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Neurokinin-α Injected Into the Ventral Tegmental Area Elicits a Dopamine-Dependent Behavioral Activation in the Rat

LINDSAY H. BURNS AND ANN E. KELLEY[1]

Department of Psychology, Harvard University, Cambridge, MA

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BURNS, L. H. AND A. E. KELLEY. *Neurokinin-α injected into the ventral tegmental area elicits a dopamine-dependent behavioral activation in the rat.* PHARMACOL BIOCHEM BEHAV 31(2)255–263, 1988.—Neurokinin-alpha (NKA) and substance P (SP), neuropeptides of the tachykinin family, have been identified in dopaminergic areas of rat brain. It has previously been shown that SP microinjected into the ventral tegmental area (VTA), site of the dopaminergic A10 (DA-A10) cell bodies, causes a behavioral activation characteristic of dopamine agonists. The present experiment measured open field behavior following bilateral VTA injections of NKA (0.02, 0.2, 2.0 μg/0.5 μl). NKA induced a dose-dependent behavioral activation at lower concentrations of NKA than previously reported with SP. Medium and high doses of NKA produced significant increases in locomotion and rearing in both the center and periphery of the open field. Grooming decreased with dose, although this effect was not significant. In a second experiment, the behavioral activation by NKA (2.0 μg) was blocked by pretreatment with haloperidol (0.2 mg/kg), confirming that the NKA-induced effect is mediated by dopamine. Although the VTA contains both SP and NKA, receptors binding NKA exist here in greater density than those binding SP. Thus NKA may be the tachykinin in this region that preferentially interacts with DA-A10 neurons mediating behavioral arousal.

Neurokinin-α Tachykinins Ventral tegmental area Dopamine Open field behavior

ONE major group of peptides that are biologically active in the nervous system is the tachykinin family, of which substance P (SP) is the best known (27). These peptides have similar pharmacological activity and share a common carboxy-terminal sequence (23). Neurokinin-α (NKA) is a novel member of this group, characterized over the past several years. First known as substance K, NKA was discovered in the search for an endogenous tachykinin ligand for the SP-E receptor. In the early 1980s, it was proposed that mammalian brain contained two different tachykinin receptors differentially sensitive to various tachykinins (20). Tachykinins with an aliphatic residue at position X (such as physalaemin) are more potent at the "SP-P" receptor type, while tachykinins with an aromatic residue (such as kassinin and eledoisin) are more potent in stimulating "SP-E" receptors (3, 10, 20, 30, 35). Maggio and co-workers isolated a peptide with a high affinity for the SP-E receptor type (10,24). This peptide was similar in structure and activity to kassinin and became known as NKA (23). Immunocytochemical mapping studies have indicated that NKA is distributed widely throughout the neuraxis, and closely parallels substance P (21,24). Cloning methods have demonstrated that SP and NKA are derived from a common prohormone, β-preprotachykinin, and thus it is likely that the two peptides are localized within the same neurons (26).

A considerable amount of interest has focused on the nature of the interaction between tachykinins and dopaminergic neurons. Significant levels of both SP and NKA are found in the ventral midbrain, in close proximity to the dopaminergic (DA) cell bodies (13, 21, 22, 36). In an electrophysiological study, NKA excited DA neurons (11). Of considerable behavioral interest is the network of tachykinin fibers surrounding the DA cell bodies in the ventral tegmental area (VTA). These DA neurons (known as DA-A10) project to a wide range of limbic and cortico-limbic structures thought to be important in controlling many aspects of

[1]Requests for reprints should be addressed to Dr. Ann E. Kelley, Department of Psychology, Harvard University, William James Hall, 33 Kirkland Street, Cambridge, MA 02138.

