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Luteinizing Hormone Releasing Hormone-Induced Conditioned Place-Preference in Male Rats

RENÉ DE BEUN,*†[1] NORA E. GEERTS,* ELLIS JANSEN,*
JEF L. SLANGEN† AND NANNE E. VAN DE POLL*

**Netherlands Institute for Brain Research, Meibergdreef 33 1105 AZ Amsterdam, The Netherlands*
†Faculty of Pharmacy, University of Utrecht, Sorbonnelaan 16 3584 CA Utrecht, The Netherlands

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DE BEUN, R., N. E. GEERTS, E. JANSEN, J. L. SLANGEN AND N. E. VAN DE POLL. *Luteinizing hormone releasing hormone-induced conditioned place-preference in male rats.* PHARMACOL BIOCHEM BEHAV **39**(1) 143–147, 1991.—Conditioned place-preference induced by intraperitoneal injections of luteinizing hormone releasing hormone (LHRH) was studied in male rats. In Experiment 1, dose-dependent effects (doses: 0, 0.2, 1 and 5 μg/kg) were observed in gonadectomized males provided with a subcutaneous silastic implant containing testosterone. Animals injected with 1 or 5 μg LHRH developed reliable preference for the LHRH-associated compartment of a two-compartment preference box. The 0 and 0.2 μg doses were without effect. Experiment 2 further studied the place-preference effects induced by 5 μg LHRH, by varying the sex steroid baseline condition of the animals. A significant effect of LHRH on place-preference was found in gonadectomized males with a testosterone or estradiol implant and in gonadally intact males. Differences between these groups were not found. However, in gonadectomized males without steroid substitution, LHRH did not induce place-preference. These data indicate that rewarding properties related to LHRH treatment can be observed in male rats, provided that the males are additionally exposed to sufficient levels of circulating sex steroids.

Conditioned place-preference LHRH Male rats Dose-dependent Steroid baseline

NUMEROUS studies have now shown that conditioned place-preference (CPP) is a suitable test procedure for the assessment of motivational properties of drugs, thus revealing appetitive or aversive aspects of drug treatment (8, 11, 12). In this procedure, typically, subjects are differentially treated with the drug and the vehicle within two distinct environmental contexts. Environmental stimuli may thus become associated with either drug treatment or vehicle treatment and preference and aversion are manifested by approach and withdrawal tendencies respectively, when animals are subsequently given the choice between the two environments under nondrug condition.

In addition to establishing affective properties of drugs, it might be of particular relevance to determine appetitive or aversive properties of endogenously produced substances. If these substances indeed have intrinsic affective properties then physiological fluctuations of these compounds may influence behavior by mechanisms of classical conditioning. CPP effects in rats have already been reported for neuropeptides as substance P (appetitive properties) (4) and vasopressin (aversive properties) (2). Recently, it was also shown that peripheral treatment with the decapeptide luteinizing hormone releasing hormone (LHRH) induces CPP in male rats (1). LHRH is the key mediator in the neuroregulation of the secretion of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH) (15, 16, 18). By regulating plasma gonadotropin and sex steroid levels, LHRH may modify sexual and aggressive behavior. Furthermore, LHRH itself can facilitate feminine and masculine sexual behavior in rats primed with gonadal hormones (3, 6, 7). Rewarding properties of LHRH, as established with the CPP procedure, may be of particular relevance for behavioral changes since hormone-behavior relationships have been shown to be reciprocal: changes in hormonal conditions affect behavior and the ensuing results of this altered behavior in turn affect endocrine functioning (9,10).

In a preceding study CPP induced by a single dose of LHRH was established in gonadectomized (GDX) male rats with a subcutaneous (SC) testosterone (T) implant. In GDX female rats with an estradiol (E_2) implant no CPP effect of LHRH was found (1). The aim of the present paper was two-fold. Firstly, to extend the findings related to GDX males by studying the effects of several doses of LHRH (Experiment 1) and secondly, to investigate whether or not the CPP effect induced by LHRH is dependent on sex steroid baseline condition of the males (Experiment 2). To dissociate fluctuations of LHRH from LHRH-induced changes in T levels, CPP was initially investigated in GDX males. These males received a T-implant to guarantee suf-

[1]Requests for reprints should be addressed to R. de Beun, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

ficient negative feedback on endogenous LHRH release, resulting in low and relatively stable baseline levels of LHRH. The lack of effect of LHRH in females, GDX and provided with an E_2-implant, may have been due to high levels of circulating E_2. Therefore, in Experiment 2, CPP effects of LHRH in GDX males with a T-implant were compared with LHRH effects in GDX males with an E_2-implant. Both T and E_2 have a negative feedback effect on the LHRH release and the E_2-implant will thus also result in low and stable endogenous levels of LHRH. The relevance of these conditions was further established by studying CPP effects of LHRH in intact males without a steroid implant (fluctuating LHRH and T levels) and GDX males without steroid replacement (elevated LHRH release and extremely low levels of circulating T).

