European Journal of Pharmacology 658 (2011) 16–21

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural Pharmacology

Intraventricular administration of neuropeptide S has reward-like effects

Junran Cao [a,1], Luis de Lecea [b], Satoshi Ikemoto [a,*]

[a] Behavioral Neuroscience Research Branch, National Institute on Drug Abuse, National Institutes of Health, US Department of Health and Human Services, 251 Bayview Blvd., Suite 200, Baltimore, MD 21224, USA
[b] Department of Psychiatry and Behavioral Sciences, Stanford University, 701B Welch Road, Palo Alto, CA 94304, USA

ARTICLE INFO

Article history:
Received 5 November 2010
Received in revised form 15 February 2011
Accepted 16 February 2011
Available online 22 February 2011

Keywords:
Self-administration
Place conditioning
Locomotion
Neuropeptide S
SB 334867
SCH 23390

ABSTRACT

Neuropeptide S (NPS) is an endogenous brain peptide produced by neurons located in the lower brainstem, and functional studies suggest that NPS has arousing effects. Because its receptors are found in reward-associated regions throughout the brain, we evaluated whether intraventricular NPS injections elicit reward-related effects in rats. Rats increased lever presses that led to intraventricular administration of NPS (0.34–34 pmol per infusion) in a dose dependent manner, with a cue-assisted procedure. Cue-assisted self-administration of NPS was decreased by systemic administration of the dopamine receptor antagonist SCH 23390 (0.025 mg/kg, i.p.) or the hypocretin-1 (orexin-1) receptor antagonist SB 334867 (20 mg/kg, i.p.). In addition, intraventricular NPS injections (1000 pmol) induced conditioned place preference, whereas a lower dose (100 pmol) of NPS induced conditioned place aversion. Finally, NPS injections (100–1000 pmol) acutely facilitated locomotor activity, whereas repeated NPS injections did not lead to locomotor sensitization. Our data suggest that intraventricular NPS injections have reward-like effects in that NPS weakly facilitates seeking and induces positive reinforcement. These effects may depend on intact dopamine and hypocretin systems.

Published by Elsevier B.V.

1. Introduction

Neuropeptide S (NPS) is a recently identified endogenous ligand of an orphan G protein coupled receptor (Xu et al., 2004). This peptide appears to have arousing properties in such that intraventricular NPS injections increase locomotor activity and wakefulness and activate the HPA axis (Reinscheid, 2008; Rizzi et al., 2008; Smith et al., 2006; Xu et al., 2004). In addition, NPS injections increase c-Fos in lateral hypothalamic hypocretin (also known as orexin) neurons (Niimi, 2006), which play a critical role in arousal (Boutrel et al., 2010; Sutcliffe and de Lecea, 2002).

The NPS receptor appears to be expressed throughout the brain (Leonard and Ring, 2011; Xu et al., 2007), including in the regions that are associated with reward processes (Ikemoto, 2010): the ventral tegmental area, olfactory tubercle, bed nucleus of stria terminalis, diagonal band, paraventricular thalamic nucleus, preoptic area, lateral and posterior hypothalamic areas, periaqueductal gray, median and dorsal raphe nuclei and parabrachial nucleus. This pattern of receptor expression led us to ask whether arousing effects of intraventricular NPS administration are reward-related.

In this study, we examined whether intraventricular NPS was self-administered and induced conditioned place preference in rats. We also examined effects of dopamine receptor antagonists and hypocretin receptor antagonists on NPS self-administration. Finally, we examined whether repeated NPS injections have sensitizing effects on locomotor activity.

2. Materials and Methods

2.1. Animals

Male Wistar rats (N = 98) (Harlan, Dublin, VA, USA) were maintained in a temperature and humidity controlled room on a reversed 12-h light–dark cycle (lights on at 21.00 h) with unlimited access to food and water except during testing. They weighed 280–350 g at the time of surgery and were individually housed after surgery. All experimental procedures were approved by the Animal Care and Use Committee of the Intramural Research Program, National Institute of Drug Abuse, and were consistent with the National Institutes of Health guidelines. We made every effort to minimize animal suffering and to reduce the number of animals used in this study.

2.2. Drugs

NPS was synthesized at the de Lecea laboratory (Stanford University) and dissolved in artificial cerebrospinal fluid consisting of (in millimolars): 148 NaCl, 2.7 KCl, 1.2 $CaCl_2$, and 0.85 $MgCl_2$, pH adjusted to 6.5–7.5. The D_1 receptor antagonist R (+)-SCH 23390 (7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-o; Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.7% saline.

