly from uninfected control rats, where 11% had died after 2 weeks (9 days vs controls: $P<0.001$, Chi^2-test; 10 days vs controls: $P<0.001$, Chi^2-test). Inoculation at 11 days of age led to death in 14% of the animals (11 days vs controls: non-significant, Chi^2-test), and when 12- to 17-day-old rats were inoculated, 9% died within 2 weeks ($\geq$ 12 days vs controls: non-significant, Chi^2-test).

tests were performed between 9 a.m. and 2 p.m. These cages were equipped with two rows of photocells which registered both locomotion (horizontal activity) and rearing (vertical activity). Four activity cages were used at the same time. The animals were placed in the cages and the activity was registered every 10 min during 1 h. After a 1-h habituation period, half of the animals received a subcutaneous injection of 1 mg/kg *d*-amphetamine sulphate (Apoteksbolaget, Stockholm, Sweden), while the other half received a subcutaneous injection of saline. The rats were then placed in the activity cages, and the motor activity was registered every 10 min for a period of 90 min. The resulting data were analysed by a two-way analysis of variance (ANOVA), and the Tukey test was used for *post hoc* comparisons.

RESULTS

Clinical course of disease

All rats inoculated with virus at 9 days of age underwent a significant weight loss as compared to uninfected controls, and died within 1 week p.i. Rats inoculated at 10 days of age showed a mortality of 68% (Fig. 1), while those aged 11 days or more at the time of inoculation displayed a mortality rate not significantly higher than that of controls. The rats infected at 11 days of age did not exhibit any significant loss of body weight, with the exception of five rats (out of the 40 infected rats used in the age-dependency studies), which suffered from a severe weight loss of about 30–40% before death. At the time of the behavioural test, the mean weights of the female rats were 280 ± 5 g, and 277 ± 5 g for VSV-infected and uninfected, respectively. The corresponding figures for the males were 450 ± 11 g and 450 ± 10 g.

Virus spread

The studies of virus spread and motor activity were performed on the rats which showed no significant loss of body weight when infected at day 11 postnatally. Virus antigen was detected at day 3 p.i. in neurons in the anterior olfactory nucleus, the anterior part of the olfactory cortex, the horizontal and vertical limbs of the diagonal band, and scattered in the hypothalamus (anterior, dorsal, lateral and paraventricular). In the diagonal bands, an average of 55–70% of the neurons were immunoreactive for virus antigen. The horizontal band appeared to be somewhat more affected than the vertical band. The spread of virus at 5 days p.i. is depicted in Fig. 2. A large number of virus-positive neurons could then be seen in the central, median and dorsal raphe nuclei (Fig. 3A). No virus antigen could be seen in the nucleus raphe magnus, obscurus and pallidus. In the locus coeruleus only a few neurons, not exceeding 5%, were labelled. Virus antigen could not be detected in other nuclei of the brainstem, nor in the basal ganglia, the substantia nigra, the ventral tegmental area, the hippocampus or the neocortex. Rats examined at day 7 p.i. showed immunoreactivity for virus antigen in the brain, with a similar distribution pattern as seen at day 5 p.i. The amount of virus antigen was markedly reduced 9 days p.i., due to the cytocidal effects of this virus (Wagner, 1990). After 11 days, only a few cells containing antigen could be seen in these areas.

Three of the five rats infected at day 11 postnatally and displaying a severe weight loss, were examined and showed an extensive spread of virus in the brain. Thus, many clusters of infected neurons could be seen in cortical as well as in subcortical areas throughout the brain, including hippocampus and neocortex, and in the cerebellum. A similar spread of virus throughout the central nervous system was observed in four rats inoculated at 9 days of age which had survived until day 5 p.i. These rats also suffered from a severe weight loss.

Quantification of infected neurons

The volume of the entire dorsal raphe nucleus was about $1.8 \pm 0.1 \times 10^9$ μm^3 (mean $\pm$ SEM), and there was no significant difference between infected and control rats. The V_v of *infected* neurons (at days 5 and 7 p.i.) as compared to the total volume of neurons in the dorsal raphe nucleus was 0.67 ± 0.10 (mean $\pm$ SEM), thus representing 67% of the total cell volume. There was no significant difference between infected and control animals in the V_v of