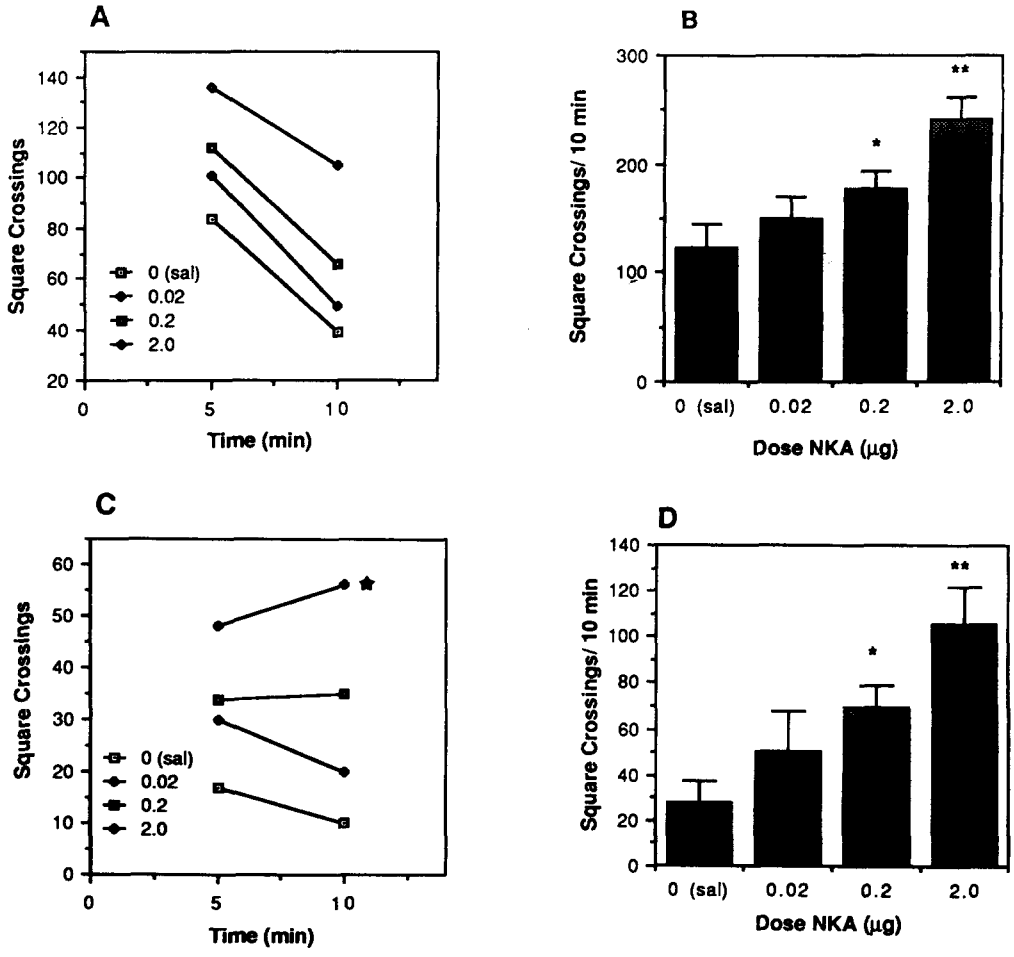

FIG. 1. The dose-dependent effect of NKA injected into the VTA on locomotion. (A) Time course in the periphery of the open field, (B) total square crossings in the periphery in the 10 min session, (C) time course in the center of the open field, (D) total square crossings in the center. *$p<0.05$, **$p<0.01$, ★$p<0.025$, dose × time interaction with respect to saline.

motivated and motor behavior (32). It has previously been shown that microinjections of SP into the VTA elicit a psychomotor activation mediated by DA release in the nucleus accumbens (18,37). In addition, a number of other studies have provided biochemical evidence for SP-induced activation of DA neurons (6, 9, 39). The discovery of NKA, a second major tachykinin in the brain, has prompted several investigations of its behavioral and neurochemical properties. The present work characterizes the behavioral effect of NKA infused into the VTA in an open field experiment, and tests the possible involvement of dopamine in the observed effects.

METHOD

Animals and Surgery

Twenty-three male Sprague-Dawley rats were used for the two experiments; 12 rats were in the first group and 11 rats in the second. (It should be noted that the first group originally contained 14 rats. At the end of the experiment, two rats with cannula tracks outside of the VTA were eliminated from the experiment; see the Histology section below.) All subjects were housed individually in clear plastic cages with food and water freely available. The day-night cycle was lights on between 8:00 and 20:00 hr. At the time of surgery, rats weighing approximately 275 g were anaesthetized with Nembutal anaesthesia (1 ml/kg) and placed in a Kopf stereotaxic apparatus. Bilateral stainless steel cannula guides (23 gauge) were aimed 2.5 mm above the VTA using the following coordinates (in mm): −3.0 from bregma, ±0.5 from superior sagittal sinus, and −6.4 from skull. The guides were fixed to the skull with stainless steel screws and dental acrylic cement. Wire stylets were placed in the guides to prevent occlusion. Animals recovered for at least three days before behavioral testing.

