Table 2. Effects of bromocriptine (CB-154) injections on testicular testosterone (T) levels and lipid fractions in mature rats (means ± SD)

Parameter	Concentration/g testis			Content/total testes		
	CB-154-injected	Control	Difference (%)	CB-154-injected	Control	Difference (%)
T (ng)	229 ± 55	292 ± 49	− 21.5*	710 ± 183	922 ± 38	− 23.0**
Total lipids (mg)	11.28 ± 1.60	9.2 ± 3.40	+ 23.6	34.5 ± 3.04	28.36 ± 8.22	+ 21.8*
Phospholipids (mg)	0.89 ± 0.09	1.27 ± 0.36	− 29.0**	2.70 ± 0.46	3.81 ± 1.27	− 29.0*
Cholesterol						
Esters (mg)	0.080 ± 0.019	0.052 ± 0.032	+ 54.0*	0.248 ± 0.061	0.163 ± 0.102	+ 51.7*
Free (mg)	0.812 ± 0.054	0.792 ± 0.041	+ 2.4	2.507 ± 0.248	2.544 ± 0.324	− 1.4

*Significant at 5% level; **significant at 1% level.

The decreased androgen levels in the testes are consistent with the decreased peripheral T titers and suggest that the reduction in plasma T in CB-154-treated animals was not due to increased metabolic clearance of T.

During active spermatogenesis and germ cell development, the testicular phospholipids have been reported to increase, with a decrease in neutral lipids[19-21]. Thus, the observed increase in total lipids content with decrease in the phospholipids fraction would suggest that CB-154 treatment can produce conditions unfavorable for normal spermatogenesis.

In summary, bromocriptine treatment of adult male rats resulted in a decrease in testicular T formation with a consequent reduction in plasma androgen levels. These effects were accompanied by changes in testicular total lipid, phospholipid and cholesterol content and seemed related to reduced testicular responsiveness to LH, since plasma LH levels were not suppressed.

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Influence of substance P and fragments on passive avoidance behavior

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Summary. N-terminal and C-terminal fragments of substance P (SP) have been shown to exert opposite effects on antinociception, grooming and fighting in mice. The present experiments explored whether these findings could be generalized to passive avoidance behavior. Substance P (SP-(1-11)) and the C-terminal fragment pyroglutamyl-SP-(7-11) attenuated passive avoidance behavior when picogram amounts were injected into the nucleus accumbens. In contrast, the N-terminal fragment SP-(1-7) had an opposite effect and facilitated passive avoidance behavior.

Brain peptides are precursor molecules of neuropeptides with different, opposite and selective CNS activities. For example, β-endorphin is a precursor of γ- and α-endorphin and their respective fragments (DTγE, DEγE, DTαE) which exert opposite effects on extinction of active and passive avoidance behavior[1]. Recently, Hall and Stewart[2] reported that the N-terminal SP-(1-7) and C-terminal pyroglutamyl-SP-(7-11) fragments of substance P exert opposite effects on several behavioral paradigms in mice. The present experiments were carried out to explore whether substance P and the N- and C-terminal fragments would also exhibit opposite effects in a learning paradigm.

Materials and methods. Animals. Male Wistar rats weighing 130-140 g were used. They were maintained under controlled conditions with a 12:12 light/dark cycle (light on between 07.00 h and 19.00 h), and received food and water ad libitum.

Implantation of cannulae into the brain. Rats were anesthetized with Hypnorm® and were secured in a stereotaxic instrument. Stainless steel cannulae (0.6 mm outer diame-

ter, 0.3 mm inner diameter) were implanted on the right side of the brain and aimed at the nucleus accumbens. The coordinates according to Pellegrino and Cushman[3] were 2.6 mm anterior to bregma, 2.7 mm lateral to the midline, 6.1 mm below the dura at the point of penetration and the cannulae were inserted at an angle of 12°. After operation the rats were housed in separate cages and allowed to recover from the operation for at least 7 days.

Passive avoidance behavior. Animals were trained in a step-through type one-trial learning passive avoidance test[4]. The training was started between 09.00 h. and 14.00 h. The experimental apparatus consisted of an illuminated platform attached to a large, dark compartment equipped with a grid floor. After habituation to the dark compartment (2 min), rats were placed on the platform and allowed to enter the dark compartment; since rats prefer dark to light, they normally enter within 15 sec. On the next day after 3 more trials, an unavoidable scrambled footshock (0.35 mA, 2 sec) was delivered through the grid floor of the dark compartment (learning trial). The median entrance latencies before the learning trial for the different groups in the various experiments ranged from 3 sec to 10 sec, and no differences were present among the various groups. Rats were removed from the shock box 10 sec after the termination of the footshock. Passive avoidance latencies were tested 24 h and 48 h after the learning trial; the rat was placed on the platform and the latency to enter the dark compartment was measured up to a maximum of 300 sec.

