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Reinforcing Properties of Substance P in the Lateral Hypothalamus Revealed by Conditioned Place Preference

M.-S. HOLZHÄUER-OITZL, K. BOUCKE AND J. P. HUSTON

Institute of Physiological Psychology, University of Düsseldorf, Universitätsstrasse 1 D-4000 Düsseldorf, F.R.G.

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HOLZHÄUER-OITZL, M.-S., K. BOUCKE AND J. P. HUSTON. *Reinforcing properties of substance P in the lateral hypothalamus revealed by conditioned place preference.* PHARMACOL BIOCHEM BEHAV **28**(4) 511–515, 1987.—Reinforcing properties of substance P (SP) were investigated in rats using a conditioned place preference paradigm. After three baseline trials one conditioning trial of 10 min duration was performed. Either SP (100 pg, 1 ng, 100 ng) or saline (SI) was injected unilaterally into the lateral hypothalamus or a sham injection (OC) was given. Pairing one compartment with SP (100 pg, 1 ng) significantly increased the time spent in this compartment. Microinjections of 100 ng SP, saline or sham injection had no effect. Locomotor activity was not influenced by either treatment. These data are discussed in terms of (a) the possibility that SP has a role in mediating reinforcement and (b) the relationship between reinforcing effects and post-trial effects on learning and memory of SP applied into the lateral hypothalamus.

Substance P Lateral hypothalamus Positive reinforcement Conditioned place preference

SEVERAL studies have shown that intracranial and peripheral injections of the neuropeptide substance P (SP) can influence performance of avoidance and appetitive learning tasks [10, 19, 20]. For example, facilitation of performance was observed after post-trial injection of SP into the lateral hypothalamus/medial forebrain bundle (LH/MFB) but not ventromedial hypothalamus [9,21]; the injection of SP into this brain area has effects in learning paradigms comparable to the post-trial electrical stimulation of the LH/MFB. The LH/MFB is also known to support electrical self-stimulation. It has been suggested that a relationship exists between mnemonic and reinforcing effects of electrical brain stimulation [8]. We hypothesized that this is also the case for SP, i.e., that at brain sites where SP facilitates performance in learning paradigms it would also have positive reinforcing properties. Evidence that SP might play a role in central reward processes was first obtained with a multi-trial T-maze learning paradigm with which brain-site dependent reinforcing effects (either facilitating or mixed/aversive) were found [22].

The aim of the present study was to assess the reinforcing properties of SP microinjected into the LH/MFB by use of the conditioned place preference (CPP) technique. With the CPP technique used in the present experiment the positive reinforcing property of a substance is reflected in the greater amount of time the animal spends in the one compartment in which it had received the treatment.

METHOD

Animals

Male Wistar rats weighing 180 to 220 g at the time of surgery were used. They were housed two per cage with free access to food and water under a 12 hr light/12 hr dark regime. Behavioral observations were performed between 10.00 and 15.00 hr.

Surgery

Under Equi-Thesin anesthesia (3 ml/kg IP) the animals were unilaterally implanted with a guide cannula (stainless steel; gauge 26; 0.46 mm o.d.; 0.25 mm i.d.; 16.0 mm long) with the tip placed 2.0 mm above the injection site in the lateral hypothalamus. For injection the following coordinates were used [16]: AP −1.1/L ±1.6/V 8.0 mm. To prevent clogging of the cannula a wire mandrel that protruded from the guide cannula by 0.3 mm was inserted.

Apparatus

The apparatus for behavioral testing was a rectangular Plexiglas box divided into two compartments of equal size (25×35×30 cm) by a center alley. The black compartment had a rough wire mesh floor (3.5 mm squares), the white compartment a fine wire mesh floor (2.0 mm squares). The compartments were separable by guillotine doors (7×8 cm)

