The action of kisspeptin-13 on passive avoidance learning in mice. Involvement of transmitters

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Abstract

Kisspeptins are G protein-coupled receptor ligands originally identified as human metastasis suppressor gene products that have the ability to suppress melanoma and breast cancer metastasis and recently found to play an important role in initiating the secretion of gonadotropin-releasing hormone at puberty. Kisspeptin-13 is an endogenous isoform that consists of 13 amino acids.

The action of kisspeptin in the regulation of gonadal function has been widely studied, but little is known as concerns its function in limbic brain structures. In the brain, the gene is transcribed within the hippocampal dentate gyrus.

This paper reports on a study the effects of kisspeptin-13 on passive avoidance learning and the involvement of the adrenergic, serotonergic, cholinergic, dopaminergic and GABA-A-ergic, opiate receptors and nitric oxide in its action in mice. Mice were pretreated with a nonselective α-adrenergic receptor antagonist, phenoxybenzamine, an α_2-adrenergic receptor antagonist, yohimbine, a β-adrenergic receptor antagonist, propranolol, a mixed $5\text{-}HT_1$/$5\text{-}HT_2$ serotonergic receptor antagonist, methysergide, a nonselective $5\text{-}HT_2$ serotonergic receptor antagonist, cyproheptadine, a nonselective muscarinic acetylcholine receptor antagonist, atropine, D_2,D_3,D_4 dopamine receptor antagonist, haloperidol, a γ-aminobutyric acid subunit A ($GABA_A$) receptor antagonist, bicuculline, naloxone, a nonselective opioid receptor antagonist and nitro-L-arginine, a nitric oxide synthase inhibitor. Kisspeptin-13 facilitated learning and memory consolidation in a passive avoidance paradigm. Phenoxybenzamine, yohimbine, propranolol, methysergide, cyproheptadine, atropine, bicuculline and nitro-L-arginine prevented the action of kisspeptin-13 on passive avoidance learning, but haloperidol and naloxone did not change the effects of kisspeptin-13. The results demonstrated that the action

of kisspeptin-13 on the facilitation of passive avoidance learning and memory consolidation is mediated, at least in part, through interactions of the α_2-adrenergic, beta-adrenergic, 5-HT$_2$ serotonergic, muscarinic cholinergic and GABA-A-ergic receptor systems and nitric oxide.

Keywords: Kisspeptin-13; receptors; passive avoidance learning.

1. Introduction

Kisspeptin (KP), formerly known as metastin, was originally identified as a human metastasis suppressor that inhibits the metastasis of melanoma and breast cancer [1]. This G protein-coupled receptor ligand was recently found to play important roles in the maturation and functioning of the reproductive axis, including the sexual differentiation of the brain, the timing of puberty, the regulation of the gonadotropin-releasing hormone (GnRH) at puberty, and the control of fertility by metabolic and environmental cues [2]. KP-13, one of the endogenous isoforms, consists of 13 amino acids [3]. In the central nervous system, KP is transcribed within the hippocampal dentate gyrus [4-6]. Little is known concerning the mechanisms and pathways of the action of KP-13 on the brain functions. We recently reported that KP-13 elicits antidepressant action in a forced swimming test in mice [7]. However no data have been published regarding the action of kisspeptin-13 on the cognitive function.

In the present investigation, the effects of KP-13 were studied on passive avoidance learning and the potential involvement of the adrenergic, serotonergic, cholinergic, dopaminergic and gabaergic, opiate and nitric oxide receptors in mice. Mice were pretreated with a nonselective α-adrenergic receptor antagonist, phenoxybenzamine, an α_2-adrenergic receptor antagonist, yohimbine, a β-adrenergic receptor antagonist, propranolol, a mixed 5-HT$_1$/5-HT$_2$ serotonergic

receptor antagonist, methysergide, a nonselective 5-HT$_2$ serotonergic receptor antagonist, cyproheptadine, a nonselective muscarinic acetylcholine receptor antagonist, atropine,a D$_2$,D$_3$,D$_4$ dopamine receptor antagonist, haloperidol, or a γ-aminobutyric acid subunit A (GABA$_A$) receptor antagonist, bicuculline, naloxone, a nonselective opioid receptor antagonist, and a nitric oxide synthase inhibitor, nitro-L-arginine.

2. Materials and methods

2.1. Animals

CD$_1$ (Charles Dawley) male mice (Bioplan Isaszeg, Hungary) were kept and handled during the experiments in accordance with the instructions of the University of Szeged Ethical Committee for the Protection of Animals in Research. Each animal was used in the experiments only once. The animals were six week old, weighed between 28- 35 g. They were housed in cages (5 animals/cage) in a room maintained at constant temperature (25 ± 1 °C) and on a 12-h d ark–light cycle (lights on at 06:00–18:00 h) with free access to tap water and standard laboratory food. One week of recovery from surgery was allowed before the experiments.

2.2. Surgery

The mice were anesthetized with sodium pentobarbital (Nembutal 35 mg/kg i.p.) implanted with a cannula introduced into the right lateral brain ventricle in order to allow intracerebroventricular (*i.c.v.*) administration. The polystyrene cannula was inserted stereotaxically into the ventricle at the coordinates 0.2 mm posterior, 0.2 mm lateral to the bregma, and 2.0 mm deep from the dural surface . The cannula was secured with cyanoacrylate (Ferrobond) (Budapest, Hungary). The mice were allowed 7 days to recover from surgery before any *i.c.v.* administration. The correct location of the cannula was checked by dissecting the brain following completion of the

experiments. Only animals with the correct location of the cannula were used in the evaluation of the experiments. All experiments were performed in the morning period.

2.3. Drugs

KP-13 was from Bachem (Basel, Switzerland); phenoxybenzamine hydrochloride from Smith Kline & French (Herts, UK); yohimbine hydrochloride from Tocris (Cologne, Germany); propranolol hydrochloride from ICI Ltd. (Macclesfield, UK); methysergide hydrogenmaleate from Sandoz (Cologne, Germany); cyproheptadine hydrochloride from Tocris (Bristol, UK); atropine sulfate from EGYS (Budapest, Hungary); haloperidol from G. Richter (Budapest, Hungary); and bicuculline methiodide from Sandoz (Basel, Switzerland). Nitroω-L-Arginine methylester hydrochloride (Sigma St Louis USA). Naloxone hydrochloride (Endo Labs, Wilmington USA).