Behavioral Testing

Spontaneous behavior was measured in a white open field (1 × 1 m) divided into 16 squares. Testing was carried out in a quiet room with fluorescent overhead lighting. Five categories of behavior were scored: locomotion (square crossings) in the periphery (the squares along the wall), locomotion in the center (inner squares), rearing in the pe-

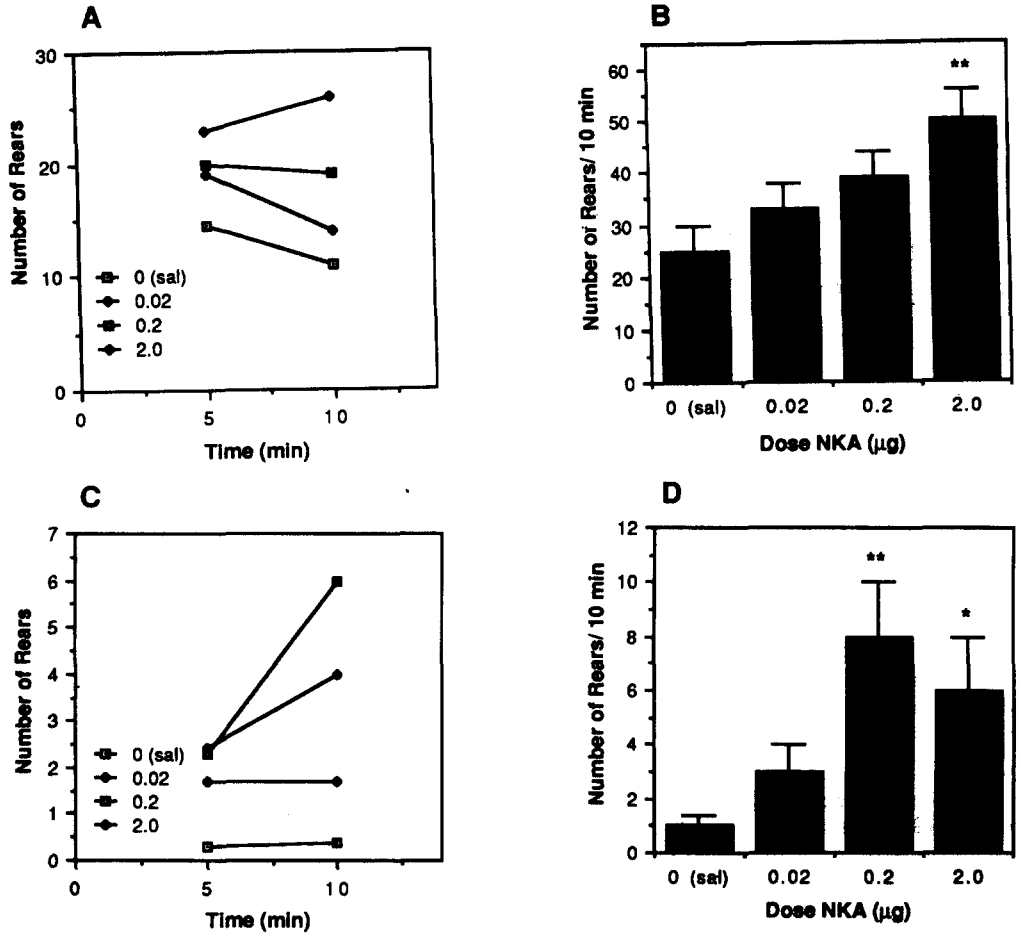

FIG. 2. The dose-dependent effect of NKA injected into the VTA on the frequency of rearing. (A) Time course in the periphery of the open field, (B) total number of rears in the periphery in the 10 min session, (C) time course in the center of the open field, (D) total number of rears in the center in the 10 min session. $^{*}p<0.05$, $^{**}p<0.01$.

riphery, rearing in the center, and grooming. For rearing and grooming, both the frequency (number of occurrences) and total duration (total time spent engaged in that behavior) were recorded. The criterion for one square crossing was three legs in the square. An observer sat next to the open field and scored behavior with an event recorder linked to a Eurobeeb microcomputer (Paul Fray LTD, Cambridge, England). Before the actual experiment, animals were habituated to the apparatus for two 20-min sessions. All experimental test sessions lasted 10 minutes.

Microinjections and Materials

All intracerebral injections were administered by means of a microdrive pump (Harvard Apparatus). Before the beginning of experimental testing, all rats were given one preliminary saline infusion to adapt them to the procedure. Bilateral injection cannulae (30 gauge) was lowered to the VTA (−8.9 mm from skull) and 0.5 μl isotonic sterile saline was infused over 1.5 min. One minute of diffusion was allowed before removing the injector and replacing the stylets. Commercially purchased NKA (Bachem, Torrance, CA) was divided into aliquots, freeze-dried and stored at −20°C. For experimental infusions, sterile saline was added to the NKA to attain selected concentrations (see doses below).