* Corresponding author at: National Institute on Drug Abuse, 251 Bayview Blvd., Suite 200, Baltimore, MD 21224, USA. Tel.: +1 443 740 2722; fax: +1 443 740 2827.
E-mail address: satoshi.ikemoto@nih.gov (S. Ikemoto).
[1] Present address: Department of Psychiatry and Neurobehavioral Sciences, University of Virginia, 1670 Discovery Drive, Suite 110, Charlottesville, VA 22911, USA.

0014-2999/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.ejphar.2011.02.009

The hypocretin-1 (also known as orexin-1) receptor antagonist SB 334867 (1-(2-methylbenzoxazol-6-yl)-3-[1,5]naphthyridin-4-yl urea; Tocris Cookson, Ellisville, MO, USA) was dissolved in 10% (2-hydroxypropyl)-β-cyclodextrin/2% dimethyl sulfoxide solution (Sigma-Aldrich).

2.3. Stereotaxic Surgeries

Under sodium pentobarbital (31 mg/kg, i.p.) and chloral hydrate (142 mg/kg, i.p.) anesthesia, a single unilateral guide cannula (24-gauge) was implanted in each rat aiming at the lateral ventricle and permanently fixed to the skull with acrylic dental cement. Stereotaxic coordinates for the lateral ventricle were 0.0 mm anterior to bregma, 1.5 mm lateral to the midline, 4.0 mm ventral to the skull surface. Rats were left undisturbed for several days after the surgery.

2.4. General Procedures

2.4.1. Procedure for Intraventricular Experimenter-administered Injections

Three days before the start of experiments, each rat was taken to a testing room and placed in a 30 cm diameter cylinder. An injection cannula (31 gauge) was inserted into the implanted guide cannula. The tip of the injection cannula extended beyond the tip of the guide by 1.0 mm. Each rat received an angiotensin solution (50 ng in 1 μl) that was delivered by a syringe pump at a constant rate over 60 s, with an additional 30 s before the injection cannula was removed. The rat was then returned to its home cage. We performed this intraventricular angiotensin injection, known to cause immediate and intense water consumption, to validate the placement of the cannula assembly aimed into the lateral ventricle. Rats that did not immediately begin drinking were eliminated from the following experiments involving NPS injections. NPS was delivered into the lateral ventricle with a 2.2 μl volume at a constant rate over 60 s, with an additional 30 s before the injection cannula was removed.

2.4.2. Procedure for Intraventricular Self-administration

Experiments were conducted in standard operant chambers (30×22×24 cm; Med Associates, St Albans, VT, USA) equipped with a lever (45 mm wide×2 mm thick protruding 20 mm from the wall) and a cue light located just above the lever. The intraventricular guide cannula was inserted with an injection cannula (31 gauge), which was connected by polyethylene tubing to a micropump consisting of a drug reservoir and step motor (Ikemoto and Sharpe, 2001) that hung a few millimeters above the rat's head. When activated, the micropump's step motor turned its shaft in six incremental steps (9° per step) over 5 s, driving its threaded shaft into the drug reservoir and pushing a 75 nl volume out of the reservoir into the brain. A response on the lever activated the micropump and the cue light for 5 s followed by a 55 s time-out period, during which lever pressing produced no programmed consequence. In addition, we monitored each rat's gross somatomotor activity (referred to as locomotion) during self-administration sessions by quantifying the rotation (Roto-Rat, Med Associates) of the electrical swivel for the microinjection pump. Each session lasted 90 min, and sessions were separated by a day.

2.5. Experiment 1: Concentration-dependent Effects of NPS Self-administration

Experimentally-naïve rats (N=9) received vehicle (75 nl/infusion) in sessions 1 and 5, and NPS (3.39 pmol/infusion) in sessions 2–4 (acquisition phase), followed by vehicle (session 5) and increasing doses of NPS (0.339, 3.39 and 33.9 pmol/infusion) in sessions 6–8, respectively (dose–response test). During self-administration sessions, locomotor activity was assessed with the Roto-Rat device, except that of the first rat we tested, since the device had not been set up then.

2.6. Experiment 2: Effects of the Dopamine Receptor Antagonist SCH 23390 on NPS Self-administration

A group of rats (N=6) was first trained to self-administer NPS (3.39 pmol/injection) in sessions 1 and 2. Then, they were pretreated with SCH 23390 (0.025 mg/kg, i.p.) or saline (1 ml/kg, i.p.) 30 min before a self-administration session. They self-administered for vehicle in sessions 3 and 4 and NPS (3.39 pmol/infusion) in sessions 5 and 6. The order of testing the effects of SCH 23390 or saline was counterbalanced among the rats.