KP-13 was lyophilized in a quantity of 10 μg per ampoule and stored at –20 ℃. Immediately before the experiments, the KP-13 was dissolved in sterile pyrogen-free 0.9% saline and administered *i.c.v.* via the cannula in a volume of 2 μl.

2.4. Treatments

For the i.c.v administration of kisspeptine-13 and nitro-L arginine, the previously inserted polystyrene canulla was used

The receptor blockers were dissolved in 0.9 % saline and were administered intraperitoneally (i.p.). The effective doses of the receptor antagonists were selected on the basis of previous experiences in which the minimal doses were effective (in other tests), but did not themselves influence the tests [7,8]. The receptor blockers were administered Immediately following the learning trial i.p and 30 min later the Kisspeptin-13, the control mice were treated with 0.9% saline (on each occasion with

2 μl i.c.v). Immediately following the learning trial, the kisspeptin-13 group was treated with 0.9% saline (2 μl/ i.c.v) which was followed 30 min later by kisspeptin-13 (1 μg/2 μl i.c.v). All groups were tested at 24 h. The same protocol was followed in all treated groups. For the combined treatment of kisspeptin-13 and antagonists, only the most effective dose (1 μg/2 μl) was used (see dose-response in kisspeptin-13 treatment).

2.5. Behavioral testing

 Passive avoidance test

One-trial learning, step-through passive avoidance behavior was measured according to Ader et al.(1972) [9] in Ugo Basile passive avoidance apparatus (Italy.) Briefly, mice were placed on an illuminated platform and allowed to enter a dark compartment. Since mice prefer dark to light, they normally entered within 5 s. Two additional trials were delivered on the following day. After the second trial, unavoidable mild electric footshocks (0.75 mA, 2 s) were delivered through the grid floor. Having entered the box, the animals could not escape the footshock. After this single trial, the mice were immediately removed from the apparatus and were treated. The consolidation of passive avoidance behavior was tested 24 h later. In the 24-h testing, each animal was placed on the platform and the latency to enter the dark compartment was measured up to a maximum of 300 s.

2.6. Statistical analysis

The analysis of variance (two-way ANOVA) test was followed by Tukey's test for multiple comparisons with unequal cell size using Origin 7.5 software. Probability values (P) of less than 0.05 were regarded as indicative of significant differences.

3. Results

Kisspeptin-13 in a dose of 0.5 and 2 µg/2 µl) i.c.v. had no action on the consolidation of passive avoidance learning, while 1 µg i.c.v. significantly facilitated the consolidation of passive avoidance memory [F(3,18)= 22.52]; p<0.05 (Fig. 1).

In the phenoxybenzamine-pretreated group, kisspeptin-13 (1 µg/2 µl i.c.v.) facilitated the consolidation of passive avoidance learning [F(3,18)=4.03]; p<0.05. Phenoxybenzamine (2 mg/kg i.p.) itself had no action, but fully blocked the action of kisspeptin-13 (Fig. 2).

In the yohimbine pretreated group yohimbine (0.5 mg/kg i.p.) itself had no action, while fully blocked the action of kisspeptin-13 (1 µg/2 µl i.c.v) [F(3,35)=3.83],p<0.05 (Fig.3).

In the propranolol-pretreated group kisspeptin-13 (1 µg/2 µl i.c.v.) facilitated the consolidation of passive avoidance learning [F(3,20=4.03], p<0.05. Propranolol (10 mg/kg i.p.) itself had no action, however fully blocked the action of kisspeptin-13. (Fig.4).

In the methysergide-pretreated group, kisspeptin-13 (1 µg/2 µl i.c.v.) facilitated the consolidation of passive avoidance learning.[F(3,20)=8.67), p<0.05. Methysergide (5 mg/kg i.p.) alone had no action in the doses used, but fully blocked the action of kisspeptin (Fig.5.).

In the cyproheptadine-pretreated group, kisspeptin-13 (1 µg/2 µl i.c.v.) facilitated the consolidation of passive avoidance learning [F(3,20)=12.27]; p<0.05. Cyproheptadine (3 mg/kg i.p.) itself had no action, but fully blocked the action of kisspeptin-13 (Fig. 6).

In the atropine-pretreated group, kisspeptin-13 (1µg/2 µl i.c.v.) facilitated the consolidation of passive avoidance learning [F(3,18)=4.68];p<0.05. Atropine (2 mg/kg i.p.) itself had no action, but fully blocked the action of kisspeptin-13 (Fig. 7).

In the haloperidol-pretreated group, kisspeptin-13 (1 µg/2 µl i.c.v.) facilitated the consolidation of passive avoidance learning [F(3,19)=4.34];p<0,05. Haloperidol (10 µg/kg i.p.) itself had no action, attenuated but did not fully block the action of kisspeptin-13 (Fig. 8).

In the bicuculline-pretreated group, kisspeptin-13 (1 µg/2 µl i.c.v.) facilitated the consolidation of passive avoidance learning. [F(3,20)=21.02] ; p<0.05. Bicuculline (2 mg/kg i.p.) itself had no action, but fully blocked the action of kisspeptin-13 (Fig. 9).

In the naloxone-pretreated group kisspeptin-13 (1 µg/2 µl i.c.v.) facilitated the consolidation of passive avoidance learning.[F(3,20)=17,20 ;P<0.05. Naloxone 0.3 mg/kg i.p) had no action on kisspeptin-13 induced facilitation of passive avoidance learning (Fig. 10)

In the nitro-L-arginine- pretreated group, kisspeptin-13 (1 µg/2 µl i.c.v.) facilitated the consolidation of passive avoidance learning [F(3,19=7.09)] ; p<0.05. Nitro-L-arginine (10 µg/2 µl i.c.v.) itself had no action, but blocked the action of kisspeptin-13 (Fig.11).

4. Discussion

In the central nervous system, KP-expressing neurons are located in the anteroventral periventricular nucleus (AVPV), the periventricular nucleus (PVN), the anterodorsal preoptic nucleus and the arcuate nuceus (Arc) [10]. In close relationship with the PVN, the neurons of the AVPV and Arc project fibers into the

preoptic area rich in GnRH cell bodies. It is now known that KP stimulates the secretion of GnRH, which is sensitive to steroid levels [3].