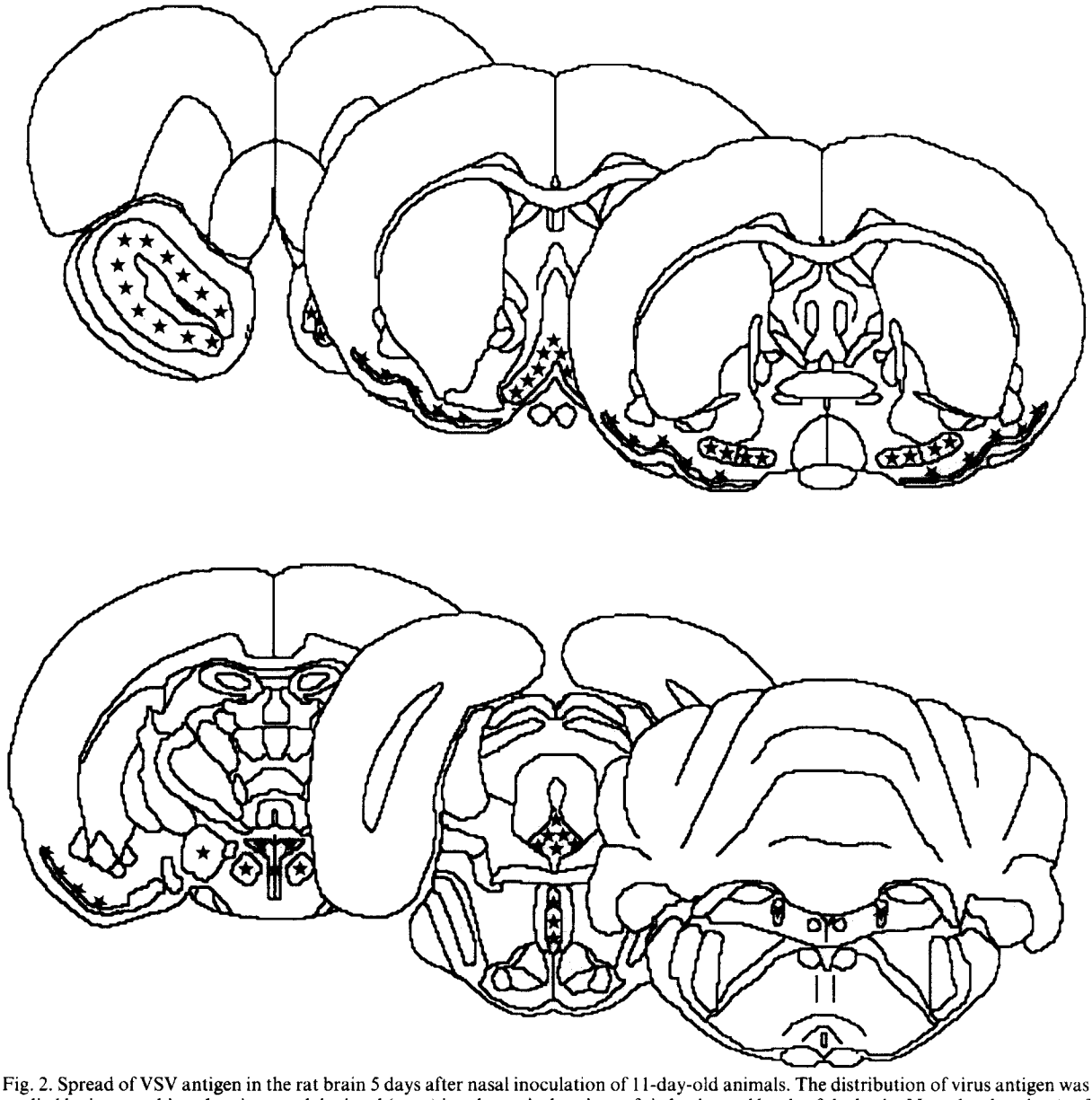

Fig. 2. Spread of VSV antigen in the rat brain 5 days after nasal inoculation of 11-day-old animals. The distribution of virus antigen was studied by immunohistochemistry and depicted (stars) in schematic drawings of six horizontal levels of the brain. Note the abundance of virus in the olfactory nucleus, diagonal bands and the anterior raphe nuclei.

the total number of neurons in the dorsal raphe nucleus, indicating that there was no major cell loss in the infected rats at days 5 and 7 p.i.

Virus infection in 5HT- and substance P-immunoreactive neurons

The majority of the neurons in the raphe nuclei were 5HT-positive (Fig. 3B). The substance P-immunoreactive cells could be seen after colchicine treatment, and were scattered in the raphe nuclei. Double-labelling (5HT/virus antigen, substance P/virus antigen) revealed the occurrence of virus antigen in most 5HT- (Fig. 3A, B) and substance P-immunoreactive neurons, respectively, in the raphe nuclei. A small proportion of the virus-positive neurons were 5HT-negative and some 5HT-immunoreactive neurons were virus-negative in double-stained sections, indicating that the observation of staining for virus proteins in 5HT-immunoreactive neurons was not due to 'fluorescence bleeding'.