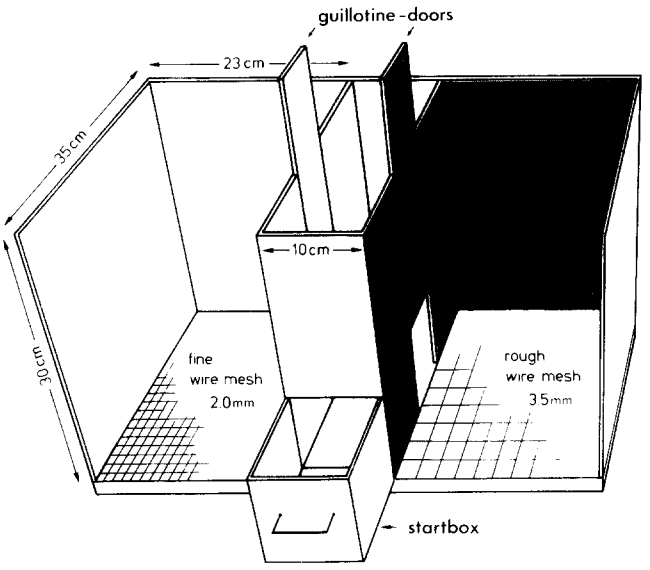

FIG. 1. Three-compartment box. Apparatus for evaluating place preference behavior in rats. A detailed description of the test box is provided in the text.

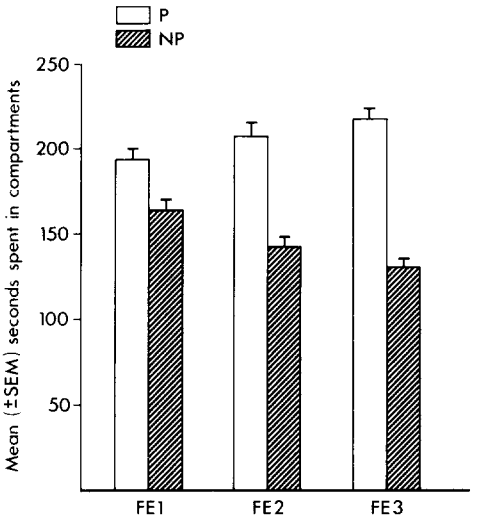

FIG. 2. Development of preference (preferred compartment: white columns, non-preferred compartment: hatched columns) over three baseline trials (FE1, FE2, FE3) for all animals (n=55). The time spent in each compartment was registered for 10 minutes (mean±SEM).

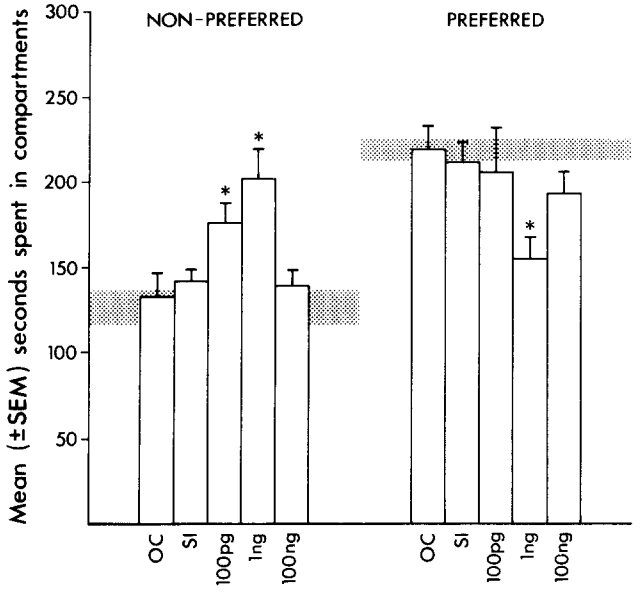

FIG. 3. Mean time (seconds) spent in the non-preferred and preferred compartments during the third baseline trial of all animals (dotted area depicts the mean with the range of the SEM; n=55) and on the day of testing for each group (white columns; mean±SEM). Animals were treated in their non-preferred compartment receiving one injection of substance P (100 pg, 1 ng or 100 ng) or saline (SI) in 0.5 μl volume unilaterally into the lateral hypothalamus; operated control animals (OC) were sham treated. (Mann Whitney U-test: *$p<0.0125$).

from the gray center alley with a transparent Plexiglas floor (9×35×30 cm; Fig. 1).

The animal was introduced into the apparatus by slowly moving the startbox into the gray center alley. The alley served as a choice point between the black and white compartments. After each trial the apparatus was swept out with water containing 0.1% acetic acid.

The testing device was set up in a closed experimental chamber, which was dimly lit. Masking noise (68 dB) was provided by a noise generator. The behavior of the rat could be monitored with the aid of a video camera mounted 100 cm above the testbox. Video monitor, registration device and observer were located outside the experimental chamber.