2.7. Experiment 3: Effects of the Hypocretin-1 Receptor Antagonist SB 334867 on NPS Self-administration

In Session 1, experimentally-naïve rats (N=6) received SB 334867 (a hypocretin-1 receptor antagonist; 20 mg/kg, i.p.) or vehicle (10% 2-hydroxypropyl-β-cyclodextrin/2% dimethyl sulfoxide; 1 ml/kg, i.p.) 30 min before they were placed in the chamber for self-administration of NPS (3.39 pmol/infusion). In session 2, the i.p. injections of SB 334867 and vehicle were reversed among the rats. The order of testing the effects of SB 334867 or vehicle was counterbalanced among the rats. In this experiment, rats were not trained to self-administer NPS prior to these tests, because we found in experiments 1 and 2 that rats reliably initiate self-administration of NPS without prior training.

2.8. Experiment 4: Place Conditioning Induced by Intraventricular NPS

Experimentally naïve rats were used for this experiment. The place conditioning chamber consisted of two compartments (21×21×28 cm) linked by a connecting area (21×21×12.5 cm), and a sliding door separated each compartment from the connecting area (Med Associates). The two compartments differed in wall color (black vs. white), floor type (net vs. grid) and lighting; the amount of light was adjusted so that the rats did not prefer one compartment over the other prior to place conditioning. We used an "unbiased" procedure, in which drug and vehicle injections were randomly assigned between the two compartments without consideration of rats' original place preference. In sessions 1 (pre-conditioning) and 10 (post-conditioning), rats were not given injections and were confined individually in the connecting area of the chamber for 30 s. They were then allowed to freely move about the chamber for 15 min with the sliding doors open. Their time spent in each compartment was recorded. Rats (N=10–11) were assigned to one of the three groups of NPS doses (10, 100 or 1000 pmol/injection). They received intraventricular injections of NPS or vehicle just before conditioning sessions 2–9. Then, each rat was confined in one of the compartments for 20 min with sliding doors closed. NPS injections were paired with one compartment and vehicle with the other. The order of injection treatments and the assignment of the compartments for injection treatments were counterbalanced among the rats in each dose group. Sessions were separated by a day. The place preference score was derived by subtracting the time spent in vehicle-paired compartment from that in drug-paired compartment.

2.9. Experiment 5: Locomotor Activity Facilitated by Intra-ventricular NPS

Tests were performed in locomotor chambers (40×40×30 cm; AccuScan Instruments, Columbus, OH, USA). Locomotion (horizontal distance traveled) was detected by 32 photocell pairs separated by 2.5 cm and placed 3 cm above the floor on all sides of the chamber. Rearing (frequency of vertical movements) was detected by 16 photocell pairs separated by 2.5 cm and placed 20 cm above the floor on two opposite sides of the chamber. Rats (N=10–13) were assigned to one of four dose groups (0, 10, 100 or 1000 pmol). Each rat received an intraventricular injection of vehicle on the first day and an injection of NPS on each of 4 consecutive days. Immediately after each of these injections, each rat was placed in the locomotor

chamber for 30 min during which locomotor distance and rearing frequency were recorded.

2.10. Data Analysis

Data were analyzed with either t-tests or ANOVAs followed by appropriate post-hoc tests. Details are provided in the Results section.

3. Results

3.1. Experiment 1: Concentration Dependent Effects of NPS Self-administration

We used a self-administration procedure assisted by visual cues. This procedure facilitates lever pressing without training rats and has been found to be useful for initial screening of potential rewarding effects of drugs. In session 1, rats obtained about 20 vehicle infusions, which were accompanied by the presentation of cue light just above the lever. Rats increased self-administration in sessions 2–4 when infusions contained NPS (3.39 pmol/infusion) (Fig. 1A). Although the levels of NPS self-administration were modest, self-administration was reliable, as indicated by a significant session effect with one-way repeated measures ANOVA on infusions over sessions 1–4 ($F_{3,\ 24} = 5.86$, $P<0.01$). The numbers of NPS infusions tended to decrease over sessions 2–4, although this trend was not statistically significant.

Rats self-administered NPS in a dose-dependent manner in sessions 6–8 (Fig. 1A). While the lowest dose of NPS (0.339 pmol/infusion) in session 6 was not reliably self-administered more than vehicle in session 5, the higher doses of 3.39 and 33.9 pmol/injection in sessions 7 and 8 were self-administered more than vehicle in session 5. Event records suggest that NPS-experienced rats began lever-pressing as soon as the session started in sessions 5–8 (Fig. 1B). However, these rats stopped responding after a few minutes when their responses were rewarded with infusions of vehicle or 0.339 pmol NPS. Lever-pressing was prolonged when the doses of NPS increased. These observations were confirmed by a significant dose effect with one-way repeated measures ANOVA on infusions over sessions 5–8 ($F_{3,\ 24} = 4.98$, $P<0.01$).