Peptides and injection procedure. Substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-amide) SP) and the fragments SP-(1-7) (Arg-Pro-Lys-Pro-Gln-Gln-Phe) and SP-(7-11) (pyroGlu-Phe-Phe-Gly-Leu-Met-amide) were used. Peptides were synthesized and purified by previously described methods[5,6]. They were dissolved in saline (0.9% NaCl; pH 6.5-6.7). The rats were injected using a Hamilton syringe by inserting a needle (0.25 mm outer diameter) into the guide cannula. 1 µl containing peptide or saline was given 1 h before the first retention test. Doses ranging from 1 pg/rat to 30 pg/rat were used.

Histological control. After experimentation the injection sites were evaluated histologically. The rats were sacrificed and the brains fixed in 4% formalin. Serial sections with a thickness of 100 µm were cut on a cryostat. The sites of injections were determined microscopically using the atlas of Pellegrino and Cushman[3]. Data from animals with injection sites outside the nucleus accumbens were discarded from the analyses.

Statistical analysis. Passive avoidance latencies were analyzed with ANOVA-testing (Kruskal-Wallis) and subsequently with Mann-Whitney non-parametric tests.

Results. Both SP and pyroglutamyl-SP-(7-11) attenuated passive avoidance behavior, while SP-(1-7) facilitated passive avoidance behavior following injection of picogram amounts into the nucleus accumbens 1 h prior to the retention test (table). The lowest dose of SP which significantly attenuated passive avoidance behavior at the 24 h (first) retention test was 3 pg. A dose of 10 pg had a somewhat stronger effect which was also demonstrable at the 2nd retention test. The fragment pyroglutamyl-SP-(7-11) was somewhat less potent than SP. The only dose of pyroglutamyl-SP-(7-11) which significantly attenuated passive avoidance behavior was 10 pg. SP-(1-7) facilitated passive avoidance behavior both at the 24 h and the 48 h. retention. Again only the 10-pg dose exerted a statistically significant effect. Passive avoidance behavior was not affected in non-shocked rats following injection of 10 pg of SP, SP-(1-7) or pyroglutamyl-SP-(7-11) into the nucleus accumbens (table). Thus, the peptides did not exert effects on locomotion in untrained animals.

Effect of graded doses of substance P and fragments on retention of one trial learning passive avoidance response, following unilateral injection into the nucleus accumbens 1 h before the first retention test

Treatment and dose per rat	Number of animals	Latency (median sec) 1st retention test 24 h[a]		2nd retention test 48 h	
Substance P-(1-11)					
1 pg	6	42	(18- 72)[c]	43	(15-69)
3 pg	12	28*	(17- 59)	36	(12-77)
10 pg	6	20*	(8- 28)	25*	(3-37)
30 pg	6	41	(15- 71)	37	(12-82)
Placebo[b]	18	80	(36-101)	69	(31-79)
Substance P-(1-7)					
3 pg	8	100	(16-137)	83	(11- 87)
10 pg	6	120*	(54-183)	155*	(70-300)
30 pg	6	101	(27-170)	83	(21-147)
Placebo	14	76	(38-131)	59	(24-102)
Substance P-(7-11)					
3 pg	8	37	(7- 87)	36	(6- 73)
10 pg	6	13**	(8- 21)	38	(3- 63)
30 pg	6	49	(22- 60)	39	(30- 53)
Placebo	14	76	(38-131)	59	(24-102)
No shock, 10 pg					
Substance P-(1-11)	6	5	(2-7)	3	(2-5)
Substance P-(1-7)	6	4	(3-6)	3	(2-4)
Substance P-(7-11)	6	4.5	(4-8)	3.5	(3-6)
Placebo	6	6.5	(4-7)	3	(2-5)

[a] h after learning trial; [b] 1 µl saline; [c] latency values are given as median in sec, within brackets the 25th and 75th percentile; * different from placebo-treated rats (*$p < 0.05$; **$p < 0.02$; Mann-Whitney U-test).

Discussion. Substance P and the C-terminal fragment pyroglutamyl-SP-(7-11) attenuated passive avoidance behavior. Hecht et al.[7] failed to find an effect of SP on avoidance acquisition of Wistar rats but a C-terminal hexapeptide analog normalized slow avoidance acquisition in spontaneously hypertensive rats.

The N-terminal part SP-(1-7) facilitated passive avoidance behavior. Whether this effect is exerted through the same mechanism or not cannot be determined as yet. However, SP-(1-7) is the first peptide which we found to facilitate passive avoidance behavior after microinjection into the nucleus accumbens. Peptides like α-endorphin, β-endorphin and vasopressin facilitate passive avoidance behavior following systemic or i.c.v. administration or following microinjection into various brain sites. Nevertheless, they are inactive after microinjection into the nucleus accumbens[8,9].