A number of excellent reviews have summarized the role of KP in the regulation of the reproductive function, concentrating mainly on the hypothalamus.[11-19], but little is known as regards its in limbic brain areas outside the hypothalamusIn the central nervous system, KP is transcribed within the hippocampal dentate gyrus [5-6]. KP-13, one of the endogenous isoforms, consists of 13 amino acids [3]. KP1r has been found in the amygdala and hippocampus [13, 20-24] and the bed nucleus of the stria terminalis [25], and is expressed in the granular cells of the dental gyrus [20],and KP treatment increases the excitability of these cells [4-6].

A few data indicate that KP has other actions   besides that on   reproduction. The hyperalgesic action of KP has been reported in mice [26]. KP directly regulates neuropeptide Y synthesis in hypothalamic neurons [27]. Central administration of KP reduces the food intake [28]. KP-10 evoked a dose-dependent increase in edema formation and led to microvascular constriction in the cutaneous vasculature [29].

Central administration of KP-10 also inhibits sodium excretion and the urine flow, presumably by increasing the plasma vasopressine concentration [30].

The interaction of kisspeptin with other neurotransmithers has been reported.

KP excites anorexigenic pro-opiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by enhancing GABA-mediated inhibition [31].

KP neurons co-express met-enkephalin and galanin in the periventricular region of the female mouse hypothalamus [32].

It has been suggested that KP may play a role in various neurological processes apart from reproduction, such as cognitition and epilepsy [6], and presumably in some so far unkown central nervous action (e.g. Oklay 2009, [33]).

We have demonstrated that KP-13 elicits antidepressant action in a modified forced swimming test in mice. This action is mediated by an interaction of α_2-adrenergic and $5HT_2$ serotonergic receptors [7].

The present study related to the action of KP-13 on passive avoidance learning and the possible involvement of neurotransmitters in this action. To clarify the mechanisms of action of KP-13 on passive avoidance learning, various receptor blockers were applied before KP-13 administration. The receptor blocker doses were selected so that the blockers *per se* were ineffective, but were able to block the action of a neuropeptide as described previously [7,8]. The findings observed with the receptor blockers indicated that the action of KP-13 is mediated by a number of receptors.

The nonselective α-adrenergic receptor antagonist phenoxybenzamine, the α_2-adrenergic receptor antagonist yohimbine, the nonselective 5-HT_2 serotonergic receptor antagonist cyproheptadine. the β-adrenergic receptor antagonist propranolol, the mixed $5\text{-HT}_1/5\text{-HT}_2$ serotonergic receptor antagonist methysergide, the nonselective muscarinic acetylcholine receptor antagonist atropine, and the $GABA_A$ receptor antagonist bicuculline and nitric-L-arginine, a nitric oxide synthase inhibitor, prevented the effects of KP-13 on passive avoidance learning and consolidation. The D_2,D_3,D_4 dopamine receptor antagonist haloperidol and naloxone did not block the effects of KP-13.

These results demonstrated that the effects of KP-13 are mediated, at least in part, through interactions of the α_2-adrenergic, 5-HT$_2$ serotonergic, beta-adrenergic, muscarinic, cholinergic and GABA-A-ergic , receptors and nitric oxide.