Unlike self-administration, we did not observe reliable changes in locomotor activity during sessions 5–8 ($F_{3,\ 21} = 1.99$, $P = 0.15$, a one-way repeated measures ANOVA) in which rats displayed increasing lever pressing for NPS (Fig. 1C). This result suggests that general arousal or hyperactivity does not explain the self-administration effects of NPS.

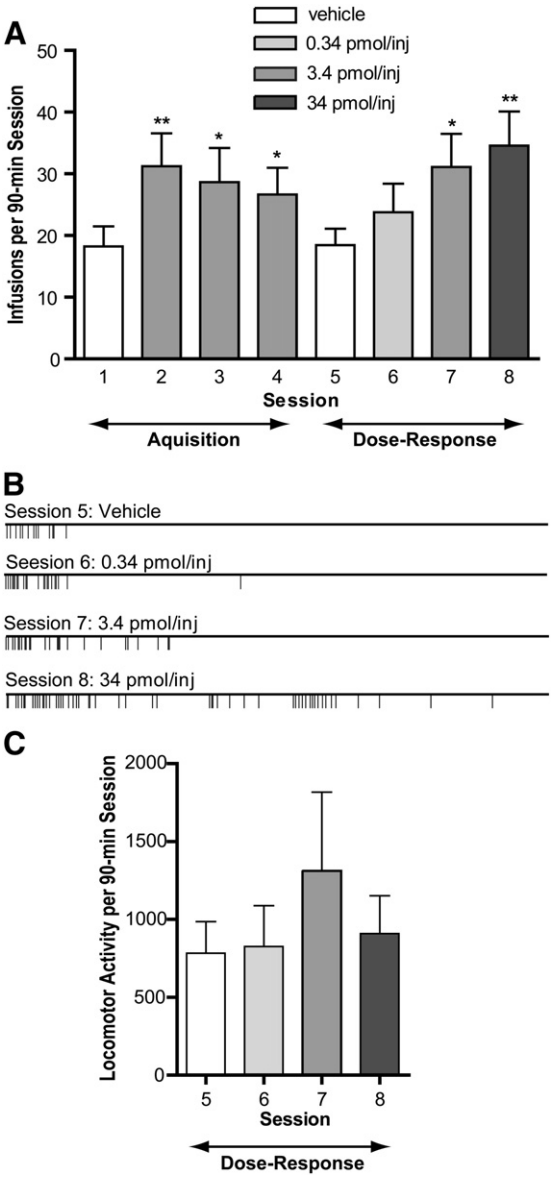

Fig. 1. Intraventricular self-administration of NPS. The acquisition of NPS self-administration was evaluated over sessions 1–4 and its dose dependent effects over sessions 5–8 (N = 9 per group). (A) Data are means with S.E.M. ** $P<0.01$, * $P<0.05$, significantly different from vehicle values in session 1 or 5 (Dunnett's). (B) NPS self-administration of a representative rat is shown as a function of dose. In event records, each vertical line indicates the time of an infusion and each horizontal line indicates the 90 min session length. (C) Data are means with S.E.M. Locomotor activity counts during self-administration sessions 5–8 are shown.

3.2. Experiment 2: Effects of the Dopamine Receptor Antagonist SCH 23390 on NPS Self-administration

Rats quickly increased lever pressing for NPS administration in sessions 1 and 2 (data not shown), replicating acquisition of NPS self-administration in experiment 1. Pretreatment with the D_1 receptor antagonist SCH 23390 significantly reduced NPS self-administration (Fig. 2). Although pretreatment with SCH 23390 also tended to reduce self-administration of vehicle, this was not statistically significant. A one-way repeated measures ANOVA on infusions shows a significant main session effect ($F_{3,\ 15} = 10.57$, $P<0.001$). Similar patterns of change were observed for locomotor counts as a function of the SCH

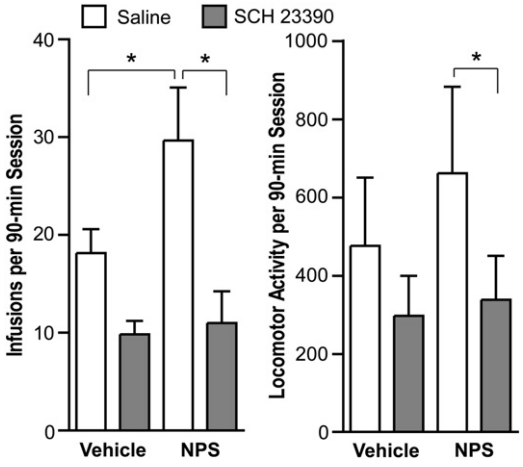

Fig. 2. Effects of the dopamine receptor antagonist SCH 23390 on NPS self-administration. After pretreatment with the D_1 receptor antagonist SCH23390 (0.025 mg/kg, i.p.) or saline, rats (N = 6) self-administered for vehicle and NPS (3.39 pmol/infusion). Data are means with S.E.M. * $P<0.05$, ** $P<0.01$, significantly different with a Newman–Keuls multiple comparison test.