METHOD

Subjects

Forty-eight male Wistar rats were used (HSD/CPB; Zeist, the Netherlands) in Experiment 1 (12 animals per condition). In addition, 48 male rats were used for Experiment 2 (12 animals per condition). All rats were 5 weeks of age at arrival at the laboratory and were maintained in groups of 4 per cage under a reversed light/dark cycle (lights off from 07.00 to 19.00 h). After arrival they were handled and weighed during 3 weeks. Food (standard pellets, Hope Farms B.V., Woerden, The Netherlands) and tap water were supplied ad lib. Room temperature was kept constant at 19.5–21°C. At the age of 6 weeks subjects were gonadectomized under hypnorm anesthesia and, where appropriate, immediately received a silastic SC T-implant or a SC E_2-implant. Behavioral tests took place during the dark phase and started when subjects were 8 weeks old (mean body weight of all subjects taken together was 232 g) and were terminated at the age of 10 weeks (mean body weight of all subjects taken together was 272 g).

Apparatus and Experimental Conditions

Adaptation session and preference test took place in a two-compartment preference box ($91 \times 41.5 \times 38$ cm) made of polyvinylchloride. The walls of one side of the box were black, whereas the other side had white walls. The black and white parts of the box were of equal size ($41.5 \times 41.5 \times 38$ cm) and were separated by an area of $8 \times 41.5 \times 38$ cm with grey walls. The floor of all parts of the box was grey. Frequencies of entrance and duration of time spent on the three different locations were registered by infrared beam interruption, and automatically recorded. To prevent direct contact with the infrared beam equipment a transparent Plexiglas inner-box was fitted within the apparatus. Association sessions were run in separate association boxes ($41.5 \times 41.5 \times 38$ cm) which were similar to the black or white compartment of the test box. To mask sudden noises, a radio was always tuned on a station broadcasting popular music, providing a background noise in the experimental room of 65 to 75 dB(A).

Drugs

Synthetic LHRH (LHRH acetate salt, peptide content approximately 87%, Sigma Chemical Company, St. Louis, MO), was dissolved in 0.9% NaCl. Solutions of LHRH or an equal volume of vehicle were injected intraperitoneally (IP, 1 ml/kg). Solution samples were stored at −80°C and daily warmed to room temperature just prior to experimentation. For the silastic T-implants (length 1.1 cm, i.d. 1.6 mm, o.d. 2.5 mm, Dow Corning Corp., Midland, MI), 4-androsten-17 β-ol-3-one testosterone was used (Steraloids Inc., Wilton, CT). For the E_2-implants (length 1.1 cm, i.d. 1.0 mm, o.d. 2.2 mm), 1,3,5(10)-estratriene-3,17 β-diol was used (Diosynth B.V., Oss, The Netherlands).

Procedure

Three weeks after arrival at the laboratory, and 2 weeks after gonadectomy, behavioral testing started with an adaptation session. The experimentally naive animals were placed in the grey zone of the preference box and allowed free access to the black and white compartment of the box for 60 min (in order to establish the baseline preference ratio for the two compartments under nondrug condition). The boxes were cleaned thoroughly between each individual test. From the next day onwards, subjects were treated daily with LHRH or vehicle and after 15 min they were placed in one of the two association boxes for 30 min. In Experiment 1, 4 doses of LHRH were used (one dose per group of animals): 0, 0.2, 1 and 5 μg/kg. In Experiment 2 only the 5 μg/kg dose was used. LHRH and vehicle treatment were alternated during 8 days, LHRH treatment being paired 4 times with one of the association boxes and vehicle treatment 4 times with the other box. For half of the animals of each group ($N = 6$), LHRH treatment was paired with placement in the black box, for the other half it was paired with the white box. Half of the animals in each subgroup ($N = 3$) started their association sessions in the white box (and consequently finished in the black box), whereas the other animals were treated first in the black box and finished in the white box. Twenty-four hours after the last association session, the animals were tested in the preference box. Similar to the adaptation session, animals were not injected prior to the preference test and were allowed free access to the white and black compartment for 60 min. Time spent on the side of the box associated with LHRH treatment, before (adaptation session) and after (preference test) the LHRH environment pairing, was compared as an index for LHRH-induced place-preference. Number of entrances of the two sides of the box was taken as an index for locomotor activity.