Acknowledgments

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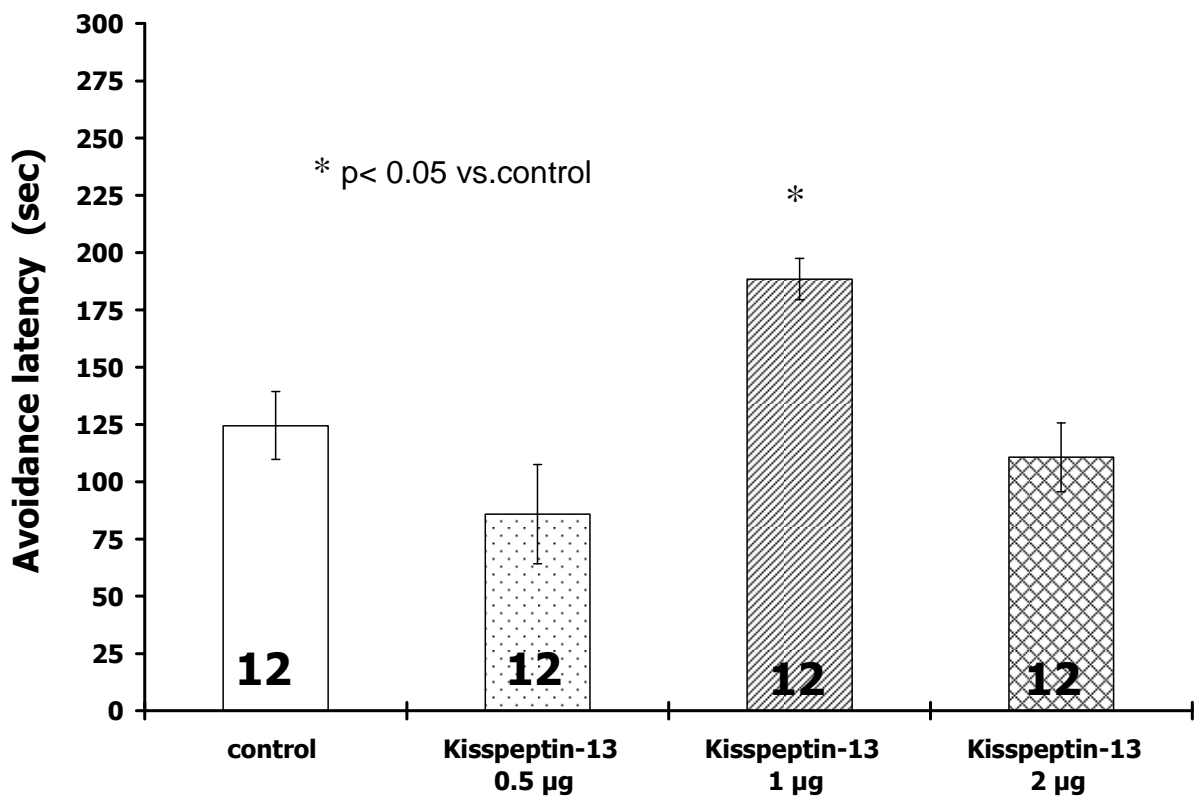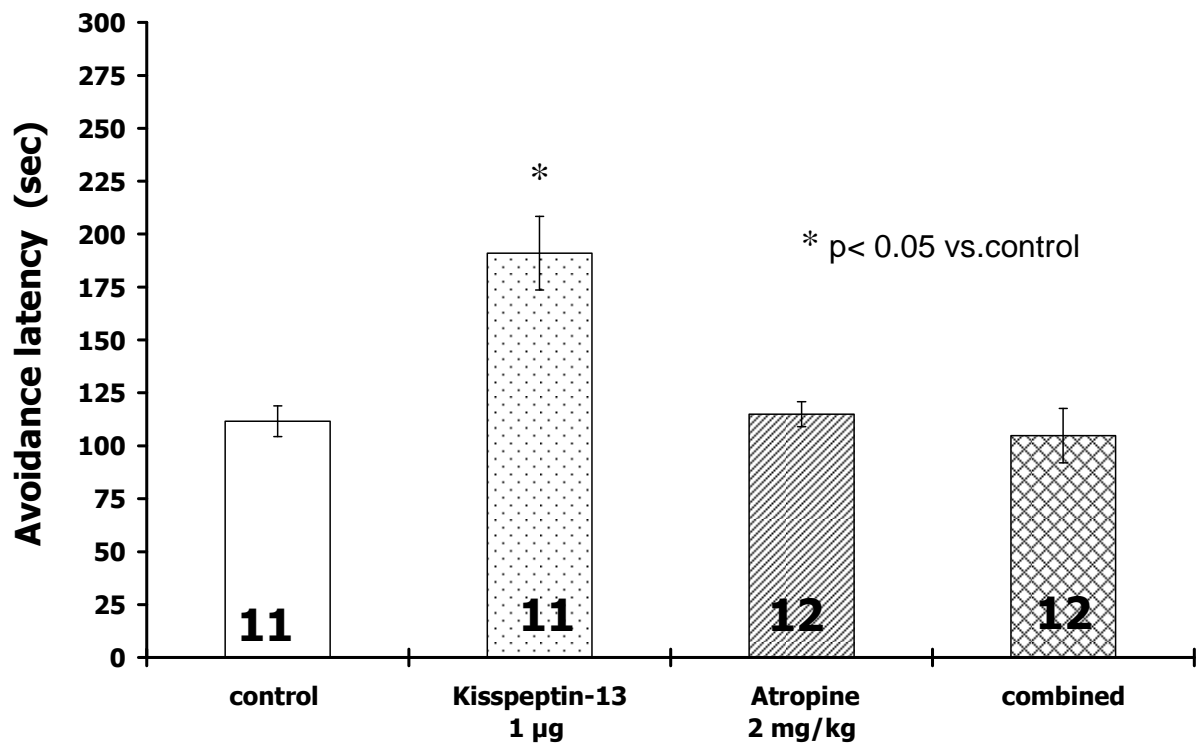
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