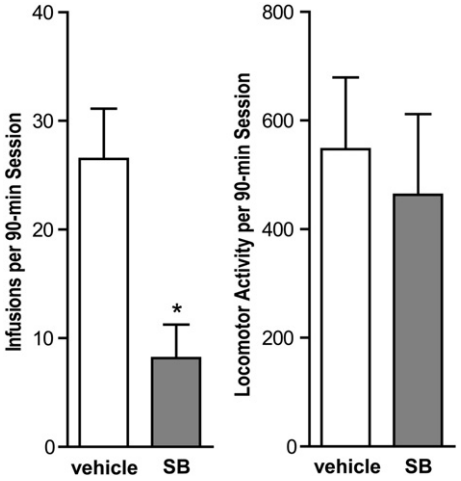

Fig. 3. Effects of the hypocretin-1 receptor antagonist SB 334867 (20 mg/kg, i.p.) on NPS self-administration. Data are means with S.E.M. (N = 6). * $P<0.05$, significantly different from vehicle values.

23390 treatment (a significant main treatment effect, $F_{3,\ 15} = 6.41$, $P<0.01$, Fig. 2).

3.3. Experiment 3: Effects of the Hypocretin/Orexin Receptor Antagonist SB 334867 on NPS Self-administration

Pretreatment with SB 334867 (20 mg/kg i.p.) significantly decreased NPS self-administration ($t_5 = 4.04$, $P<0.01$), whereas it did not reliably decrease locomotor activity during self-administration sessions (Fig. 3).

3.4. Experiment 4: Place Conditioning Induced by Intraventricular NPS

To examine whether NPS injections have a positive affective effect, we employed a place conditioning procedure in which compartments' contextual cues were paired with arousing effects induced by intraventricular NPS injections. The rats receiving the high dose of NPS (1000 pmol) spent more time in the drug-paired compartment, suggesting conditioned approach, whereas the rats received the medium NPS dose (100 pmol) spent more time in the vehicle-paired side, suggesting conditioned avoidance (Fig. 4). The low dose (10 pmol) of NPS had no reliable effect. These observations were confirmed with a significant interaction with a 3×2 (dose×before-and-after conditioning) mixed ANOVA ($F_{2,\ 28} = 9.32$, $P<0.001$).

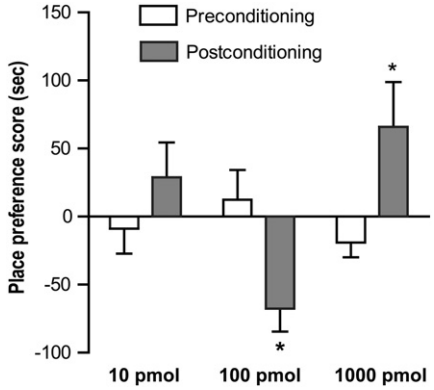

Fig. 4. Conditioned place preference and aversion induced by intraventricular NPS. Data are mean place preference scores with S.E.M. (N = 10–11). * $P<0.05$, significantly different from preconditioning values with Bonferroni corrected t-tests.

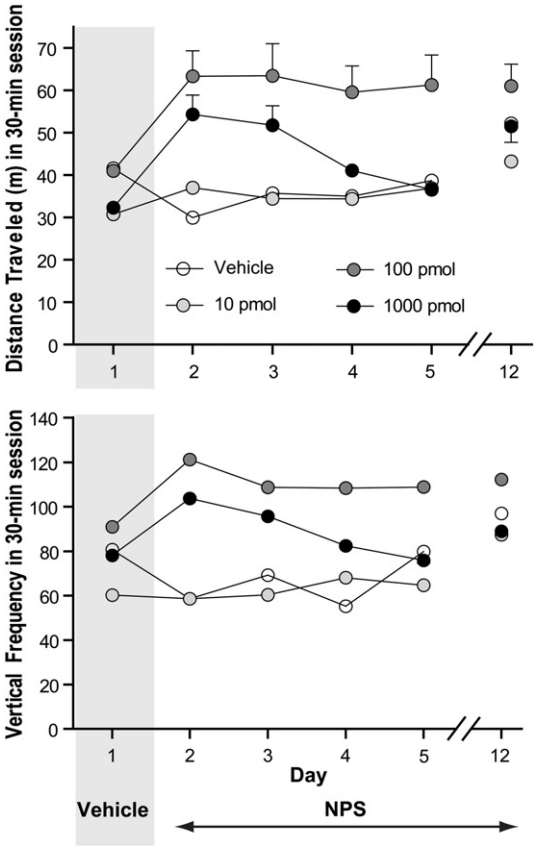

Fig. 5. Effects of repeated NPS injections on locomotion and rearing. All rats received intraventricular vehicle injections on day 1. Then, rats were assigned to one of four dose groups (0, 10, 100 and 1000 pmol; N = 10–13 per group) and received the same intraventricular treatment on days 2–5 and day 12. Data are means. Data points with error bars (S.E.M.) are significantly different from respective vehicle values on day 1 ($P<0.05$, Newman–Keuls).