Behavioral Procedure

Experiment I. For this experiment, 12 rats received three doses of NKA and saline in a counterbalanced order, on four separate test days. The doses of NKA were 2.0, 0.2, 0.02 μg (1.76 nmol, 0.176 nmol, 17.6 pmol), and saline, always administered bilaterally. The volume of injectate in each cannula was always 0.5 μl. Rats were injected with the given dose for that day and placed immediately in the open field.

Experiment II. In this experiment, eleven rats received either NKA or saline in the VTA in combination with either saline or a neuroleptic intraperitoneally (IP). Thus, four treatments were administered on four separate days in the counterbalanced order. The dose of NKA was fixed at 2.0 μg bilaterally. Haloperidol (Sigma), a DA receptor blocker, was

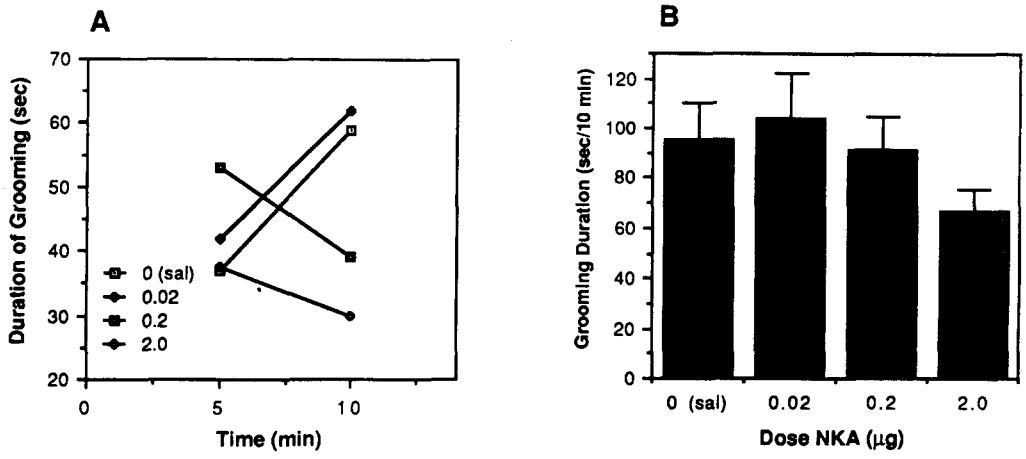

FIG. 3. The dose-dependent effect of NKA injected into the VTA on duration of grooming. (A) Time course, (B) total time spent grooming in the 10 min session.

injected 30 min before the intracerebral injection in a dose of 0.2 mg/kg distilled water. Rats were tested in the open field immediately after the intracerebral injection.

Histology

Following completion of behavioral testing, rats were injected with an overdose of Nembutal anaesthesia and perfused through the heart with isotonic saline followed by 10% formalin. The brains were removed and stored in formalin. Later, the brains were frozen and sectioned into 60 μm coronal sections. The sections were defatted, stained with cresyl violet and examined under a projection microscope for anatomical localization of cannulae tracks.

Statistical Analysis

All data were analyzed by a two-factor analysis of variance (dose or treatment and time), followed by post hoc comparisons between means (Newman-Keuls) where appropriate, or analysis of simple main effects for interactions. A square root transformation was used for center rearing in Experiment II. Data were analyzed on an IBM PC using the CRISP statistical package (Crunch Software, San Francisco).

RESULTS

Experiment I

Figures 1–3 show the effect of VTA-infused NKA on open field activities. NKA elicited a dose-dependent increase in locomotion in the periphery of the open field, $F(3,33)=11.26$, $p<0.001$. Post hoc analysis with Newman-Keuls (N-K) comparisons between means indicated that both the 0.2 and 2.0 μg doses significantly increased locomotion in the periphery. Figure 1A and B show the time course and total scores for this effect. NKA also augmented locomotion in the center of the open field; overall analysis of variance indicated a significant effect of dose, $F(3,33)=12.22$, $p<0.001$. Locomotion in the center increased significantly with both the high and middle doses, as seen in Fig. 1C and D.

Rearing behavior was also affected by VTA infusions of NKA. Figure 2A and B show that NKA significantly increased rearing in the periphery [overall dose effect, $F(3,33)=6.80$, $p<0.001$]. In this case, only the highest dose differed significantly from saline. Rearing in the center, which has a very low frequency in the control rats, was greatly increased by NKA [overall, $F(3,33)=5.2$, $p<0.005$]. There was also a dose × time interaction which almost reached statistical significance, $F(3,33)=2.77$, $p<0.057$. Figure 2C shows this interaction: NKA-treated rats engage in more rearing in the center towards the end of the session. The duration of rearing was similar to rearing frequency and is not presented.

Grooming was not significantly affected by NKA treatment (Fig. 3A and B), although rats treated with the two higher doses of NKA tended to groom less in the last 5 min, when other motor activity was most elevated. Only the duration of the grooming is presented.