The present results agree with observations summarized by Hall and Stewart[2]. These authors found that SP and SP-(1-7) reduced isolation-induced fighting in mice while pyroglutamyl-SP-(7-11) had an opposite effect. Grooming in male mice was increased by SP and pyroglutamyl-SP-(7-11) while SP-(1-7) decreased grooming behavior. SP and SP-(1-7) induced mild antinociception. These authors noted that the C-terminal peptide generally has an excitatory action, while the N-terminal peptide is inhibitory. The present study shows the opposite in that the C-terminal had an inhibitory action in passive avoidance behavior although the effect could be due to a stimulatory effect on DA autoreceptors in the nucleus accumbens[9].

The nucleus accumbens seems to be highly sensitive to γ-type endorphins, ceruletide, sulphated CCK-8 and desulphated CCK-8 as determined by attenuation of passive avoidance behavior and inhibition of apomorphine-induced hypolocomotion[8,10]. These effects have been attributed to the neuroleptic-like influence of these neuropeptides presumably directed towards DA autoreceptors in this structure. It is surprising that SP and pyroglutamyl-SP-(7-

11) exert the same effect as γ-type endorphins, which suggests that these peptides may also interact with DA auto-receptors in the nucleus accumbens. Some evidence for an influence on dopamine transmission is available, since Glowinski et al.[11] have shown that SP and SP-(4-11) stimulate the release of DA from nerve terminals in the caudate nucleus of the rat, and SP inhibits apomorphine induced hypolocomotion in rats (Van Ree et al., personal communication).

The biotransformation of β-endorphin or vasopressin and oxytocin has been shown to generate powerful neuropeptides with different, opposite, selective and more potent effects[12]. It is possible that SP requires processing by enzymatic cleavage to active moieties which elicit the various behavioral effects that have been found in the present experiments and those reported by Stewart et al.[6].

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Effect of cadmium chloride on steroidogenic enzymes in the Bidder's organ of the toad *(Bufo melanostictus)*

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Summary. Injection of cadmium chloride in a toad increases both the Δ^5-3β-hydroxy-steroid dehydrogenase and 17β-hydroxysteroid dehydrogenase activities in Bidder's organ.

Although Bidder's organ in the male toad has been considered as a rudimentary ovary containing corpus luteum[1] and follicle[2], little is known about the occurrence of steroid-forming enzymes in this organ in *Bufo melanostictus.* In vitro studies have shown that the Bidder's organ of *Bufo vulgaris* can synthesize steroids like the ovary of the same species[3]. Previous studies also indicate that the removal of both testes stimulates of Bidder's organ to change into a functional ovary[4] while administration of testosterone results in atrophy of the same organ[2].

Cadmium chloride is known to inhibit spermatogenesis[5] and testicular 17β-hydroxysteroid dehydrogenase activity in toads[6]. The present experiments were undertaken to demonstrate the activities of Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) and 17β-hydroxysteroid dehydrogenase(17β-HSD) in the Bidder's organ of normal toads and those treated with cadmium chloride.

Materials and methods. For the present investigation, 330 male toads *(B. melanostictus)* of average weights 50-60 g were collected from their natural environment during the breeding season (August). The animals were divided equally into 2 groups. A single s.c. injection of 0.5 mg of cadmium chloride, dissolved in 0.2 ml amphibian saline, was given to one group of animals while the remaining group received vehicle only. All the animals were provided with food (ant eggs) ad libitum every other day and were sacrificed after 7 days. 320 animals were used for biochemical estimation of Δ^5-3β-HSD and 17β-HSD activity, and 10 animals were used for histochemical demonstration. For estimation of Δ^5-3β-HSD and 17β-HSD, both control and treated groups were divided equally and the Bidder's organs of 10 animals were pooled in both control and treated animals for estimation of each enzyme activity.

For assay of Δ^5-3β-HSD activity, the Bidder's organs were removed and dropped into ice cold homogenizing medium consisting of equal parts of 0.65% sodium chloride and 0.1 M sodium phosphate buffer, pH 7.4, to give a tissue concentration of 5 mg/ml. The enzyme was assayed by spectrophotometric measurement of the production of Δ^4-androstenedione from dehydroepiandrosterone (DHEA)[7].

The activity of 17β-HSD was measured by the method of Jarabak et al.[8]. Pooled Bidder's organs were homogenized in 20% spectroscopic grade glycerol, 5 mM potassium phosphate and 1 mM EDTA and centrifuged at 10,000 × g for 30 min. 1 ml of the supernatant was mixed with 440 µmoles sodium pyrophosphate buffer, pH 10.2; 25 mg crystalline bovine serum albumin and 0.3 µmoles testosterone. The enzyme activity after addition of 1.1 µmoles NADP was measured spectrophotometrically at 340 nm against a blank (without NADP). One unit of enzyme

Effect of cadmium chloride on Δ^5-3β-HSD and 17β-HSD activities in Bidder's organ of toad

Treatment	Δ^5-3β-HSD activity nmoles/mg tissue/h	17β-HSD activity units/mg tissue/h
Control	3.9 ± 0.44	16.84 ± 1.64
Cadmium	6.62 ± 0.88	26.68 ± 1.98

Each value represents mean ± SD. $p < 0.01$ (Wilcoxon test) control vs cadmium, where N = 8.