3.5. Experiment 5: Locomotor Activity Facilitated by Intraventricular NPS

To examine whether repeated injections of NPS are behaviorally sensitizing, we monitored locomotion and rearing after NPS injections over a few days. The medium (100 pmol) and high (1000 pmol) doses of NPS injections acutely increased locomotion and rearing, while the low dose (10 pmol) had no detectable effect (Fig. 5). Instead of being sensitizing, the high dose of NPS injections decreased locomotion and rearing over days 2–5. When the rats received the same injection manipulation on day 12, none of the NPS doses increased locomotion or rearing more than on previous days. Thus, we did not observe any evidence of behavioral sensitization. These observations were confirmed by significant group×session interactions on locomotion ($F_{15,210} = 3.94$, $P<0.001$) and rearing ($F_{15,210} = 2.66$, $P<0.005$) with 4×6 (group×session) ANOVAs.

4. Discussion

4.1. Reward-like Effects of Intraventricular NPS

We observed that untrained rats increased cue-assisted self-administration of intraventricular NPS when they received it for the first time, and maintained NPS self-administration for the next two sessions. In subsequent sessions, rats self-administered NPS in a dose-dependent manner. These observations need to be carefully interpreted, particularly because our self-administration procedure involved not only NPS infusions, but also visual cues.

Our results on self-administration of NPS suggest that intraventricular NPS facilitates seeking responses. Sensory cues accompanied

by drug administration serve as "bridges" between responding and pharmacological actions of the drug (Sorge et al., 2009). Cue-assisted procedures of drug self-administration make it easier for animals to learn the relationships between manipulators, actions and drug effects. Moreover, because brief presentation of visual cues is positively salient and triggers seeking responding in rats (Shin et al., 2010; Stewart and Hurwitz, 1958), this procedure was used to facilitate lever pressing, thereby, learning the association between lever pressing and the delivery of NPS. However, this procedure is only useful as an initial screening tool for seeking facilitation properties of drugs, because this procedure does not clearly indicate what rats respond for, in this case, NPS infusions or visual cues. In addition, the fact that intraventricular NPS promotes locomotor activity and wakefulness (Xu et al., 2004) makes it unclear whether increased lever pressing was due to enhanced seeking or "general" arousal. In our previous study, however, we demonstrated that as far as amphetamine is concerned, seeking facilitation effects can be dissociated from general arousal: Relatively high doses of amphetamine that elicited intense locomotor activity diminished seeking responses (Shin et al., 2010), using the identical lever press procedure employed in the present study. Therefore, it is tempting to interpret that increased lever pressing is seeking-related. Consistent are recent findings that intraventricular injections of NPS facilitate seeking for alcohol or cocaine (Cannella et al., 2009; Kallupi et al., 2010; Paneda et al., 2009).

Because our self-administration effects of NPS are rather weak, we did not further use self-administration procedures to determine if rats can learn to seek NPS infusions. Instead, we conducted the place conditioning experiment. Our place conditioning results provide partial support for intraventricular NPS being sought by rats (i.e., positive reinforcement). Rats increased time in the compartment paired with intraventricular injections of 1000 pmol NPS, suggesting conditioned approach for NPS, whereas they decreased time in the compartment paired with 100 pmol NPS, suggesting conditioned aversion for NPS. It is difficult to compare effects between self-administration and place conditioning, since NPS were delivered with different procedures between them. Having that said, the 3.4-pmol dose was self-administered roughly 30 times in average, adding up to roughly 100 pmol, which was the dose that induced conditioned aversion. The 34-pmol dose was also self-administered roughly 30 times, adding up to 1000 pmol, which was the dose that induced conditioned approach. Therefore, it is difficult to reconcile the data between self-administration and place conditioning procedures with respect to affective properties of NPS. To understand mechanisms through which NPS exert positive and negative affective (i.e., reinforcing) effects, additional research is needed.