Experiment II

Administration of the haloperidol effectively blocked the behavioral activation induced by 2.0 μg NKA in the VTA (Figs. 4, 5). For locomotion in the periphery (Fig. 4A and B), overall analysis of variance of the four treatments resulted in a significant treatment effect, $F(3,30)=14.3$, $p<0.001$. Post hoc comparisons showed that the NKA-saline group differed from NKA-haloperidol, saline-haloperidol, and saline-saline. A similar profile of behavior was obtained for locomotion in the center [Fig. 4C and D; $F(3,30)=14.3$, $p<0.001$].

The effect of neuroleptic treatment on NKA-induced rearing is shown in Fig. 5. Figure 5A and B illustrate the blockade by haloperidol of NKA-induced rearing in the periphery [overall treatment effect, $F(3,30)=6.4$, $p<0.002$]. Post hoc analysis indicated that the NKA-saline treatment differed significantly from NKA-haloperidol and saline-haloperidol, although the NKA-saline treatment was not significantly higher than the saline-saline treatment in this experiment. However, there was an overall dose × time interaction, $F(3,30)=5.24$, $p<0.05$. Further analysis of simple main effects revealed dose × time interactions between the saline-

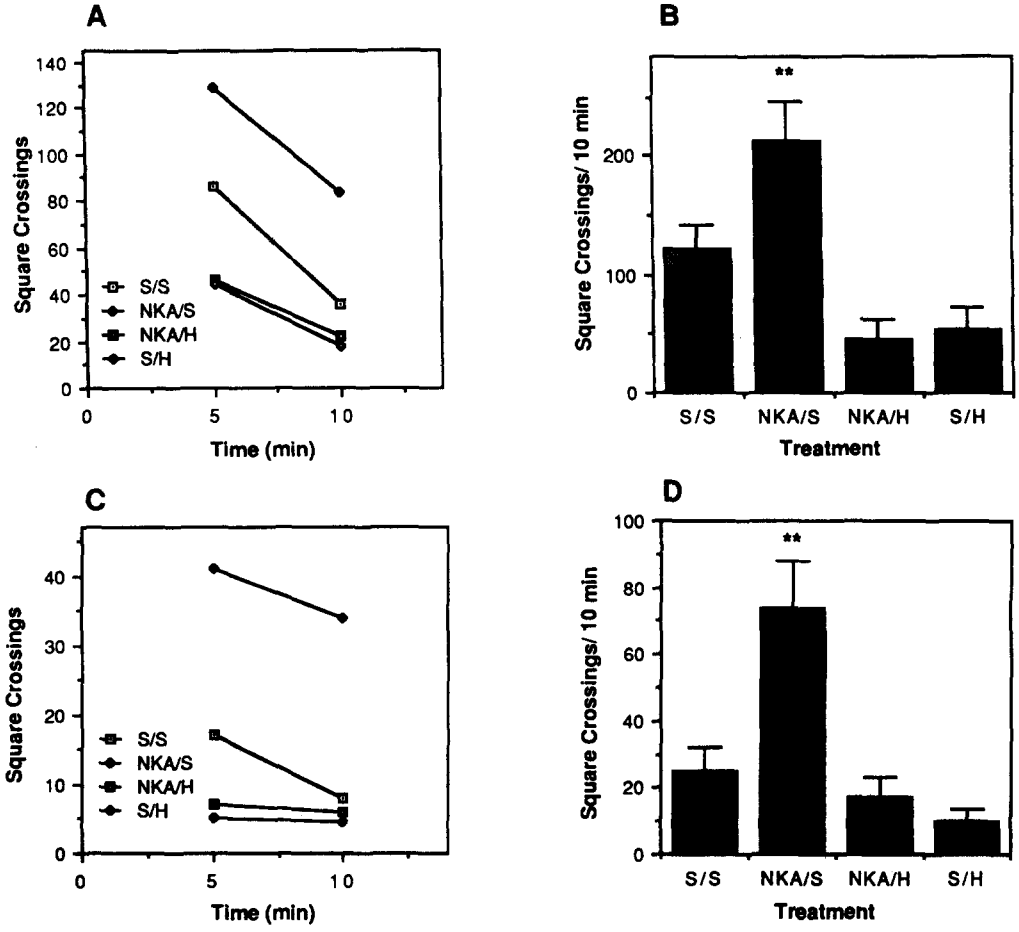

FIG. 4. The effect of NKA (2.0 μg) injected into the VTA on locomotion, suppressed by haloperidol (0.2 mg/kg) injected IP. (A) Time course in the periphery of the open field, (B) total square crossings in the periphery in the 10 min session, (C) time course in the center of the open field, (D) total square crossings in the center in the 10 min session. S/S=saline-VTA, saline IP; NKA/S=NKA-VTA, saline IP; NKA/H=NKA-VTA, haloperidol IP; S/H=saline-VTA, haloperidol IP. $^{**}p<0.01$.

saline and the other three treatments ($df=1,30, p<0.01$, for all comparisons). Rearing in the center of the open field displayed an overall effect of treatment, $F(3,30)=3.52, p<0.03$, and post hoc analysis indicated that the NKA-saline treatment differed significantly from the other three treatments (see Fig. 5C and D).