Motor activity

Locomotion

Males: Locomotion counts are shown in Fig. 4A. Two-way ANOVA revealed a significant main effect of group ($P<0{\cdot}0001$), and a significant group × period interaction ($P=0{\cdot}0003$). Tukey test showed that the VSV-infected saline-injected animals had slightly higher locomotor scores than the uninfected saline-injected animals, but no significant differences between these two groups were

obtained. Nor did the amphetamine-treated groups differ significantly from each other. The two groups given amphetamine (infected and uninfected) had significantly higher locomotor scores than the groups that received saline, and the locomotor scores of the uninfected amphetamine-treated group were significantly higher than those of the uninfected saline-injected group at all the 10-min time periods, whereas the locomotor scores of the VSV-infected amphetamine-treated group differed significantly from the VSV-infected saline-injected group only at the 50-, 60- and 70-min periods.

Females: Fig. 4B shows the results on locomotion counts for the female rats. Two-way ANOVA showed a significant group effect ($P<0.0001$) and a significant group × period interaction effect ($P<0.0001$). Tukey test showed that there was no difference in locomotor scores between uninfected and infected female rats. Similar to the male rats, the locomotor scores of the amphetamine-treated groups (infected and uninfected) were significantly different from the saline-injected groups (infected and uninfected) at all time periods.

Rearing

Males: Results from the rearing frequency counts are shown in Fig. 5A. Two-way ANOVA indicated a significant group effect ($P<0.05$). Subsequent Tukey test showed that for uninfected rats, amphetamine-treated animals had significantly higher rearing scores than the saline-injected rats. In infected rats, there was no significant increase in rearing caused by amphetamine treatment.

There was a significant interaction effect also on rearing duration (Fig. 5B; $P<0.05$). *Post hoc* test showed that for the uninfected groups, amphetamine-treated animals had a significantly longer duration of rearing at 60, 70 and 80 min. After VSV infection, the amphetamine-treated animals did not differ significantly from the saline-treated ones.

Females: Fig. 6A shows the results on rearing frequency. Two-way ANOVA revealed a significant group effect ($P<0.0001$) and a significant group × period interaction effect ($P<0.0001$). Tukey test showed that the VSV-infected amphetamine-treated rats had significantly lower rearing scores than the uninfected amphetamine-treated rats at the 50-, 60-, 70-, 80- and 90-min periods. Similar to the male rats, there was a significant increase in rearing scores after amphetamine treatment in the female uninfected rats ($P<0.01$), and there was a smaller but significant increase in rearing scores in the infected rats ($P<0.05$). There was in fact a significant difference in rearing scores after amphetamine treatment between infected and uninfected female rats.

The results of rearing duration are shown in Fig. 6B. A significant group ($P<0.0001$) and interaction ($P<0.0001$) effect was seen. *Post hoc* test showed significant effects on rearing duration similar to those for rearing frequency.

DISCUSSION

The spread of VSV in infant rats was remarkably age-dependent, as shown in the early study by Sabin and Olitsky (1937) and in our previous study on mice (Lundh *et al.*, 1988). The susceptibility decreased dramatically between 9 and 11 days postnatally. While the 9-day-old rats died soon after infection with an extensive spread of infection throughout the brain, the 11-day-old rats survived with an infection limited to the olfactory pathways, hypothalamus, diagonal bands and the anterior raphe nuclei. The reason for this age-dependent restriction of virus spread in the brain is unclear, but may involve factors from the host animal's immune or defence system. The maturity of the nervous system *per se* may also be of importance for the viral growth (Dubois-Dalcq *et al.*, 1982; Griffin *et al.*, 1974). During the second week of life, the anterior raphe nuclei (and the diagonal bands) are characterized by marked alterations in axonal and dendritic arborization, remodelling of cortical innervation and changes in transmitter receptivity including second messengers (D'Amato *et al.*, 1987; Jonsson and Kasamatsu, 1983; Lidow and Molliver, 1982; Zifa *et al.*, 1988).

The rats infected at day 11 postnatally had an extensive infection in the anterior raphe nuclei, i.e. the volume of infected neurons constituted 67% of the total neuronal volume in the dorsal raphe nucleus. Double-labelling experiments revealed that most of the 5HT-immunoreactive neurons and the substance P-immunoreactive neurons, respectively, were infected. Also in tracer studies on connectivity between the olfactory bulbs and the anterior raphe nuclei, it was found that a majority of the raphe neurons project to the olfactory bulbs (de Olmos *et al.*, 1978; Macrides *et al.*, 1981; Shipley and Adamek, 1984; Shipley, 1985).

