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BP101 Peptide Promotes Female Sexual Receptivity in the Rat

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ABSTRACT

Introduction: Low sexual desire is a frequent sexual problem in women, with only one drug for the condition approved by the Food and Drug Administration.

Aim: To evaluate the ability of a novel synthetic peptide, BP101, to facilitate sexual behavior after intranasal administration or infusion into certain brain areas in female rats.

Methods: Bilaterally ovariectomized female rats, primed with a suboptimal combination of estradiol benzoate (EB) and progesterone, were used as a model of low sexual motivation. Sexual behavior was tested with stud male rats after acute (experiment 1) or long-term (experiment 2) intranasal administration of BP101 or peptide infusion into the olfactory bulb, medial preoptic area, ventromedial hypothalamic nucleus, or ventral tegmental area (experiment 3).

Main Outcome Measures: Frequency of solicitations (SF), as an indicator of sexual motivation in female rats, and lordosis frequency and ratio, as measurements of female consummatory sexual behavior.

Results: Acute intranasal BP101 administration moderately increased SF, with the highest tested dose of 300 μg/kg causing an 80% increase. Female rats receiving BP101 75 or 300 μg/kg daily on days 6 to 16 of the peptide administration displayed twofold higher SF compared with the placebo-treated animals, an increase comparable to optimally hormone-primed female rats. Infusion of BP101 1 and 5 μg per rat into the medial preoptic area, but not into the olfactory bulb, ventromedial hypothalamic nucleus, or ventral tegmental area, increased SF in female rats supplemented with EB 10 or 20 μg. The effect was relatively more pronounced in female rats receiving EB 10 μg (≈300%) compared with EB 20 μg (≈50%) with direct brain infusions.

Conclusion: BP101 displays a potent stimulatory effect on sexual motivation in the female rat, and the medial preoptic area seems to be the site of its action. BP101 is effective in female rats receiving different hormone supplementations, making the present data generalizable to pre- and postmenopausal women with hypoactive sexual desire. **Andreev-Andrievskiy A, Lomonosov M, Popova A, et al. BP101 Peptide Promotes Female Sexual Receptivity in the Rat. J Sex Med 2017;14:336–346.**

Key Words: BP101 Peptide; Medial Preoptic Area; Female Rat; Sexual Behavior

INTRODUCTION

Low sexual desire is a frequent sexual problem in women.[1] Its incidence strongly depends on the age of the responders, region, and study methodology. The most comprehensive study, published in 2005, reported a prevalence of low sexual desire from 25.6% in Northern Europe to 43.4% in the Middle East.[2] Various social, psychological, and medical reasons and the use of certain medications can lead to its development.[1] Flibanserin, a mixed serotonin receptor 1A agonist and receptor 2B antagonist, was the first drug for the condition approved by the Food and Drug Administration.[3] Flibanserin was shown to be efficacious in behavioral tests of female rats[4] and marmosets[5] and a number of clinical trials with women.[6–8] Modulation of the brain monoamine balance by flibanserin enhances solicitations, an indicator of sexual motivation, in the female rat, whereas consummatory sexual behavior is not affected.[4] The action of flibanserin seems to be mediated by the mesolimbic dopaminergic pathway and hypothalamic structures involved in the integration of sexual cues related to sexual motivation.[9] Other substances, including off-label use of hormones,[10] and

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other drugs are used to ameliorate low sexual desire. New drugs for this condition currently are in clinical trials, such as bremelanotide,[11] Lybridos, and Lybrido.[12,13]

In the present article, we describe a new drug, BP101, with a strong ability to increase sexual behavior in female rats when delivered intranasally or directly into certain brain areas.

BP101 is a small peptide molecule. Its structure is not disclosed because of the commercial nature of this information.

The effects of BP101 on sexual behavior were evaluated using ovariectomized hormone-primed female rats. The effects of the peptide were investigated after single or long-term intranasal administration or after infusion into brain centers involved in female sexual behavior control.

METHODS

Study Overview

The study used three distinct experiments aimed at defining the effects of the BP101 peptide on female sexual behavior. In experiment 1, the acute effects of BP101 on female sexual behavior were studied after a single intranasal administration. In experiment 2, with another batch of animals, the pro-sexual effects of long-term intranasal BP101 administration were studied longitudinally. In experiment 3, BP101 was administered directly to brain centers involved in sexual behavior control to determine the peptide's site of action; a third batch of animals with long-term implanted cannulas were repeatedly tested in this experiment.

Ovariectomized female rats kept on suboptimal estradiol benzoate (EB) and progesterone (P) supplementation were used as a model of low (hypoactive) sexual desire in all experiments. Before the experiments, all female rats were subjected to a series of training sexual behavior sessions to induce relatively stable sexual behavior and to habituate the rats to the test paradigm and manipulations.

Animals

Male and female Sprague-Dawley rats were used in the study. Animals were 2.0 to 2.5 months old at the start of experiments and approximately 4 months old at the time of sexual behavior testing after the completion of training. Three different batches of animals were used for experiments 1 to 3 (total = 250 female and 100 male rats).

Rats were kept in groups of two to four in semitransparent plastic cages (floor area = 1,500 cm^2; Techniplast, Buguggiate, Italy) at 20°C to 26°C and 30% to 70% relative humidity under a 12-hour light-dark cycle (lights on at 9:00 AM). Wood chips (J. Rettenmaier & Söhne Group, Rosenberg, Germany) were used for bedding. Standard rat chow (Assortiment-Agro, Turacovo, Russia) and deionized water were provided ad libitum. Wooden sticks and paper tissues were provided for environmental enrichment.