Overall analysis of variance for grooming duration indicated a significant treatment × time interaction, $F(3,30)=4.6$, $p<0.009$, although no treatment effect per · se was found. Haloperidol-treated groups showed considerably less grooming toward the end of the test session. Means and standard errors for the first five minutes and the second five minutes of the test session were (in sec): NKA-saline: 46±13, 47±12; saline-saline: 52±1, 60±15; NKA-haloperidol: 42±12, 21±6; saline-haloperidol: 62±22, 21±9.

Histology

Photomicrographs of representative cannula tracks are shown in two different animals in Fig. 6A and B. Both rats were from the first experiment. Figure 6A shows a cross section with the most anterior tracks in the group, and Fig. 6B shows tracks which fell in the most posterior part of the VTA.

DISCUSSION

The above findings demonstrate that neurokinin-α, a tachykinin peptide located in nerve terminals within the VTA, elicits a dose-dependent motor activation when injected directly into this region. This work confirms recent reports that VTA microinjections of NKA increased motor activity (8, 13, 38). In addition, analysis of the pattern of behaviors as well as their spatial distribution provides a qualitative profile of NKA-induced behavioral activation. Blockade of this response with a neuroleptic suggests that the behavior is mediated via dopaminergic neurons.

The enhancement of locomotion and rearing by NKA, both in the periphery and center of the open field, closely resembles the behavioral pattern observed after substance P infusions into this area. Control animals tend to avoid the center of a large open field presumably because of fearfulness or increased emotionality (40). NKA-treated rats approached the center more often possibly due to decreased