The neuroanatomically restricted VSV infection also had functional consequences. Thus, we recently found that VSV infection leading to lesion of the raphe system in rats caused persistent learning deficits in the Morris maze and impaired exploratory behaviour in the open field test (Mohammed *et al.*, 1990, 1991). In the present study, we subjected the rats to the psychomotor stimulant amphetamine. The motor stimulatory action of amphetamine is mediated by diverse transmitter systems such as dopaminergic (Creese and Iversen, 1975; Kelly *et al.*, 1975; Shaywitz *et al.*, 1976), noradrenergic (Mohammed *et al.*, 1986) and cholinergic (Robinson, 1986) neurons. Of interest to the present work are a number of studies demonstrating that the central action of amphetamine can be modified by manipulation also of the serotonergic system. The weight of evidence indicates that the locomotor stimulant effect of amphetamine is attenuated by increased serotonergic transmission (Warbritton *et al.*, 1978; Carter and Pycock, 1978) and enhanced by blockade or destruction of the serotonin system

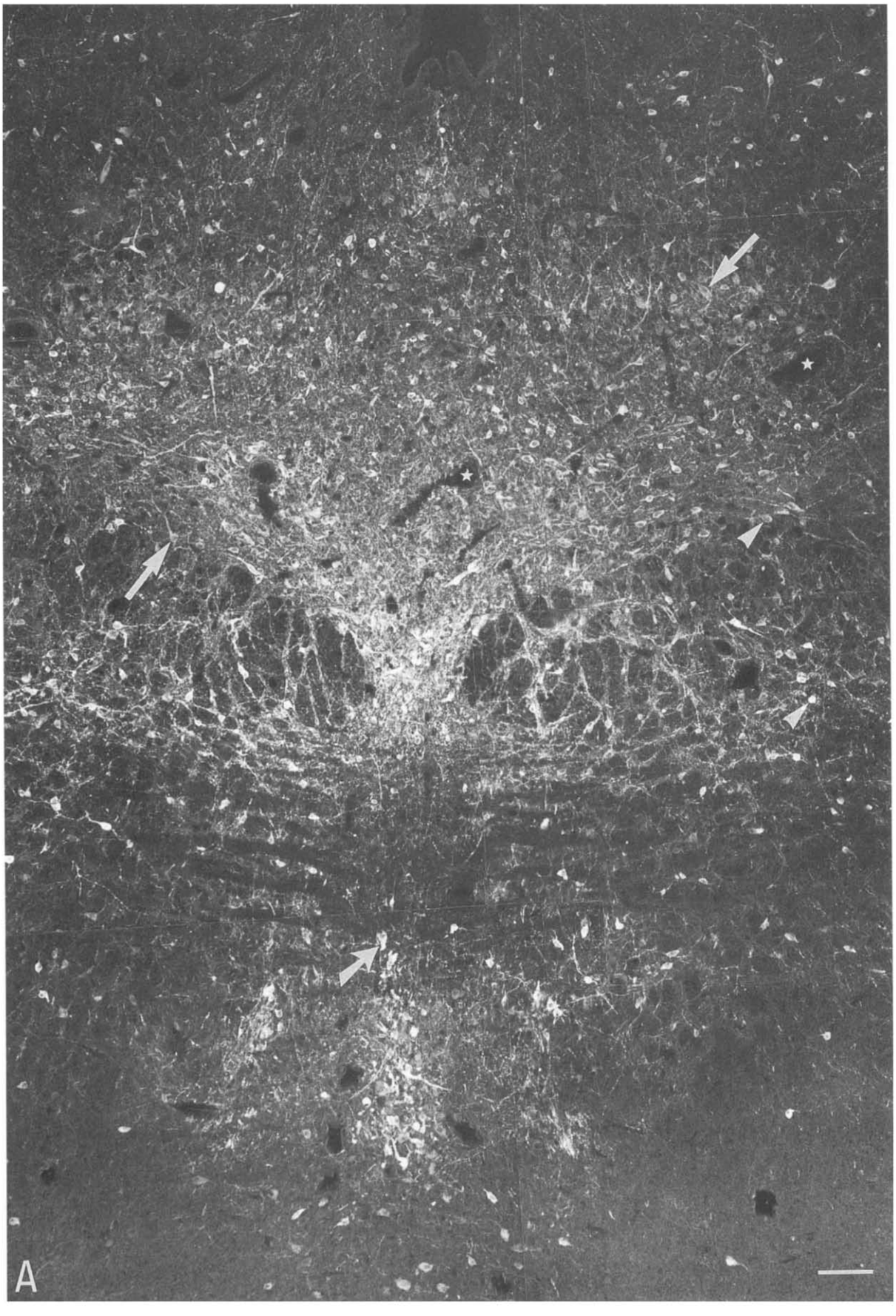
A

Fig. 3. Immunofluorescence micrographs of a section of the rat brain after incubation with antibodies against VSV antigen (A) and 5HT (B) at day 5 p.i. The section is viewed through filters for (A) fluorescein isothiocyanate and (B) tetramethylrhodamine isothiocyanate. 5HT immunoreactivity is seen in neurons in the dorsal and median raphe nuclei. VSV antigen occurs in the majority of the serotonergic neurons (arrows on a few of these). Only a small number of the VSV-containing neurons are not 5HT-positive (arrowheads in A). Bar = 100 μm.