Statistics

Data of both experiments (time spent on LHRH-associated side) were submitted to analysis of variance (ANOVA) with repeated measures. The design consisted of one between-subjects factor (the experimental groups of animals used) and two within-subjects factors (treatment with LHRH and 4 subsequent intervals within a 60-minute session). Additional ANOVA's for separate groups of animals were used and post hoc analysis took place with two-tailed paired t-tests. Results were considered significant when $p < 0.05$.

RESULTS

Experiment 1

For all 4 doses of LHRH, data obtained during the adaptation session (unconditioned preference) and preference test (conditioned preference) are presented in Fig. 1. Time spent on the side of the box paired with LHRH treatment, before and after association sessions are represented. These data were submitted to analysis of variance (ANOVA), with the between-subjects factor Dose (4 levels) and the within-subjects factors Treatment (2 levels: pretreatment adaptation session and posttreatment preference test) and Interval (four levels: preference during adaptation session and during preference test was measured for 4

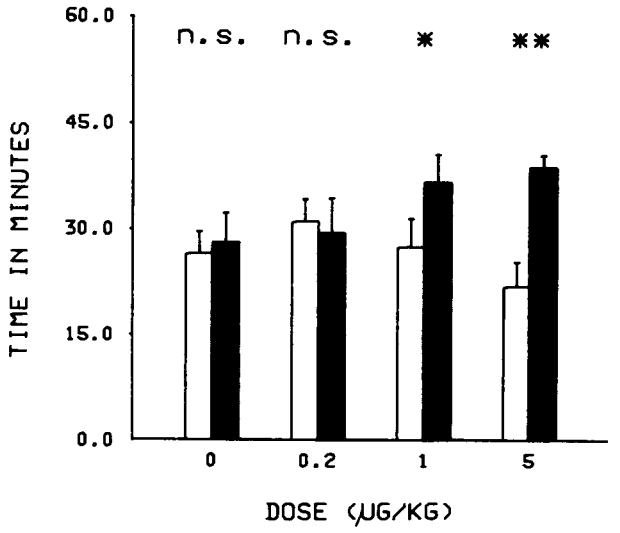

FIG. 1. Time spent on the LHRH-paired side of the test box, before (open bars) and after (filled bars) association with LHRH treatment. Represented are mean time and SEM of 4 groups of animals injected with different doses of LHRH (N = 12 per group). n.s. = nonsignificant, *$p<0.05$, **$p<0.01$ (paired samples two-tailed t-tests).

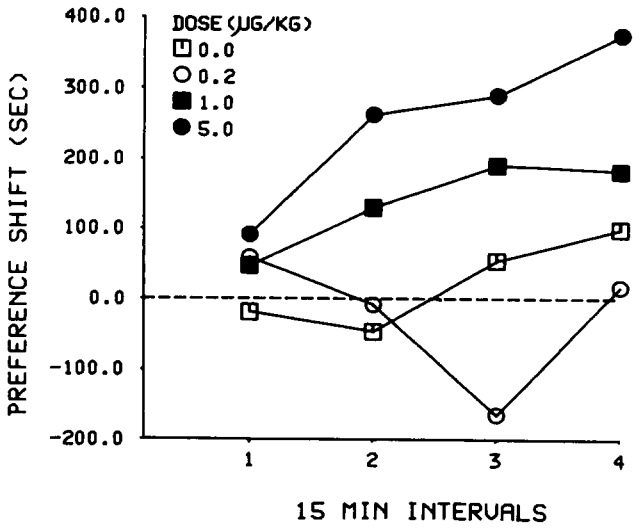

FIG. 2. Difference in time spent on the LHRH-paired side of the test box before and after association sessions, for 4 subsequent intervals of 15 min. Represented are mean preference shifts of 4 groups of animals injected with different doses of LHRH (N = 12 per group). Positive and negative values on the ordinate denote an increase and a decrease respectively in time spent on the LHRH-paired side after conditioning.

intervals of 15 min separately).