Drugs and Injection Procedure

Substance P (SP 1-11; Peninsula Labs., USA) was dissolved in physiological saline containing 0.01 M acetic acid, deep frozen in stock solutions of 200 μg SP/ml (pH 4.0) and diluted shortly before use. Materials in contact with SP were acid-washed.

Animals were assigned randomly to the five treatment groups. The following treatments were administered; 100 pg SP ($\hat{=}$0.074 pmol; n=10), 1 ng SP ($\hat{=}$0.742 pmol; n=12), 100 ng SP ($\hat{=}$74.195 pmol; n=11) or a saline control injection (SI—physiological saline containing 0.01 M acetic acid; n=12) in a volume of 0.5 μl unilaterally into the lateral hypothalamus. A further control group consisted of animals that were implanted with a cannula, but did not receive an injection (OC—sham injection; n=10).

For smooth intracranial application of substances the injection needle (gauge 33; 0.2 mm o.d.; 0.1 mm i.d.; 18.0 mm long) was connected via polyethylene tubing to a microsyringe (Hamilton 1 μl) fixed to an electrically driven pump. The injection lasted 30 sec; the needle was removed 30 sec after the end of injection. During this procedure the animal was loosely restrained by hand. Immediately afterwards the rat was placed into the testbox. Animals of the operated control group (OC) were restrained for one minute.

Behavioral Testing

On the fifth postoperative day behavioral testing was begun.

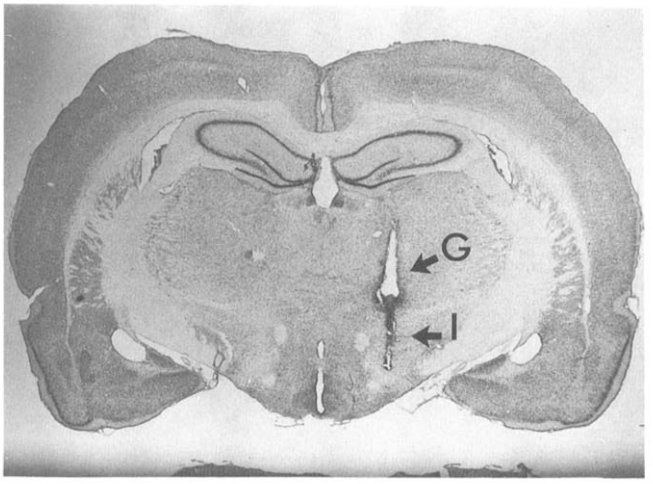

FIG. 4. Injection site in the lateral hypothalamus. Arrows point to the position of the guide cannula (G) and injection cannula (I).

(1) Baseline (FE1, FE2, FE3). On three consecutive days the animals were allowed to explore the apparatus for 10 min per day. The rat was placed into the testbox by slowly pushing the moveable startbox into the gray center alley. Both guillotine doors were opened. During the 10 min observation period the amount of time spent in the black and white compartments as well as the number of entries into the compartments were recorded. A rat was considered to be in either compartment only when all four paws were inside.

After the third baseline trial (FE3) the preference for one of the two compartments was calculated by taking the mean time spent in the compartments over the three baseline trials. The compartment in which the rat spent less time was called "non-preferred" (NP) and the other one "preferred" (P). Then the rats were assigned randomly to the treatment groups.

(2) Treatment (TR). Twenty-four hours after FE3 the treatment was adminstered: it consisted of a unilateral injection into the lateral hypothalamus of either SP (100 pg, 1 ng, 100 ng) or saline (SI) or a sham injection (group OC). Immediately afterwards the rat was placed into the center of the previously determined "non-preferred" compartment with the guillotine doors closed, and left there for 10 min.

(3) Test (TE). After 24 hours the rats were allowed to explore the apparatus with the guillotine doors opened for 10 min. Behavior was registered as during the baseline trials.

Histology

The animals were perfused with saline and a 10% formalin-sucrose solution. After postfixation of the brain in a 30% formalin-sucrose solution for 2 to 4 days frozen sections (40 μm) were taken and stained with cresyl violet. Data were used only from animals with histologically confirmed correct placement of the injection cannula and animals which had gained their preoperative weight on the day of the third baseline trial.

Statistics

Analysis of data was performed using the Mann-Whitney U-test to compare the time spent in the non-preferred compartment on the day of testing during the 10 min observation period (one tailed: groups 100 pg, 1 ng, 100 ng SP versus SI; two tailed: SI versus OC). In order to avoid Type I error, which is known to vary with the number of tests conducted, the level of significance was adjusted for four tests to $\alpha=0.05/4=0.0125$. Data of the 10 min test period were grouped into two blocks of 5 minutes. Possible time-dependent influences of the treatment were compared post hoc with the Mann-Whitney U-test (one-tailed: SP groups vs. SI).