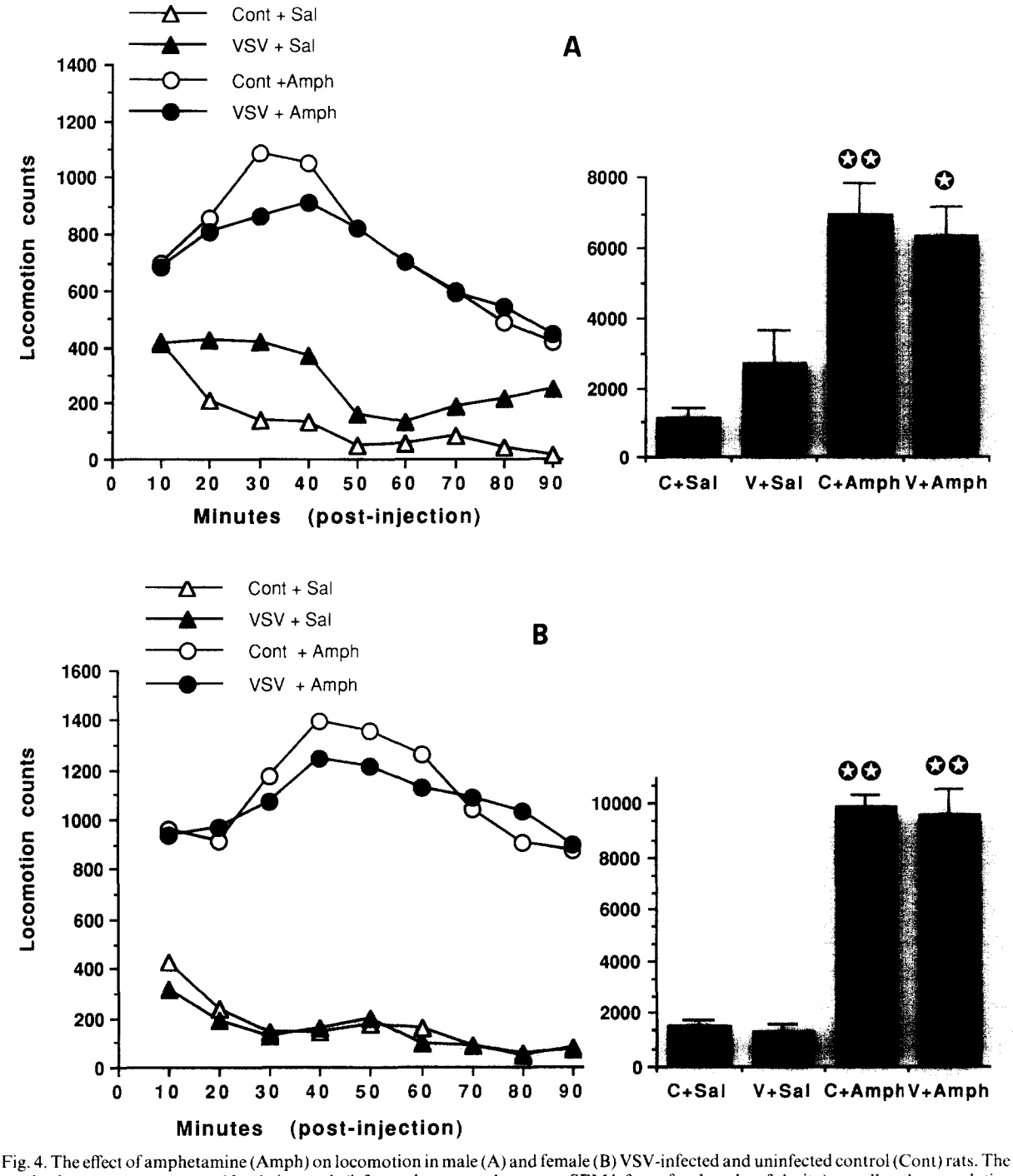

Fig. 4. The effect of amphetamine (Amph) on locomotion in male (A) and female (B) VSV-infected and uninfected control (Cont) rats. The results demonstrate counts at 10-min intervals (left panel, presented as mean; SEM left out for the sake of clarity) as well as the cumulative counts for the entire 90-min test period (right panel, presented as mean ± SEM). VSV-infected rats were more active than uninfected controls during the 60-min pre-injection habituation period. $*P<0.05$, $**P<0.01$. Statistical significance with respect to C + Sal and V + Sal.

(Breese *et al.*, 1974; Mabry and Campbell, 1974; Neill *et al.*, 1972).

In the present study, amphetamine treatment increased locomotion to some extent in uninfected and VSV-infected rats. However, with regard to rearing, there was a marked reduction of the amphetamine-induced increase in rearing scores observed in the VSV-infected animals. This was the case both for the frequency and the duration of rearing. Little is known about the neuronal basis of rearing. Rearing can be considered as a measure of exploratory behaviour (Archer, 1973), which includes cognitive function and locomotion. Amphetamine increases rearing in a dose-dependent manner (Russel *et al.*, 1987) and the stimulatory effect is larger on rearing than on