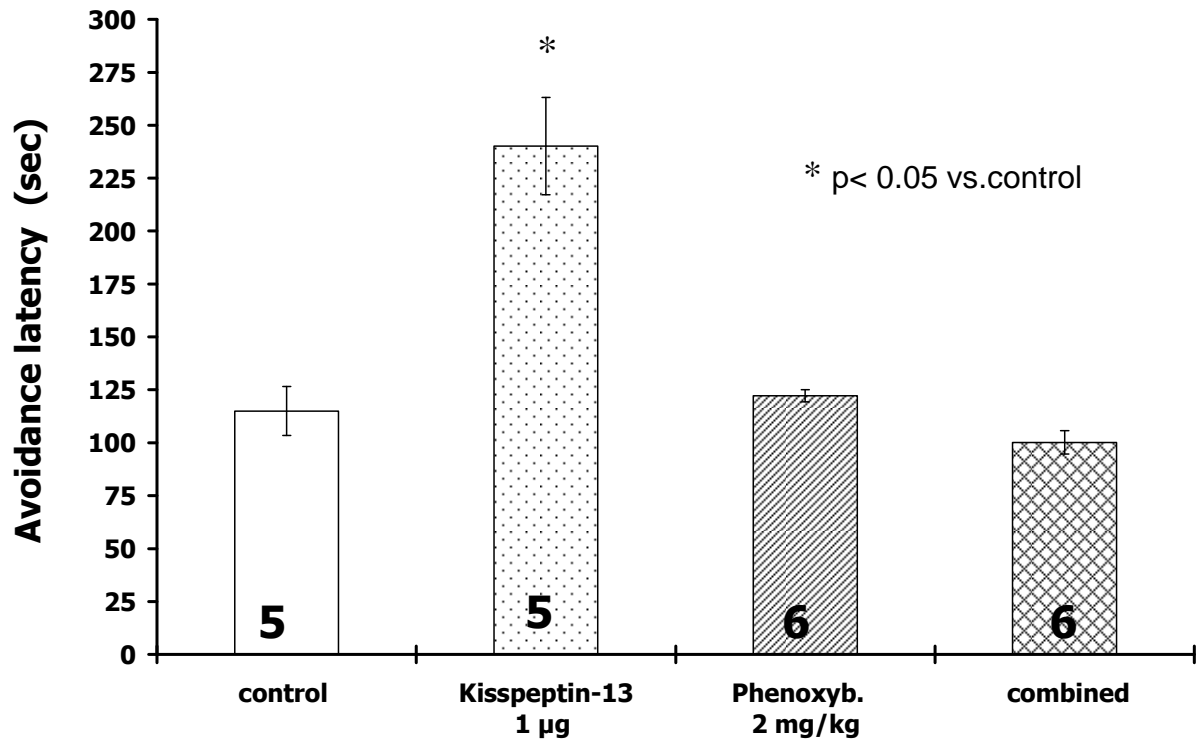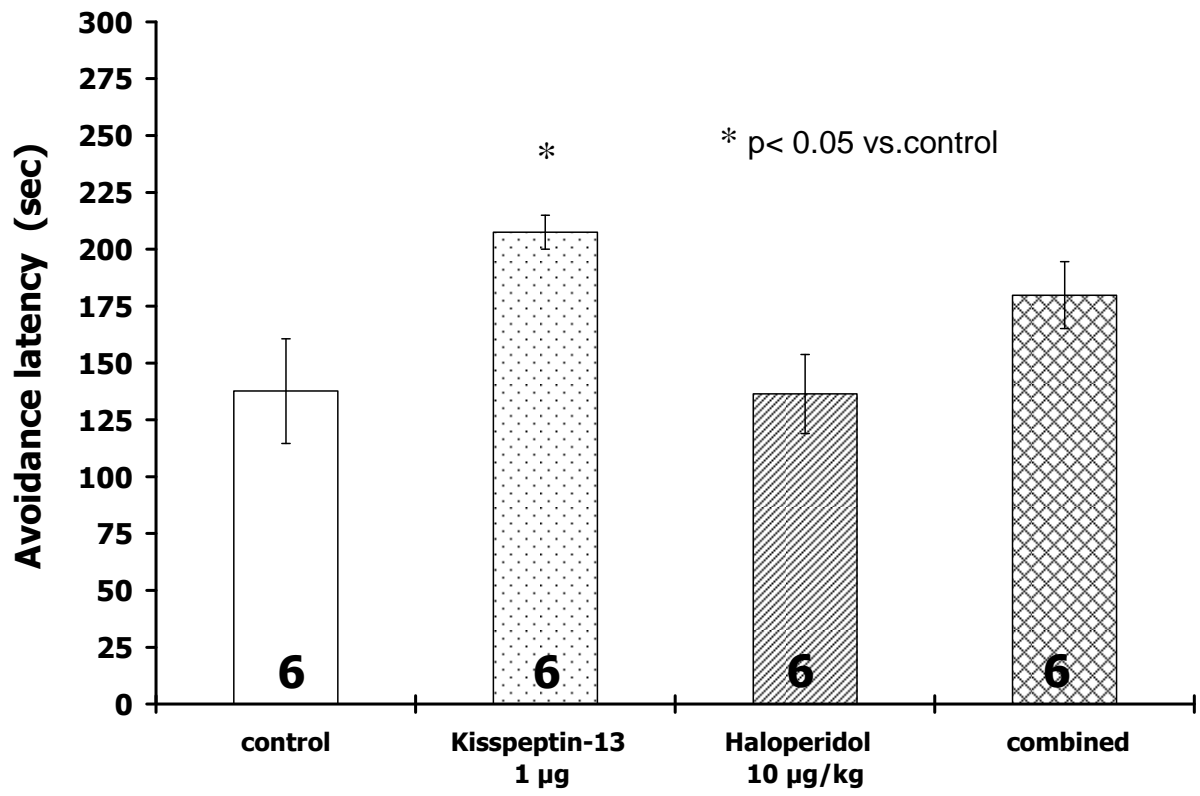
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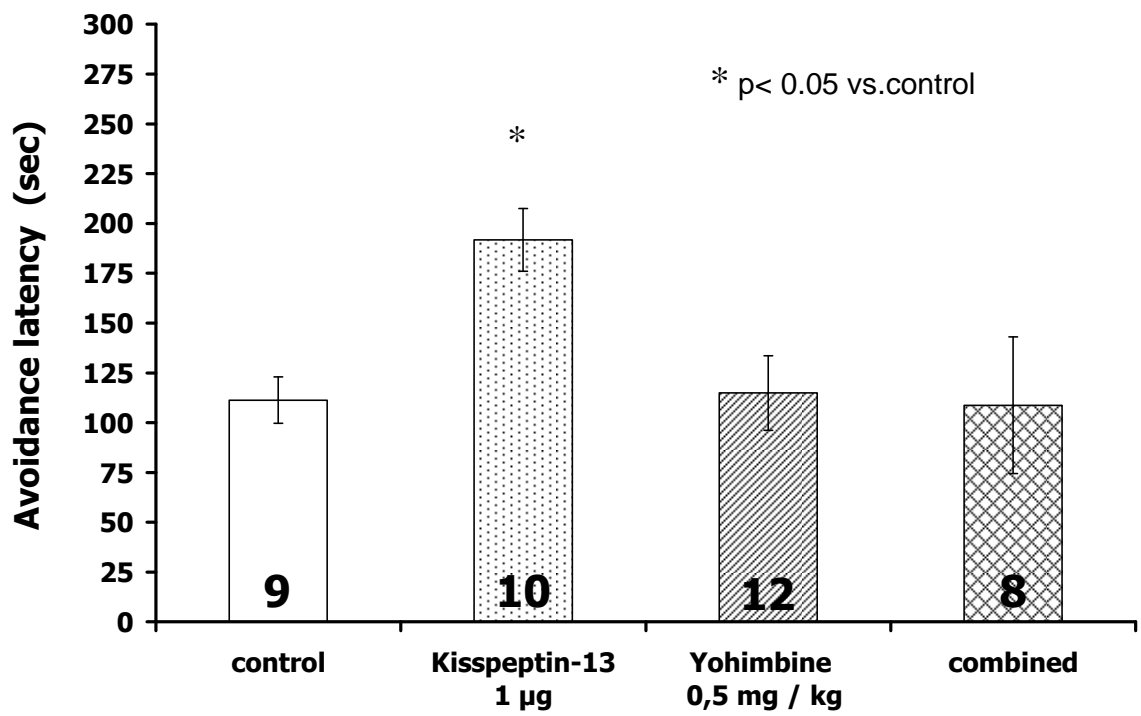