As can be seen in Fig. 1, there was a significant Treatment effect of LHRH, $F(1,44)=6.88$, $p<0.05$. This effect of LHRH was dose-dependent: the Dose × Treatment interaction was significant, $F(3,44)=3.89$, $p<0.05$. In addition, a significant effect was found for Interval, $F(3,132)=3.00$, $p<0.05$ which depended on Treatment, $F(3,132)=2.89$, $p<0.05$ (Treatment × Interval) and dose, $F(9,132)=2.25$, $p<0.05$ (Dose × Treatment × Interval).

Further analysis (ANOVA for separate doses with only the within-subjects factors Treatment and Interval) did not reveal any significant effects for the 0 and 0.2 μg doses. With 1 μg LHRH, significant effects of Treatment and Treatment × Interval were found, $F(1,11)=6.42$, $p<0.05$ and $F(3,33)=3.82$, $p<0.05$, respectively and similar effects were observed with 5 μg LHRH, $F(1,11)=14.85$, $p<0.01$ and $F(3,33)=4.55$, $p<0.01$. For the 1 and 5 μg doses, this additional analysis thus revealed an interval-dependent increase in time spent in the LHRH-paired environment during the preference test.

For the 1 and 5 μg groups separately, data of each 15-min interval obtained during the adaptation session (preconditioning) were compared with corresponding data from the preference test (postconditioning) using paired samples t-tests. The results for the 4 subsequent intervals showed an increase in difference in time spent on the LHRH-paired side of the test box between the adaptation session and the preference test (preference shift). This increase in preference shift during the 60-min test is shown in Fig. 2. For the 1 μg dose there were no significant differences in preference for the LHRH-paired side between adaptation session and preference test during the first two intervals. During the last two intervals there was a significant preference shift, $t(11)=-2.62$ and -2.54, $p<0.05$. For the 5 μg dose there were significant preference shifts within all 4 intervals, $t(11)=-2.34$, -2.92, -2.96, $p<0.05$ and, -4.31, $p<0.001$, respectively.

An additional ANOVA, without the within-subjects factor Treatment, revealed that number of entrances of the two sides of the preference box, taken as an index for locomotor activity, significantly decreased during the 60-min preference test: Interval, $F(3,132)=169.17$, $p<0.001$. This decline of locomotor activity was found in all groups, Dose × Interval, $F(9,132)=1.24$, n.s.

Experiment 2

Figure 3 shows time spent on the LHRH-paired side during the adaptation session (unconditioned preference) and preference

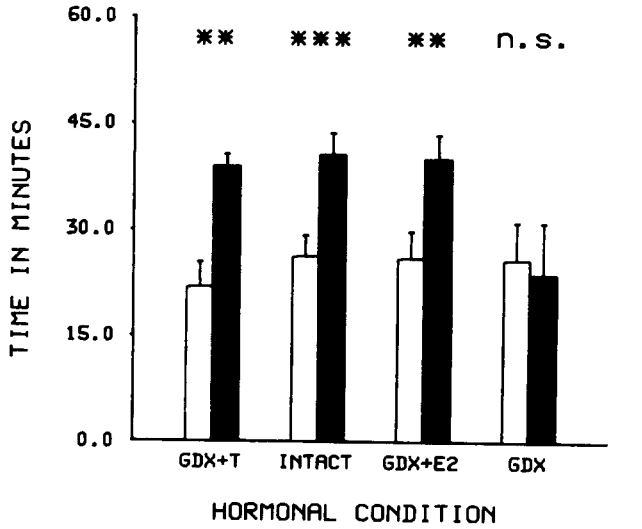

FIG. 3. Time spent on the LHRH-paired side of the test box, before (open bars) and after (filled bars) association with LHRH treatment (5 μg/kg). Represented are mean time and SEM of 4 groups of animals under different hormonal conditions (N = 12 per group, except GDX: N = 6). n.s. = nonsignificant, **$p<0.01$, ***$p<0.001$ (paired samples two-tailed t-tests).