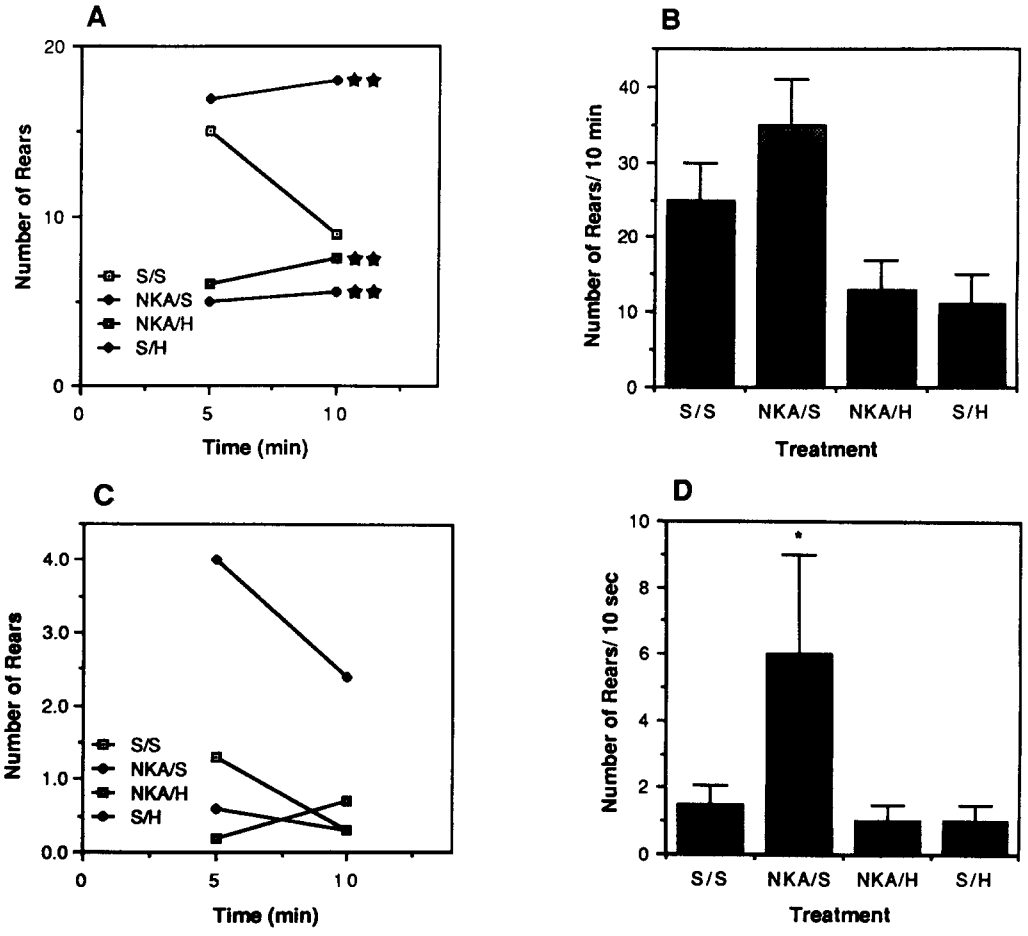

FIG. 5. The effect of NKA (2.0 μg) injected into the VTA on locomotion, suppressed by haloperidol (0.2 mg/kg) injected IP. (A) Time course in the periphery of the open field, (B) total number of rears in the periphery in the 10 min session, (C) time course in the center of the open field, (D) total number of rears in the center in the 10 min session. S/S=saline-VTA, saline IP; NKA/S=NKA-VTA, saline IP; NKA/H=NKA-VTA, haloperidol IP; S/H=saline-VTA, haloperiodol IP *$p<0.05$, ★★$p<0.01$, dose × time interaction for all three groups with respect to S/S.

fear or enhanced exploration. In agreement with this hypothesis, rats injected with SP into the VTA show more investigatory responses in a hole-board task (17). The increased output of motor responses following NKA infusions also supports the hypothesis that this peptide is activating the mesolimbic DA system. Pharmacological treatments which stimulate this system tend to enhance motor activity, particularly "approach" responses, such as locomotion, rearing and exploration. For example, a low dose of amphetamine, which preferentially releases DA in mesolimbic terminal areas, elicits open field behavior very similar to that observed after tachykinin stimulation of the VTA (12). One difference to be noted between the open field behavioral profiles of NKA and SP is that SP significantly reduces grooming (37). NKA did decrease grooming, especially in the last 5 min, although not significantly. Grooming is generally decreased in active animals since the two behaviors are negatively correlated (29).

Since NKA and SP elicit similar responses in the open field test, it is possible that both peptides act at the same tachykinin receptor. However, in comparison with previous studies, NKA is approximately 10 times more potent than SP in eliciting locomotor activity. The threshold dose for NKA-induced motor activity in this experiment is 0.176 nmol, close to the 0.1 nmol threshold for NKA found by Kalivas *et al.* and much smaller than their observed threshold of 1.0 nmol for SP (13). In addition, binding sites for NKA in the ventral mesencephalon are in much greater density than those for SP (13, 25, 33). In fact, binding sites for SP in this area, which has a very high concentration of both tachykinins, are surprisingly sparse or absent (31,34). Thus, NKA may be the tachykinin of greater physiological significance in this region. A further suggestion is that NKA interacts preferentially with DA-A10 neurons projecting to the nucleus accumbens, whereas SP, when released, may activate DA neurons projection to prefrontal cortex (1, 2, 7). This hypothesis is based on the finding that VTA-injections of SP, but not NKA, potently stimulate DOPAC formation in frontal cortex (7). In contrast, equimolar injections of NKA do affect nucleus accumbens DA metabolism. The puzzle regarding this finding is that NKA *decreases* nucleus accumbens DOPAC (an increase might be expected if NKA were