RESULTS

During baseline trials the rats developed a preference for either the black or white compartment (Fig. 2). Only 2 out of the 55 rats spent more time in the white than in the black compartment. The total time spent in both compartments did not change over days. On FE3 (i.e., trial prior to treatment) the mean amount of time spent in the non-preferred compartment (NP) ranged from 121 to 139 sec, in the preferred compartment (P) from 211 to 238 sec.

Successful place conditioning is indicated by a significant increase in the amount of time spent in the compartment in which the animals were subjected to treatment. Figure 3 depicts the time spent in NP and P for the pre- and postconditioning trials. A significant increase in time spent in the previously NP was registered in animals injected with 100 pg SP ($U=25$, $p=0.0105$) and 1 ng SP ($U=10$, $p=0.0001$). Rats treated with 1 ng SP changed their place preference; for this group a post hoc analysis revealed a decrease in time spent in the previously preferred compartment (Mann-Whitney U-test; $U=23$, $p=0.0023$). Injections of 100 ng SP ($U=51$, $p=0.1779$), saline and the sham injection did not influence the baseline preference behavior of the animals. A comparison of saline and sham injections showed no effects (SI versus OC; $U=59$, $p=0.9474$). Figure 4 shows a representative injection site in the lateral hypothalamus.

Substance P exerted a dose-dependent effect in place conditioning: 100 pg SP increased the time spent in the drug-paired compartment, 1 ng SP changed the place preference to this side and 100 ng SP had no effect.

For post hoc analysis the observation period of 10 min was divided into two blocks of 5 min (min 0–5; min 6–10; see

TABLE 1

MEAN TIME (±SEM) IN SECONDS SPENT IN THE NON-PREFERRED COMPARTMENT BEFORE (FE3) AND AFTER (TE) THE SINGLE CONDITIONING TRIAL

	Baseline (FE3)		Test (TE)	
	Min 0–5	Min 6–10	Min 0–5	Min 6–10
100 pg SP	67.00 ± 5.28	64.83 ± 9.19	94.10 ± 4.64*	81.32 ± 14.11
1 ng SP	66.31 ± 4.48	64.82 ± 8.58	89.68 ± 6.47	112.82 ± 12.02*
100 ng SP	69.58 ± 6.87	70.33 ± 8.23	87.95 ± 9.93	51.86 ± 11.14
SI	65.58 ± 5.81	65.60 ± 2.64	75.33 ± 4.66	66.84 ± 6.82
OC	60.15 ± 8.53	60.93 ± 8.79	71.42 ± 7.21	61.69 ± 9.23

The observation period of 10 min was divided into two blocks of 5 min. Abbreviations for groups: see Fig. 3.
*$p<0.05$.

TABLE 2

NUMBER OF ENTRIES INTO THE COMPARTMENTS (MEAN ± SEM) BEFORE (FE3) AND AFTER (TE) TREATING THE ANIMALS IN THEIR NON-PREFERRED COMPARTMENT

	SP 100 pg	SP 1 ng	SP 100 ng	SI	OC
FE3	21.40 ± 1.69	21.33 ± 1.66	22.18 ± 1.12	18.58 ± 1.54	19.30 ± 2.23
TE	20.00 ± 1.81	23.33 ± 2.21	21.09 ± 2.55	20.83 ± 1.51	22.80 ± 2.54

Abbreviations for groups: see Fig. 3.

Table 1). During the third baseline trial (FE3) animals of all groups spent a comparable amount of time in NP during both 5 min periods. On the day of testing (TE) all groups increased their time spent in NP during the first 5 min. Rats injected with SP spent more time in NP (100 pg SP: $U=22, p=0.0061$; 1 ng SP: $U=48, p=0.0829$; 100 ng SP: $U=43, p=0.0785$) than saline controls. In the second 5 min of the observation period rats treated with 100 ng SP decreased ($U=47, p=0.1211$) and animals injected with 1 ng and 100 pg SP increased their time spent in the drug-paired compartment (1 ng SP: $U=25$, $p=0.0023$; 100 pg SP: $U=51, p=0.2764$).