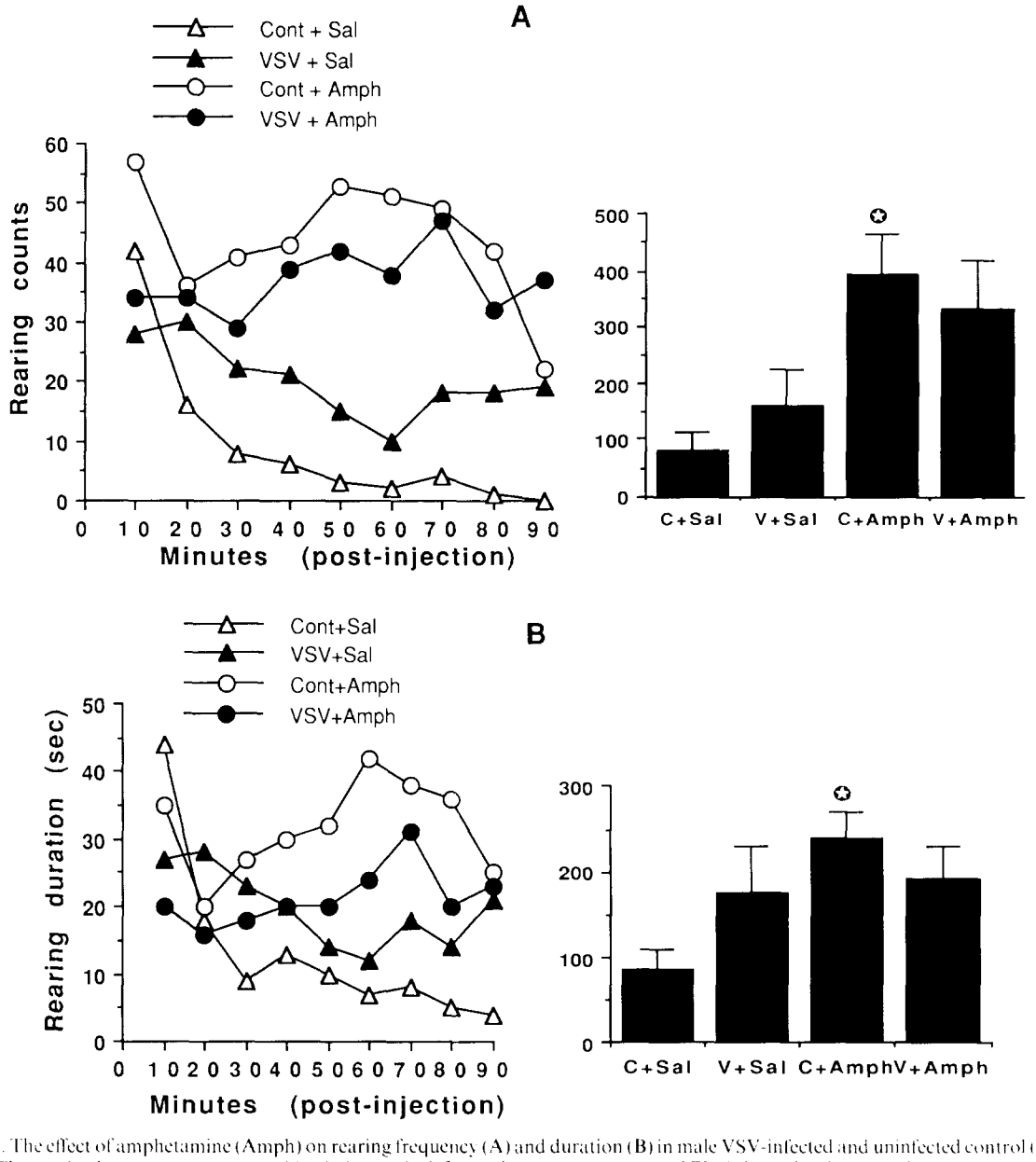

Fig. 5. The effect of amphetamine (Amph) on rearing frequency (A) and duration (B) in male VSV-infected and uninfected control (Cont) rats. The results demonstrate counts at 10-min intervals (left panel, presented as mean; SEM left out for the sake of clarity) as well as the cumulative counts for the entire 90-min test period (right panel, presented as mean ± SEM). VSV-infected rats were more active than controls during the 60-min pre-injection habituation period. $*P < 0.05$. Statistical significance with respect to C + Sal.

locomotion (Sandberg *et al.*, 1987). Most studies have considered the locomotor component of amphetamine-induced activity and, as mentioned above, it appears that this aspect of motor behaviour is mainly under the control of dopaminergic and serotonergic mechanisms. Our findings suggest that serotonergic neurons are critically involved in mediating amphetamine-induced rearing. Since substance P-containing neurons, which are involved in cognitive function (Huston and Oitzl, 1989; Hasenöhrl *et al.*, 1990), were also infected, this subpopulation of neurons may have contributed to this effect. As demonstrated earlier, the stimulant effects of amphetamine on both locomotion and rearing were more pronounced in female than in male rats, which may be due to sex differences in brain concentrations of amphetamine after systemic injections (Becker *et al.*, 1982; West and Michael, 1988).

The present study showed that virus antigen was present not only in the raphe nuclei, but also in neurons in the olfactory cortex, and the diagonal bands, and in a few noradrenergic neurons in the locus coeruleus. However, our previous biochemical study showed no consistent reduction in levels of choline acetyltransferase and noradrenaline, suggesting that the viral effects on these neurons has been compensated for by the uninfected ones. Virus antigen was also observed in neurons in different parts of the hypothalamus, which has been shown to be involved in amphetamine-induced locomotor