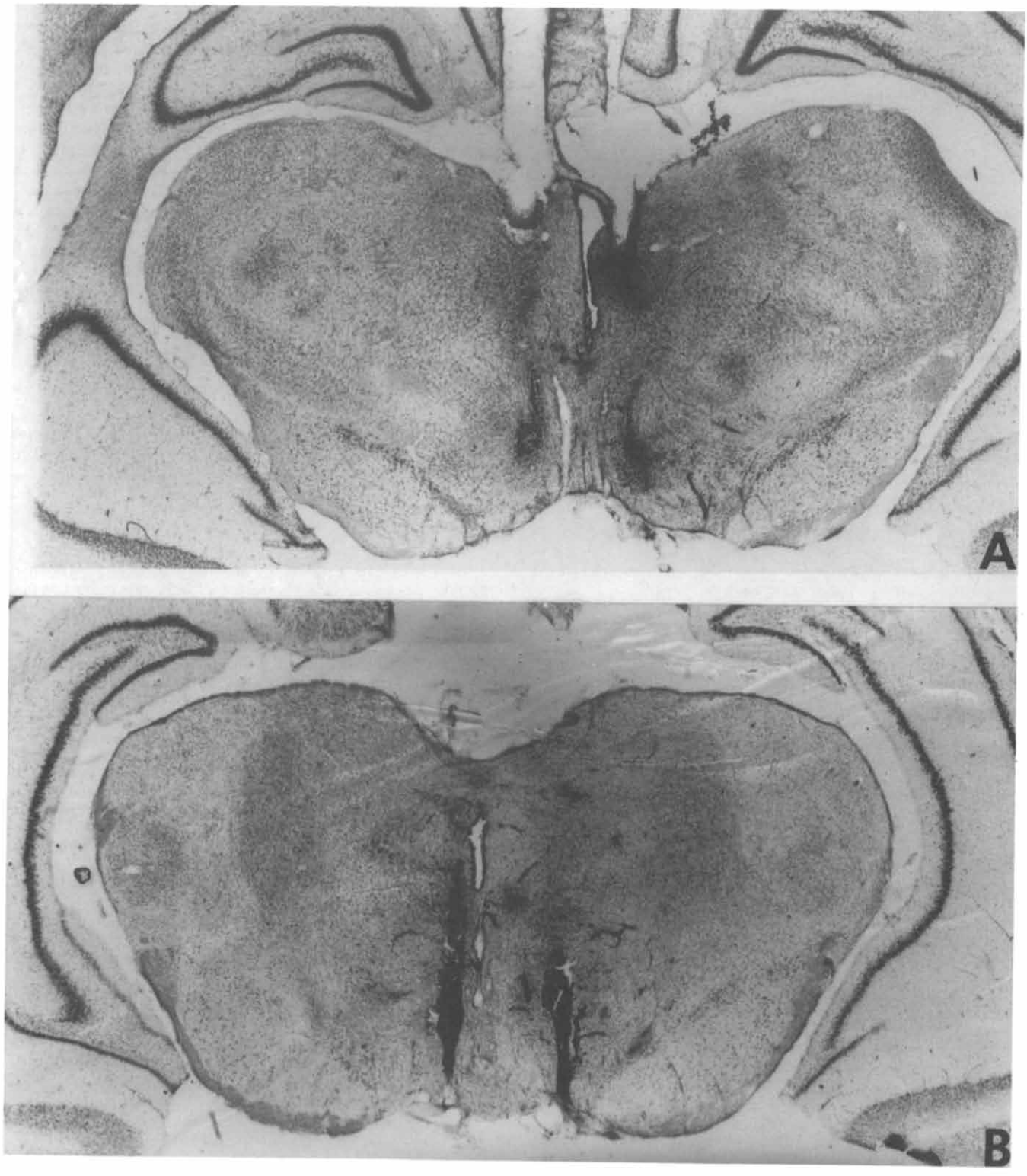

FIG. 6. Two cresyl-violet stained sections representative of the range of cannula placements in the VTA. A, anterior-most; B, posterior-most.

stimulating turnover). Deutch and co-authors have suggested that since a substantial decrease in nucleus accumbens DA was also observed after NKA injections, the peptide may induce an initial massive release of DA from this terminal site (7). Further neurochemical analysis is needed to clarify this point.

It is important to consider a third tachykinin, neurokinin-β, also called neuromedin K, which has been isolated in the mammalian central nervous system (15,19) and may exist with its own receptor type in the ventral mesencephalon (4). The potential role of neurokinin-β in DA-dependent behavioral activation has not yet been investigated.

The present study demonstrated that pretreatment with a relatively specific DA-antagonist blocks NKA-induced motor activity, in support of results reported by Takano and colleagues (38). Thus, convincing evidence suggests that this behavioral activation results from increased DA release, quite probably in the nucleus accumbens. Further experiments using DA blockade of specific anatomical sites in the forebrain could more precisely delineate which part of the DA system is being activated. It might be argued that the blockade with haloperidol is nonspecific and that a general motor impairment prevents the expression of the response. Since DA plays a role in normal spontaneous activity, rats treated with this dose of haloperidol could have a mild motor impairment. Both rearing and grooming decreased slightly; however, locomotion was not affected by haloperidol alone. Furthermore, in other studies, motor activation that appears independent of the dopamine system is not blocked by similar doses of haloperidol (14,28).

These experiments have investigated the behavioral effects of only one peptide located in the A10 dopaminergic region. In addition to the tachykinin peptides, multiple networks of other types of peptides surround the DA cells in the ventral mesencephalon. Although the roles of these peptides in relation to DA function are uncertain, evidence has emerged that they may interact differentially with the DA neurons. For example, studies of feeding and operant behavior show that VTA injections of various neuropeptides have dissociable behavioral effects (5,16). More detailed studies of these peptides and their specific effects are required in order to clarify their interactions with the dopamine systems.

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