4.2. Cue-assisted Self-administration of NPS Depends on Intact Dopamine and Hypocretin Systems

A low dose of SCH 23390 (0.025 mg/kg, i.p.) decreased lever presses for NPS. We also found a strong trend that SCH 23390 decreased lever pressing under the control condition in which responding was maintained by visual cues without NPS infusions. This effect is not surprising in light of the previous findings that responding maintained by salient stimuli is readily decreased by low doses of dopamine receptor antagonists (Bardo et al., 1989; Besheer et al., 1999; Olsen and Winder, 2009). It is probable that responding with vehicle infusions was largely maintained by visual cues and that this visual cue seeking must have depended on dopamine transmission. It is unlikely that the SCH 23390 injections disrupted behavior in a nonspecific manner, because the previous studies suggest that 0.025 mg/kg dose in rats does not impair motor process (Koob et al., 1987; Hauber, 1996). In any case, cue-assisted self-administration of NPS appears to depend on intact dopamine transmission. Because dopamine transmission occurring in the medial part of the ventral striatum plays a critical role in reward seeking (Ikemoto, 2007; Shin et al., 2010), the medial ventral striatum may play a critical role in cue-assisted self-administration of NPS.

We found that a dose of hypocretin-1 receptor antagonist SB 334867 decreased cue-assisted NPS self-administration, while having no reliable effect on locomotor activity. It is possible that NPS stimulates hypocretin neurons, and that this action of NPS is important for its seeking effects. As mentioned above, intraventricular NPS administration induces c-Fos in hypocretin neurons. Moreover, NPS receptor expression has been detected in the lateral hypothalamus, including a fraction of hypocretin neurons (Kallupi et al., 2010). Because the present result is preliminary, additional investigations are needed to understand whether and how the NPS and hypocretin systems interact.

4.3. Lack of Behavioral Sensitization

Repeated NPS injections did not lead to sensitized locomotor activity. Instead, a high dose of NPS resulted in reversible tolerance to NPS injections on locomotor activity. Therefore, unlike dopaminergic or other drugs of abuse, NPS does not seem to have any influence on sensitization-related plasticity. Although repeated injections of dopaminergic drugs lead to behavioral sensitization, the mechanism through which dopaminergic drugs acutely elicit locomotion and reward appears to be different from that leading to behavioral sensitization. Dopaminergic drugs' acute locomotor and rewarding effects are mediated via their action in the ventral striatum (Ikemoto, 2002, 2003; Ikemoto et al., 2005), while behavioral sensitization depends on their actions in the ventral tegmental area (Kalivas and Stewart, 1991; Kalivas and Weber, 1988; Vezina and Stewart, 1990). Therefore, unlike dopaminergic or other drugs of abuse, NPS does not seem to modulate the activity of this region in such a way to cause behavioral sensitization.

5. Conclusions

Intraventricular injections of NPS appear to have reward-like effects, and can facilitate seeking and elicit weak reinforcement, although these effects are not robust in any way. These effects of NPS may be mediated by its modest ability to stimulate the dopaminergic and hypocretin systems. In addition, unlike drugs of abuse, NPS appears to lack the ability to sensitize seeking-related behavior.

Acknowledgements

The present research was supported by the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health and the extramural research grant R01DA021880 (to LdL).

References

Bardo, M.T., Neisewander, J.L., Pierce, R.C., 1989. Novelty-induced place preference behavior in rats: effects of opiate and dopaminergic drugs. Pharmacol. Biochem. Behav. 32, 683–689.
Besheer, J., Jensen, H.C., Bevins, R.A., 1999. Dopamine antagonism in a novel-object recognition and a novel-object place conditioning preparation with rats. Behav. Brain Res. 103, 35–44.
Boutrel, B., Cannella, N., de Lecea, L., 2010. The role of hypocretin in driving arousal and goal-oriented behaviors. Brain Res. 1314, 103–111.
Cannella, N., Economidou, D., Kallupi, M., Stopponi, S., Heilig, M., Massi, M., Ciccocioppo, R., 2009. Persistent increase of alcohol-seeking evoked by neuropeptide S: an effect mediated by the hypothalamic hypocretin system. Neuropsychopharmacology 34, 2125–2134.
Hauber, W., 1996. Impairments of movement initiation and execution induced by a blockade of dopamine D1 OR D2 receptors are reversed by a blockade of N-methyl-d-aspartate receptors. Neuroscience 73, 121–130.
Ikemoto, S., 2002. Ventral striatal anatomy of locomotor activity induced by cocaine, d-amphetamine, dopamine and D1/D2 agonists. Neuroscience 113, 939–955.
Ikemoto, S., 2003. Involvement of the olfactory tubercle in cocaine reward: intracranial self-administration studies. J. Neurosci. 23, 9305–9311.
Ikemoto, S., 2007. Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens–olfactory tubercle complex. Brain Res. Rev. 56, 27–78.