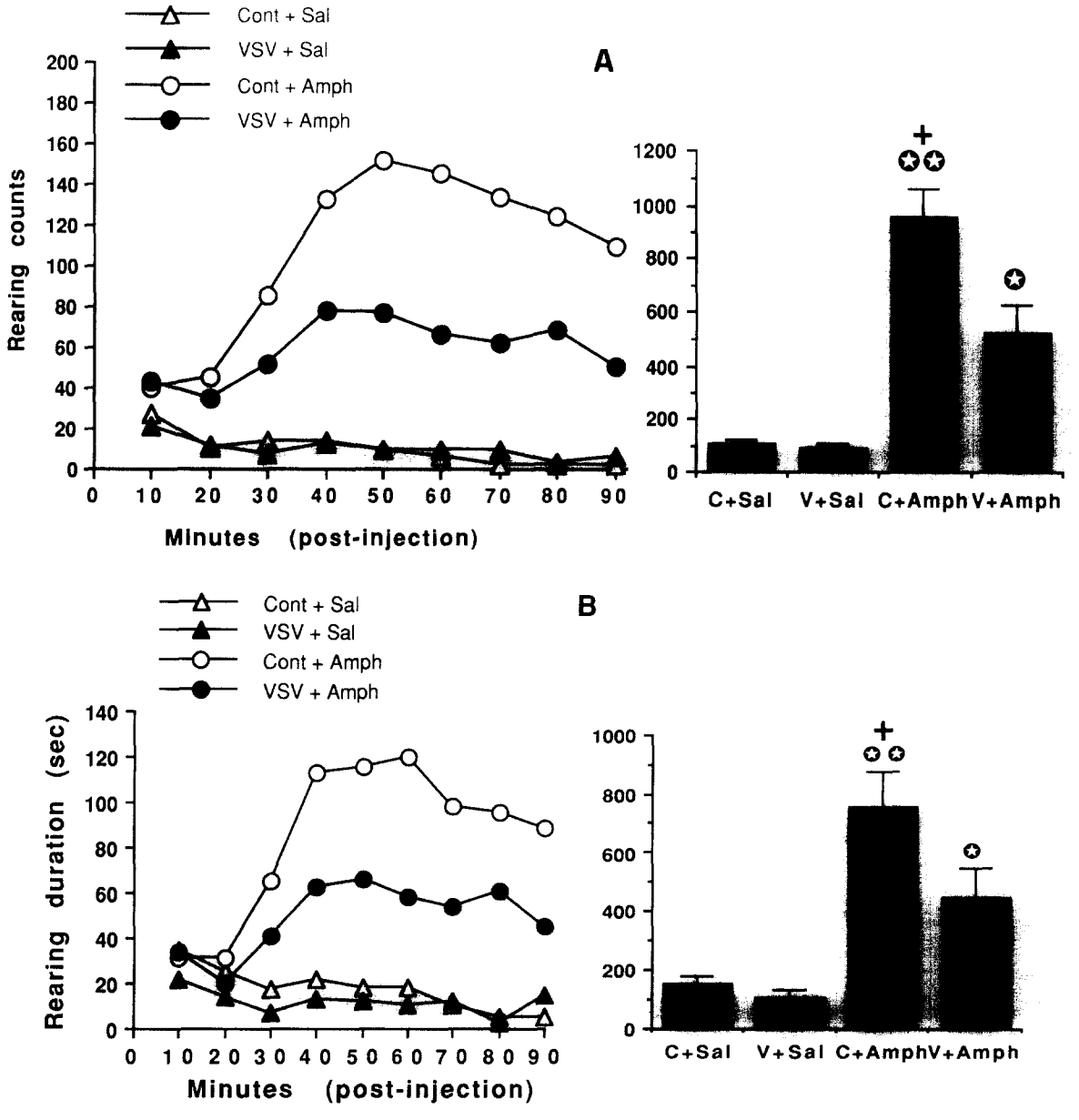

Fig. 6. The effect of amphetamine (Amph) on rearing frequency (A) and duration (B) in female VSV-infected and uninfected control (Cont) rats. The results demonstrate counts at 10-min intervals (left panel, presented as mean; SEM left out for the sake of clarity) as well as cumulative counts for the entire 90-min test period (right panel, presented as mean ± SEM. VSV-infected rats were more active than controls during the 60-min pre-injection habituation period. $*P<0.05$, $**P<0.01$. Statistical significance with respect to C + Sal and V + Sal. $+P<0.05$. Statistical significance in comparison between C + Amph and V + Amph.

stimulation in rats (Shian *et al.*, 1985). The nigro-cortical and nigro-striatal dopaminergic neurons, which are known to control motor activity (Carlsson and Carlsson, 1990) were, however, not infected. We suggest therefore that the attenuation of amphetamine-induced rearing in VSV-infected rats is mainly due to the lesion of the raphe nuclei.

Viruses can display a remarkable selectivity in their attack on nervous tissue (Johnson, 1980), due to differences in routes of infection, in viral cell surface receptors and in host cell restrictions on virus multiplication. Although cellular recognition sites for VSV are considered to be a component of virtually all cells (Emerson, 1985), this virus infects the neuroepithelia (olfactory mucosa and vomero-nasal organ) and not the respiratory epithelium in the nasal cavity (Lundh *et al.