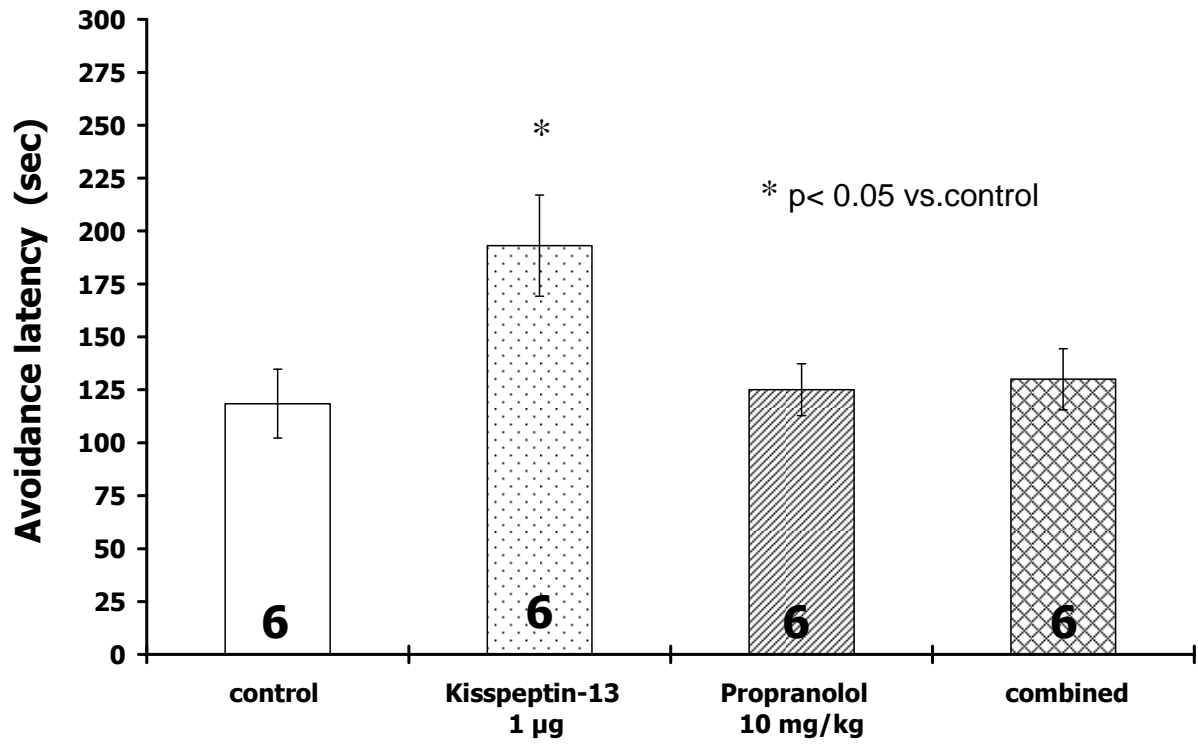
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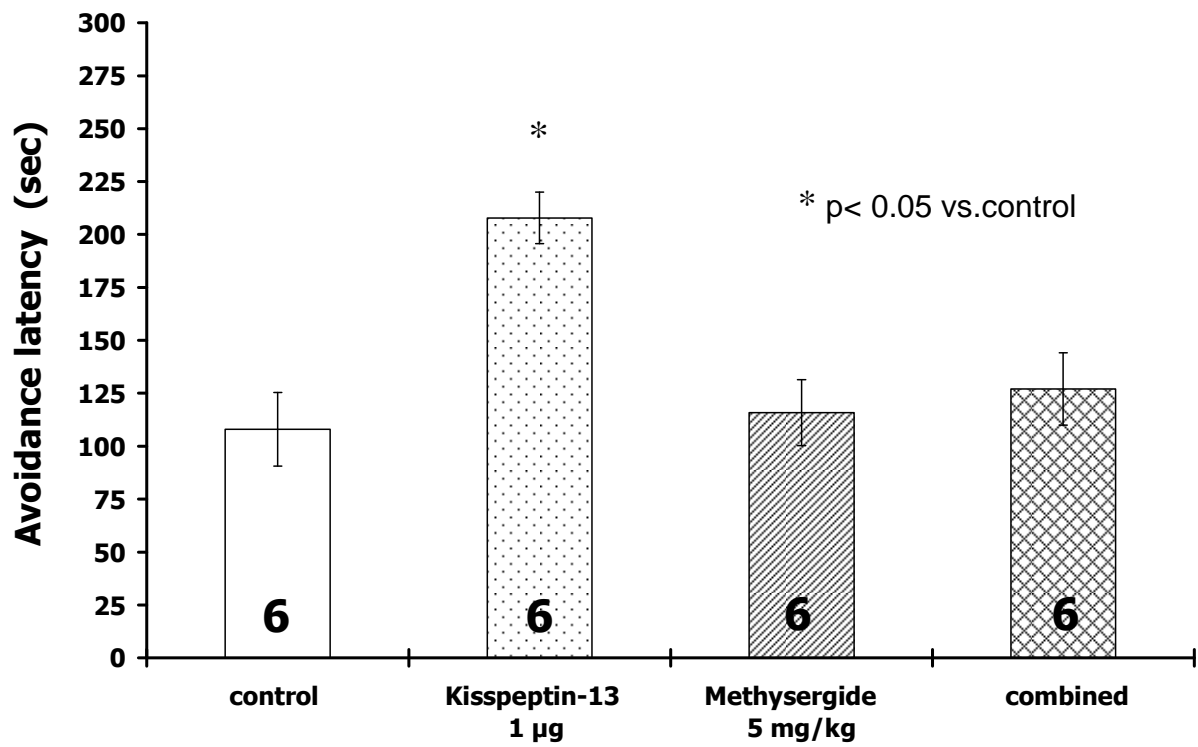
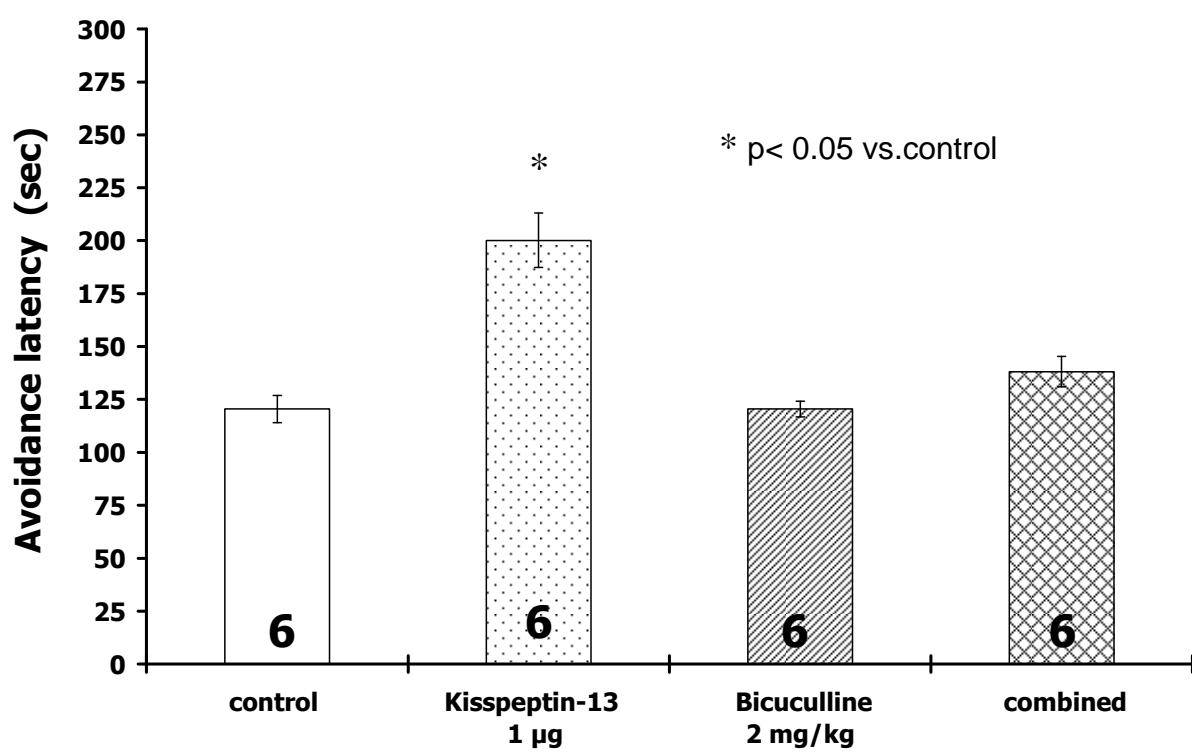