Ikemoto, S., 2010. Brain reward circuitry beyond the mesolimbic dopamine system: a neurobiological theory. Neurosci. Biobehav. Rev. 35, 129–150.
Ikemoto, S., Sharpe, L.G., 2001. A head-attachable device for injecting nanoliter volumes of drug solutions into brain sites of freely moving rats. J. Neurosci. Methods 110, 135–140.
Ikemoto, S., Qin, M., Liu, Z.H., 2005. The functional divide for primary reinforcement of D-amphetamine lies between the medial and lateral ventral striatum: is the division of the accumbens core, shell and olfactory tubercle valid? J. Neurosci. 25, 5061–5065.
Kalivas, P.W., Stewart, J., 1991. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16, 223–244.
Kalivas, P.W., Weber, B., 1988. Amphetamine injection into the ventral mesencephalon sensitizes rats to peripheral amphetamine and cocaine. J. Pharmacol. Exp. Ther. 245, 1095–1102.
Kallupi, M., Cannella, N., Economidou, D., Ubaldi, M., Ruggeri, B., Weiss, F., Massi, M., Marugan, J., Heilig, M., Bonnavion, P., de Lecea, L., Ciccocioppo, R., 2010. Neuropeptide S facilitates cue-induced relapse to cocaine seeking through activation of the hypothalamic hypocretin system. Proc. Natl Acad. Sci. 107, 19567–19572.
Koob, G.F., Le, H.T., Creese, I., 1987. The D1 dopamine receptor antagonist SCH 23390 increases cocaine self-administration in the rat. Neurosci. Lett. 79, 315–320.
Leonard, S.K., Ring, R.H., 2011. Immunohistochemical localization of the neuropeptide S receptor in the rat central nervous system. Neuroscience 172, 153–163.
Niimi, M., 2006. Centrally administered neuropeptide S activates orexin-containing neurons in the hypothalamus and stimulates feeding in rats. Endocr. 30, 75–79.
Olsen, C.M., Winder, D.G., 2009. Operant sensation seeking engages similar neural substrates to operant drug seeking in C57 mice. Neuropsychopharmacology 34, 1685–1694.
Paneda, C., Huitron-Resendiz, S., Frago, L.M., Chowen, J.A., Picetti, R., de Lecea, L., Roberts, A.J., 2009. Neuropeptide S reinstates cocaine-seeking behavior and increases locomotor activity through corticotropin-releasing factor receptor 1 in mice. J. Neurosci. 29, 4155–4161.
Reinscheid, R.K., 2008. Neuropeptide S: anatomy, pharmacology, genetics and physiological functions. Results Probl. Cell Differ. 46, 145–158.
Rizzi, A., Vergura, R., Marzola, G., Ruzza, C., Guerrini, R., Salvadori, S., Regoli, D., Calo, G., 2008. Neuropeptide S is a stimulatory anxiolytic agent: a behavioural study in mice. Br. J. Pharmacol. 154, 471–479.
Shin, R., Cao, J., Webb, S.M., Ikemoto, S., 2010. Amphetamine administration into the ventral striatum facilitates behavioral interaction with unconditioned visual signals in rats. PLoS ONE 5, e8741.
Smith, K.L., Patterson, M., Dhillo, W.S., Patel, S.R., Semjonous, N.M., Gardiner, J.V., Ghatei, M.A., Bloom, S.R., 2006. Neuropeptide S stimulates the hypothalamo-pituitary-adrenal axis and inhibits food intake. Endocrinology 147, 3510–3518.
Sorge, R., Pierre, V., Clarke, P., 2009. Facilitation of intravenous nicotine self-administration in rats by a motivationally neutral sensory stimulus. Psychopharmacology 207, 191–200.
Stewart, J., Hurwitz, H.M.B., 1958. Studies in light-reinforced behaviour III. The effect of continuous, zero and fixed-ratio reinforcement. Q. J. Exp. Psychol. 10, 56–61.
Sutcliffe, J.G., de Lecea, L., 2002. The hypocretins: setting the arousal threshold. Nat. Rev. Neurosci. 3, 339–349.
Vezina, P., Stewart, J., 1990. Amphetamine administered to the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: lack of conditioned effects. Brain Res. 516, 99–106.
Xu, Y.L., Reinscheid, R.K., Huitron-Resendiz, S., Clark, S.D., Wang, Z., Lin, S.H., Brucher, F.A., Zeng, J., Ly, N.K., Henriksen, S.J., de Lecea, L., Civelli, O., 2004. Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron 43, 487–497.
Xu, Y.L., Gall, C.M., Jackson, V.R., Civelli, O., Reinscheid, R.K., 2007. Distribution of neuropeptide S receptor mRNA and neurochemical characteristics of neuropeptide S-expressing neurons in the rat brain. J. Comp. Neurol. 500, 84–102.