*, 1987). In the brain, VSV infects the anterior optic nucleus and reticular core neurons projecting to the olfactory bulbs. In the present study, the majority of neurons in the diagonal bands and anterior raphe nuclei were attacked, and in the raphe nuclei both substance P and 5HT neurons were infected. Although the locus coeruleus was heavily infected in mice (Lundh *et al.*, 1988), this nucleus showed only minimal infection in the rat. Recently, a marked retrograde labelling of the diagonal bands, the anterior raphe nuclei and locus coeruleus was found after intranasal infection

of herpes simplex virus type I in rats (McLean *et al.*, 1989), while a mutant strain of rabies virus (AvO1) showed no infection of the raphe nuclei after intranasal instillation, although the wild type did (LaFay *et al.*, 1991). The latter study illustrates that a selectivity in virus attack on groups of neurons projecting within the olfactory system can be obtained. Neither the wild type nor the mutant rabies virus infected the granular cells in the main olfactory bulb or the locus coeruleus, and the mutant, although it failed to infect the anterior olfactory nucleus (which the wild type did), gave rise to an extensive infection of the horizontal diagonal band. These studies indicate that there may be selectivity in the attack on the reticular core neurons projecting to the olfactory bulbs by different viruses spreading from the nasal cavity. Viruses and their mutants may, therefore, have potential as valuable tools in combined neurochemical and behaviour studies aimed at evaluating the neuroanatomical correlates of behaviour.

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