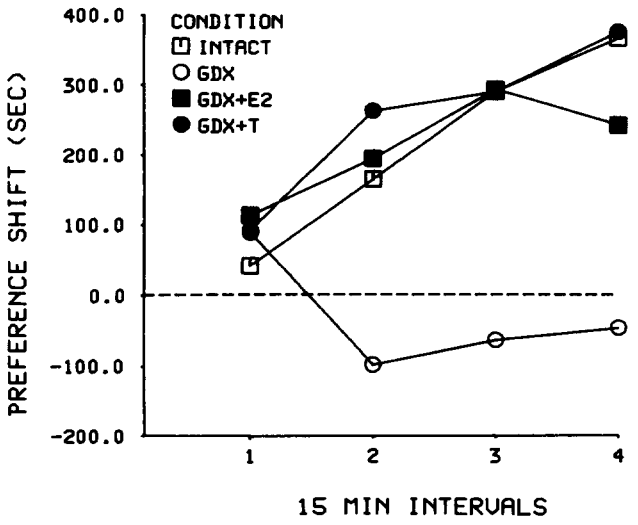

FIG. 4. Difference in time spent on the LHRH-paired side of the test box before and after association sessions, for 4 subsequent intervals of 15 min. Represented are mean preference shifts of 4 groups of animals under different hormonal conditions (N = 12 per group, except GDX: N = 6). Positive and negative values on the ordinate denote an increase and a decrease respectively in time spent on the LHRH-paired side after conditioning.

test (conditioned preference) for the 4 groups of males studied in this experiment (data of one group, GDX and T-implanted originated from Experiment 1). These data were submitted to ANOVA with the between-subjects factor Group (4 levels) and the within-subjects factors Treatment (2 levels: pretreatment adaptation session and posttreatment preference test) and Interval (4 levels: preference during adaptation session and preference test was measured for 4 intervals of 15 min separately). Due to technical problems with data acquisition during the adaptation session, data of only 6 animals of the GDX males without steroid implant could be included for this statistical analysis.

A clear effect of Treatment, $F(1,38) = 22.18$, $p < 0.001$ and a weak (but nonsignificant) interaction of Group × Treatment, $F(3,38) = 2.58$, $p < 0.1$ were found. Figure 3 suggests that the Group × Treatment interaction is due to a lack of effect of LHRH-treatment in the GDX group without steroid substitution, which was confirmed by further analysis (separate ANOVA's per group with only the within-subjects factors Treatment and Interval). No treatment effect was found in GDX animals without steroid replacement: Treatment, $F(1,5) = 0.07$, n.s. (Because of 6 missing values of the adaptation session in this group, they were also submitted to an alternative analysis by comparing time spent in the LHRH-associated side with time spent in the vehicle-associated side during the preference test, where all 12 animals could be included. The outcome of this check for possible bias was not different from the utilized analysis.) Gonadectomized males with T substitution showed a profound conditioned preference for the LHRH environment: Treatment, $F(1,11) = 14.85$, $p < 0.01$ (Experiment 1). Similar conditioned preference results were obtained with both the gonadally intact animals: Treatment, $F(1,11) = 23.58$, $p < 0.001$ and the GDX males who received E_2 replacement: Treatment, $F(1,11) = 11.96$, $p < 0.01$.

In agreement with Experiment 1, significant effects of Interval, $F(3,114) = 3.39$, $p < 0.05$ and Treatment × Interval, $F(3,114) = 3.45$, $p < 0.05$, were noticed. For all 4 groups separately, data of each 15-min interval obtained during the adaptation session (preconditioning) were compared with corresponding data from the preference test (postconditioning) using paired samples t-tests. Analogous to the results of Experiment 1 with 5 μg and 1 μg LHRH, the data of the 4 subsequent intervals showed an increase in difference in time spent on the LHRH-paired side of the test box between the adaptation session and the preference test (preference shift). This increase in preference shift during the 60-min test is shown in Fig. 4. Except for the GDX group without steroid substitution, this progression in time spent in the LHRH-associated environment was observed in all groups. For the intact males there was no significant difference in preference for the LHRH-paired side between adaptation session and preference test during the first interval. During the remaining 3 intervals there was a significant preference shift, $t(11) = -2.39$, $p < 0.05$, -3.89 and -4.11, $p < 0.01$. For the GDX males with an E_2-implant there were no significant differences in preference during the first and last interval. During the second and third interval a significant preference shift was noticed, $t(11) = -2.17$, $p < 0.05$ and -3.13, $p < 0.01$. These preference patterns closely resembled the results of the GDX males with a T-implant, $t(11) = -2.34$, -2.92, -2.96, $p < 0.05$ and -4.31, $p < 0.001$ respectively.

Locomotor activity (number of entrances of the two sides of the preference box), analyzed per 15-min period with an ANOVA without Treatment factor, decreased during the 60-min preference test, Interval, $F(3,132) = 126.99$, $p < 0.001$. This effect was not dependent on the group used, Group × Interval, $F(9,132) = 0.41$, n.s.

DISCUSSION

The present data provide unequivocal evidence that male rats develop a dose-dependent preference for environmental stimuli associated with luteinizing hormone releasing hormone treatment. Conditioned place-preference was induced by using 5 and 1 μg doses (the 5 μg dose being the more potent one); the 0.2 μg dose was not effective. Being submitted to the procedure per se had no effect on preference behavior (0 μg dose).