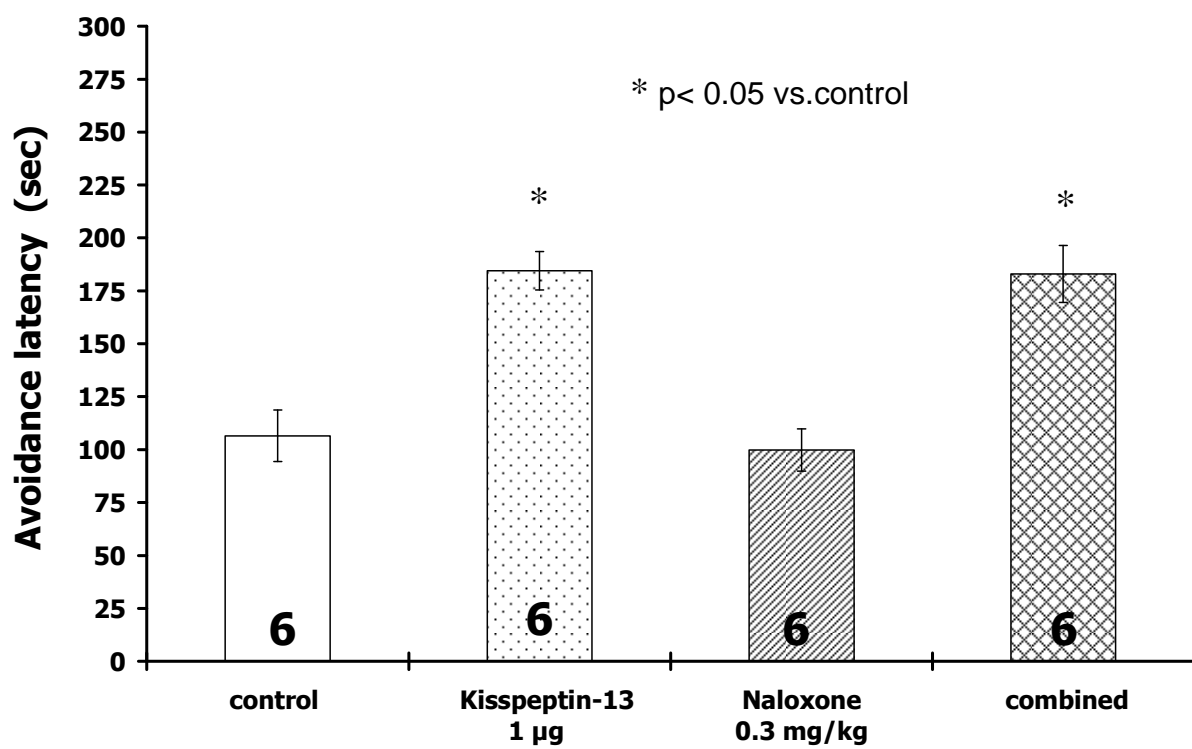Fig .5.
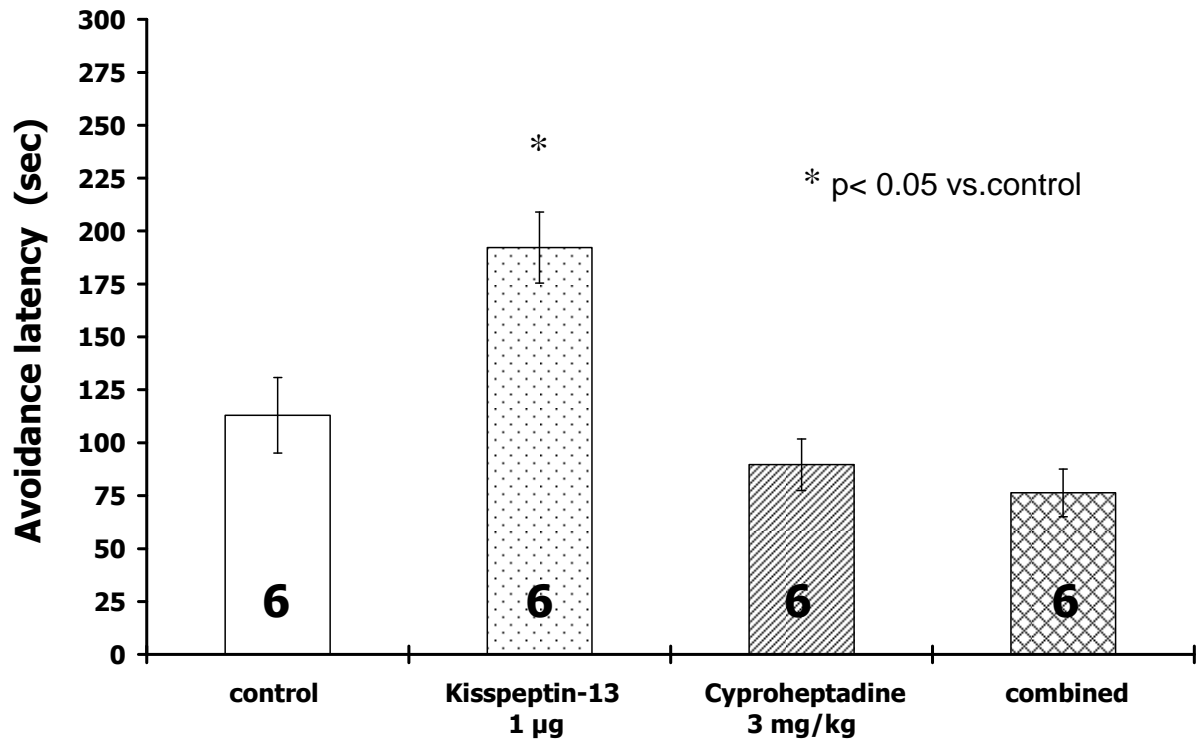
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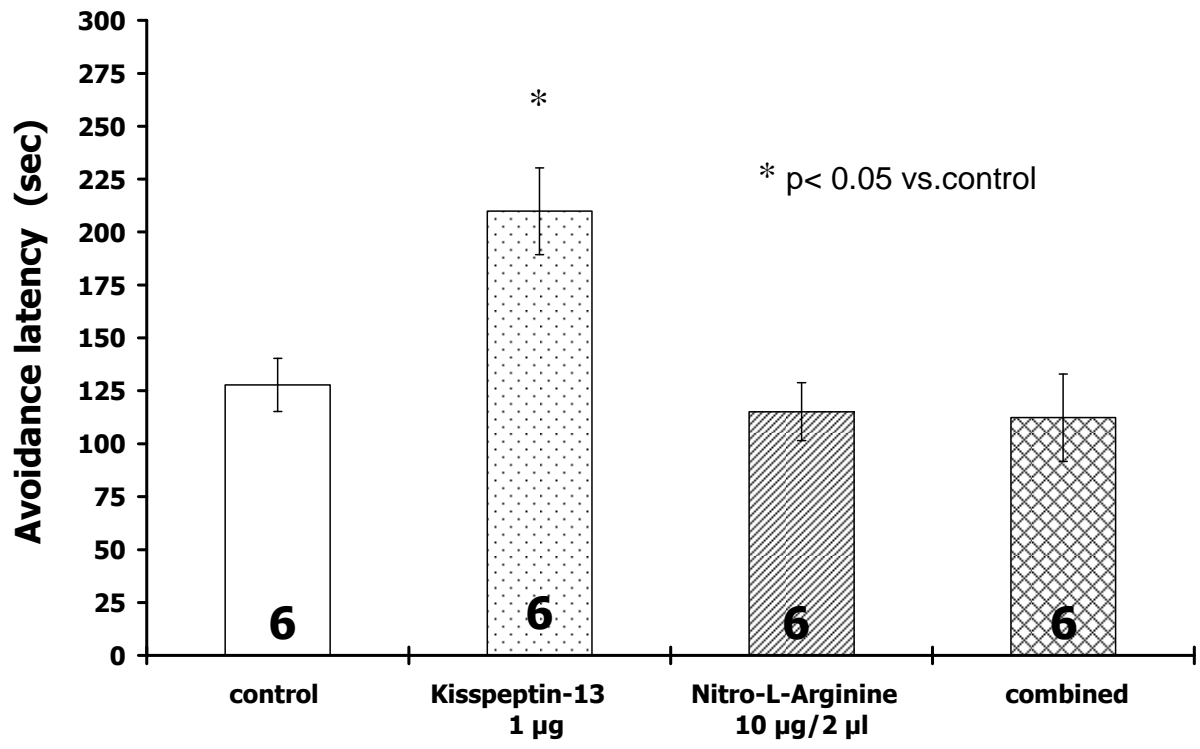