Locomotor Activity

The mean number of entries into the compartments increased over baseline trials (n=55; FE1: 17.4, FE2: 19.1, FE3:20.5). There was no difference between groups before (FE3) and after treatment (TE; see Table 2).

DISCUSSION

The results of the present study provide further evidence for a role of substance P (SP) in the mediation of positive reinforcement. Place preference could be produced by SP with a single conditioning trial; the unilateral injection of 1 ng SP into the LH/MFB of rats resulted in the animals favouring the previously non-preferred compartment. The effect of 100 pg SP was seen as an increase in time the rats spent in the drug-paired compartment whereas 100 ng SP were ineffective in influencing place preference behavior.

Although animals of all groups increased the amount of time spent in the non-preferred compartment during the first 5 min on the day of testing, this effect was more pronounced in SP-treated animals. Animals injected with 1 ng SP showed a further increase in the time spent in the drug-paired compartment, whereas the positive effect of 100 pg SP declined during the 2nd 5 min. However, animals injected with 100 ng SP tended to avoid the drug-paired compartment. It can be concluded that there exists a small critical range for the positive reinforcing property of SP in the LH/MFB; perhaps higher doses of SP are aversive. Similar dose-response effects of SP have been obtained for the performance of various inhibitory avoidance tasks [11, 19, 21, 24].

Injections of μg-amounts of SP into the brain have been reported to induce behavioral activation [4,15]. None of the doses of SP used in the present study led to observable behavioral changes after injection. Gross locomotor activity, expressed as the number of entries into both compartments and the drug-paired compartment, respectively, was not influenced by treatment conditions on the day of testing. SP-treated animals increased the amount of time spent in the drug-paired compartment during each entry, rather than increasing the number of entries into this compartment per se.

It appears that SP can have positive reinforcing as well as aversive properties depending on the site of action in the brain. SP has been discussed to play a role in the transmission of pain [6]. The stable analog of the SP-fragment SP5-11 (DiMeC7) was reported to have aversive properties after ICV administration tested in a place preference paradigm [3]. Similary, ICV self-administration of SP has not been found to be positively reinforcing [23]. Electrical self-stimulation of the LH was reduced after the injection of SP into the LH [5]. Others reported a dose-related decrease of electrical self-stimulation in the prefrontal cortex after ICV or intracortical injection of μg-amounts of SP [14]. The results of the present study are in accordance with the previous finding that the injection of SP into the LH/MFB and septum facilitated

learning of a multiple-trial T-maze task; injections of SP into the amygdala and substantia nigra had variable or aversive effects [22].

Biochemical and behavioral studies have also shown that SP interacts with other transmitter systems and/or hormonal processes [2, 7, 12, 17]. In CPP studies dopaminergic and cholinergic substances and opioids have been demonstrated to have positive reinforcing properties [1, 18, 25]. It is possible that the effects produced by SP are, at least in part, mediated via other neuropharmacological systems. However, local vasoactive effects may be related to the positive reinforcing property of SP in the LH/MFB. SP produces vasodilation of hypothalamic blood vessels resulting in increased hypothalamic blood flow. This is probably mediated via an adrenergic/cholinergic mechanism [13].

SP could play a role in reinforcement either by activating the reinforcement system directly or by activating a specific reward system. The site-dependent properties of SP, to act as reinforcer as well as to influence performance in various learning tasks, parallel the properties of electrical brain, stimulation. In this respect our data support the hypothesis of a relationship between mnemonic and reinforcing effects of SP in accordance with predictions from a model of reinforcement [8] that post-trial positive reinforcers (e.g., electrical brain stimulation or SP) facilitate learning and memory.