The CPP effect of peripheral LHRH treatment in male rats was found to be robust. CPP could not only be established in rats with high and stable levels of testosterone and low levels of LHRH, but also in gonadally intact rats with presumably more fluctuating levels of endogenous LHRH, gonadotropic hormones and T due to the highly dynamic feedback systems of the intact gonadal axis. However, as is clear from the results of the E_2-implanted males, relatively high levels of T are not a prerequisite for CPP to occur in males after LHRH treatment.

At this moment it remains unclear what mechanism is involved in the CPP effect induced by systemic injections of LHRH. Rewarding properties of LHRH treatment could result from a direct LHRH receptor-mediated mechanism (either via LHRH binding sites located in the central nervous system or in the periphery), dissociated from "triggering" the gonadal axis (the endocrine action of LHRH on pituitary LH and FSH release and consequently on sex steroid release) (5,14). An alternative hypothesis is that CPP develops to the extent that LHRH stimulates gonadotropic hormone release from the pituitary (i.e., activates the gonadal axis). Administration of 5 μg/kg IP LHRH has been found to result in a significant increase of plasma levels of T (from 7 to 67 nmol/l) (our laboratory, unpublished). This elevated level of circulating T after injection of 5 μg LHRH (taken as index for activation of the gonadal axis) is caused by an increase in LH (and to a lesser extent FSH) release. The CPP effect of LHRH may, therefore, depend on LHRH-stimulated LH release. Similarly, FSH may be involved in producing CPP effects. Current research is directed at delin-

eating the mechanisms involved in the presently described effects of LHRH treatment on CPP.

Both circulating E_2 and T result in low endogenous baseline levels of LHRH and gonadotropins due to negative feedback on both LHRH and gonadotropin release. The present data suggest that this is of importance for a CPP effect to develop. If the negative feedback of circulating sex steroids on LHRH and gonadotropin release was abolished by castration and subjects were not provided with sufficient levels of either T or E_2, a CPP effect of LHRH was not observed. Elevated release of endogenous LHRH and (or) gonadotropins may blur the distinction between LHRH condition and saline condition.

In a previous paper we reported that we were unable to induce CPP with 5 μg LHRH in GDX female rats with an E_2-implant (1). The present finding that the CPP effects of LHRH in GDX males provided with an E_2-implant resemble the results obtained with males provided with a T-implant, indicates that high circulating levels of E_2 do not interfere with the expression of LHRH-induced CPP in males. The reported lack of effect in females with an E_2-implant is thus probably not due to interference of high levels of circulating E_2.

As reported elsewhere (1), the present results confirm that a prolonged test period of 60 min provides a more sensitive measurement of conditioned preference, as compared to the conventionally used 15-min test period (11,17). A clear-cut preference was established, which would not have been found when adaptation and preference sessions had been restricted to a 15-min period. The data on locomotor activity suggest that a high level of locomotor activity interferes with the manifestation of CPP. Locomotor activity was highest during the first 15 min, about 50% of the entrances of the one or the other side of the box took place in this period. It is not clear yet whether or not time-dependent absence of preference (under nondrug condition!) and time-dependent locomotor activity are related to the affective quality (like, for instance, salience) of LHRH. It is possible that more salient stimuli show a different temporal expression of preference in CPP procedures. Besides our own experiments, we are aware of only one other paper dealing with variance in CPP in relation to session length (13). Reid et al. report that morphine-induced CPP increased when test length was prolonged (test duration between 15 and 60 min). In addition to an increase in mean time spent in the morphine-associated environment, the authors also noted a considerable increase in variance of the scores, resulting in an optimal test duration on the order of 30 min instead of 60 min. These results are in accordance with the temporal aspects of LHRH-induced CPP found in the present and previous experiment. However, an increase in variance throughout the 60-min session was not observed, presumably because testing was done during the animals' dark phase. Reid et al. observed that under their experimental conditions (with white light) animals often fell asleep after about 30 minutes, on an apparently random base on either side of the test box. This could be the crucial factor explaining the slight discrepancies found between the temporal course of manifestation of LHRH and morphine-induced preferences.

In summary, it seems reasonable to state that treatment with LHRH has affective properties for male rats. LHRH activity seems to be rewarding, capable of influencing behavior by associative learning. Future investigations should gain insight in the mechanisms responsible for the CPP effects of LHRH administration.

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