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Figure captions

Fig.1. The action of different doses of kisspeptin-13 administered i.c.v. immediatelly following the learning trial on the consolidation of passive avoidance response. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

Fig.2. The effect of a nonselective α-adrenergic receptor antagonist, phenoxbenzamine (phenoxyb, 2 mg/kg i.p) on kisspeptin-13-induced (1 µg/2 µl i.c.v.) consolidation of passive avoidance learning. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

Fig.3. The effect of an α2-adrenergic receptor antagonist, yohimbine (0.5 mg /kg i.p.) on kisspeptin-13- induced (1 µg/2 µl i.c.v.) consolidation of passive avoidance learning. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

Fig.4. The effect of a β-adrenergic receptor antagonist, propranolol (10 mg/kg i.p.) on kisspeptin-13-induced (1 µg/2 µl i.c.v.) consolidation of passive avoidance learning. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

Fig.5 The effect of a mixed 5-HT1/5-HT2 serotonergic receptor antagonist, methysergide (5 mg/kg i.p.) on kisspeptin-13-induced (1 µg/2 µl i.c.v.) consolidation of passive avoidance learning. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

Fig.6. The effect of a nonselective 5-HT2 serotonergic receptor antagonist, cyproheptadine (3 mg/kg i.p.) on kisspeptin-13-induced (1 µg/2 µl i.c.v.) consolidation of passive avoidance learning. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

Fig.7. The effect of a nonselective muscarinic acetylcholin receptor antagonist, atropine (2 mg/kg i.p.) on kisspeptin-13-induced (1 µg/2 µl i.c.v.) consolidation of passive avoidance learning. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

Fig.8. The effect of a D2,D3,D4 dopamine receptor antagonist, haloperidol (10 ug/kg i.p.) on kisspeptin-13-induced (1 µg/2 µl i.c.v.) consolidation of passive avoidance learning. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

Fig.9. The effect of a γ-aminobutyric acid subunit (GABA A)receptor antagonist ,bicuculline (2 mg/kg i.p.) on kisspeptin-13-induced (1 µg/2 µl i.c.v.) consolidation of passive avoidance learning. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

Fig.10. The effect of a nonselective opioid receptor antagonist, naloxone (0.3 mg/kg i.p.) on kisspeptin-13-induced (1 µg/2 µl i.c.v.) consolidation of passive avoidance learning. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.

Fig.11. The effect of a nitric oxide synthase inhibitor, nitro-L-arginine (10 µg/2 µl i.c.v.) on kisspeptin-13-induced (1 µg/2 µl i.c.v.) consolidation of passive avoidance learning. Data are expressed as means ± S.E.M. Number in bars are the numbers of animals used.