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REFERENCES

1. Carr, G. D. and N. M. White. Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. *Life Sci* **33:** 2551–2557, 1983.
2. Crawley, J. N., J. A. Olschowka, D. I. Diz and D. M. Jacobowitz. Behavioral investigation of the coexistence of substance P, corticotropin releasing factor, and acetylcholinesterase in lateral tegmental neurons projecting to the medial frontal cortex of the rat. *Peptides* **6:** 891–901, 1985.
3. Elliott, P. J. and S. D. Iversen. Reinforcing properties of Di-MeC7 using a place conditioning procedure in rats. In: *Substance P*, edited by P. Skrabanek and D. Powell. Dublin: Boole Press, 1983, pp. 49–50.
4. Elliott, P. J. and S. D. Iversen. Behavioural effects of tachykinins and related peptides. *Brain Res* **381:** 68–76, 1986.
5. Goldstein, J. M. and J. B. Malick. Effect of substance P on medial forebrain bundle self-stimulation in rats following intracerebral administration. *Pharmacol Biochem Behav* **7:** 475–478, 1977.
6. Henry, J. L. Substance P and pain: an updating. *Trends Neurosci* **3:** 95–97, 1980.
7. Hisano, S., Y. Okamura and S. Daikoku. Synaptic regulation of hypothalamic neurons containing ACTH by substance P. *Brain Res* **308:** 162–165, 1984.
8. Huston, J. P., C. C. Mueller and C. Mondadori. Memory facilitation by post-trial hypothalamic stimulation and other reinforcers: a central theory of reinforcement. *Biobehav Rev* **1:** 143–150, 1977.
9. Huston, J. P. and U. Stäubli. Post-trial injection of substance P into lateral hypothalamus and amygdala, respectively, facilitates and impairs learning. *Behav Neural Biol* **27:** 244–248, 1979.
10. Huston, J. P. and U. Stäubli. Substance P and its effects on learning and memory. In: *Endogenous Peptides and Learning and Memory Processes*, edited by J. L. Martinez, R. A. Jensen, R. B. Messing, H. Rigter and J. L. McGaugh. New York: Academic Press, 1981, pp. 521–540.
11. Kafetzopoulos, E., M.-S. Holzhäuer and J. P. Huston. Substance P injected into the region of the nucleus basalis magnocellularis facilitates performance of an inhibitory avoidance task. *Psychopharmacology (Berlin)* **90:** 281–283, 1986.
12. Kelley, A. E. and S. D. Iversen. Substance P infusions into substantia nigra of the rat: behavioural analysis and involvement of striatal dopamine. *Eur J Pharmacol* **60:** 171–179, 1979.
13. Klugman, K. P., F. Lembeck, S. Markowitz, G. Mitchell and C. Rosendorff. Substance P increases hypothalamic blood flow via an adrenergic-cholinergic interaction. *Br J Pharmacol* **71:** 623–629, 1980.
14. Mora, F. and J. M. R. Ferrer. Is substance P involved in self-stimulation (SS) of the prefrontal cortex in the rat. *Neurosci Lett [Suppl]* **18:** S356, 1984.
15. Naranjo, J. R. and J. DelRio. Locomotor activation induced in rodents by substance P and analogues. *Neuropharmacology* **23:** 1167–1171, 1984.
16. Pellegrino, L. J., A. S. Pellegrino and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain*. New York: Plenum Press, 1979.
17. Pernow, B. Substance P. *Pharmacol Rev* **35:** 85–141, 1983.
18. Phillips, A. G. and F. G. LePiane. Reward produced by microinjection of (D-Ala2), Met5-enkephalinamide into the ventral tegmental area. *Behav Brain Res* **5:** 225–229, 1982.
19. Schlesinger, K., D. U. Lipsitz, P. L. Peck, M. A. Pelleymounter, J. M. Stewart and T. N. Chase. Substance P enhancement of passive and active avoidance conditioning in mice. *Pharmacol Biochem Behav* **19:** 655–661, 1983.
20. Schlesinger, K., M. A. Pelleymounter, J. van de Kamp, D. L. Bader, J. M. Stewart and T. N. Chase. Substance P facilitation of memory: effects in an appetitively motivated learning task. *Behav Neural Biol* **45:** 230–239, 1986.
21. Stäubli, U. and J. P. Huston. Differential effects on learning by ventromedial vs. lateral hypothalamic posttrial injection of substance P. *Pharmacol Biochem Behav* **10:** 783–786, 1979.
22. Stäubli, U. and J. P. Huston. Central action of substance P: possible role in reward. *Behav Neural Biol* **43:** 100–108, 1985.
23. Stein, L. and J. D. Belluzzi. Brain endorphins: possible role in reward and memory formation. *Fed Proc* **38:** 2468–2472, 1979.
24. Tomaz, C. and J. P. Huston. Facilitation of conditioned inhibitory avoidance by post-trial peripheral injection of substance P. *Pharmacol Biochem Behav* **25:** 469–472, 1986.
25. Yeomans, J. S., O. Lofman and V. McFarlane. Cholinergic involvement in lateral hypothalamic rewarding stimulation. *Brain Res* **329:** 19–26, 1985.