The experiments were approved by the bioethics commission of the MSU Institute of Mitoengineering (Moscow, Russia) and conducted in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

Animal Training

To prepare the animals for experiments 1 to 3, female rats were bilaterally ovariectomized and, after 2 weeks of postsurgical recovery, 6 to 10 cycles of suboptimal hormonal supplementation and training sessions with stud male rats were conducted. After the animals were habituated to the experimental procedures, they were randomly assigned to groups and used in the experiments.

Ovariectomy

Female rats were anaesthetized with an intraperitoneal injection of a mixture containing tiletamine and zolazepam (15 mg/kg each) supplemented with xylazine 3 mg/kg. Additional doses of anesthetics (~20% of initial dose) were administered as needed. The ovaries were accessed through a midline cut in the back skin and a puncture in the muscles 1.5 cm lateral to the backbone and pulled out of the abdomen. After the uterus horn was ligated with 4-0 silk just under the oviduct, the ovary and surrounding fat pad were excised. The cuts were sutured with absorbable 4-0 sutures. During the first 2 to 3 days of postsurgical recovery, rats were administered daily with Midocalm (tolperisone; Gedeon Richter, Budapest, Hungary) 1.0 mg/kg and Bactrim (trimethoprim and sulfamethoxazole; AR Scientific, Philadelphia, PA, USA) 40 mg/kg subcutaneously. Two weeks were allowed for complete postsurgical recovery under the control of a veterinarian.

Hormonal Supplementation

After the female rats recovered from surgery, they were subjected to cyclic hormonal supplementation. EB (Sigma, St Louis, MO, USA) was administered every 5 days, 52 hours before the sexual behavior tests or training sessions, at 10 μg per rat ("low" protocol) or 20 μg per rat ("high" protocol) subcutaneously on the back or flanks as a propylene glycol solution (0.1 or 0.2 mL per rat at 1 or 2 points, respectively). P was administered 48 hours after EB administration and 4 hours before sexual behavior tests or training sessions at a dose of 500 μg subcutaneously per rat (0.1 mL of a propylene glycol solution). In experiments 1 and 2, the positive control (PC) group of female rats received P 1,000 μg per rat on the days of the sexual behavior tests to induce maximal sexual receptivity.

Sexual Behavior Sessions

To habituate the rats to the experimental procedures and ensure the animals gained sexual experience and stable sexual activity, 6 to 10 training sexual behavior sessions were performed. Animals that did not display sexual behavior (at least lordosis in response to a mount) during the two last training

sessions were discarded. The studs used in these sessions and sexual behavior tests were male rats selected for high sexual activity before use.

Sexual behavior sessions were conducted every 5 days in the evening (12:00—6:00 PM) and 4 to 8 hours after P administration. Female and male rats were brought to the experimental room and allowed 30 minutes for adaptation. Then, the female rats were placed in the square 60 × 60-cm Plexiglas test arena followed 5 minutes later by a stud male rat. Behavior was video-recorded at 30 frames/s under infrared light for 30 minutes. The training session recordings were video-scored for the presence of sexual activity of male and female rats.

Sexual Behavior Tests

Sexual behavior tests were performed as described for the training sessions. The male rats selected for high sexual activity during the training were used as studs at 2- to 3-day intervals in these tests.

Videos were analyzed by an operator blinded to the treatments. The following female behavioral acts and patterns were scored per 30-minute trial:

- Female solicitations (referred to as solicitation frequency), consisting of an approach to the male rat, followed by a hasty retreat, hops and darts (optionally), and waiting for the male rat to approach
- Lordosis reaction or its absence during a mount (lordosis frequency)
- Refusal to accept male courting
- Social behaviors: frequencies of anogenital sniffing and approaching the male rat outside the sexual context (not followed by retreat, hops, darts, or any copulatory activity)

Experiment 1: Acute Intranasal BP101 Administration

The effects of a single intranasal administration of BP101 on female rat sexual behavior were investigated. After the initial training, female rats receiving the high EB supplementation were habituated to the procedure of intranasal administration for 5 consecutive days before the test and randomly assigned to groups. BP101 was dissolved in deionized water supplemented with 0.01% benzalkonium chloride; 0.01% aqueous benzalkonium chloride was used as a placebo. The peptide was administered intranasally at 25 (n = 11), 75 (n = 12), 200 (n = 11), and 300 (n = 11) μg/kg (instillation volume = 100 μL/kg). A separate study showed that BP101 after intranasal administration is found in the blood and brain (eFigure 1). Two control groups were given placebo; one group received P 500 μg per rat (n = 9) and the other group (PC group) received P 1,000 μg per rat (n = 11) to induce maximal sexual receptivity. Thirty minutes after BP101 or placebo instillation, female rats were placed in the arena for the sexual behavior test with a stud male rat that was conducted as described earlier.

Experiment 2: Long-Term Intranasal BP101 Administration

In the second experiment, using a separate batch of animals, the effects of long-term administration of BP101 75 (n = 19) or 300 (n = 20) μg/kg for 16 days were investigated. After the training and random assignment to groups, BP101 was administered to previously habituated female rats kept on the high EB dose similarly to experiment 1 once daily for 16 days. Female rats of two control groups received placebo intranasally as in experiment 1; PC female rats were treated as in experiment 1 (n = 16 and 15, respectively). The first treatment day was matched to the day of P administration and sexual behavior testing. Thus, female rats were repeatedly tested after 1, 6, 11, and 16 intranasal peptide administrations. Sexual behavior testing was performed as described earlier. Baseline sexual behavior values were measured during the last training session (day −4) before administering the drug.

Experiment 3: Local BP101 Infusion Into Certain Brain Areas

In this experiment, the effects of BP101 infusion into the olfactory bulb (OB), medial preoptic area (MPA), ventromedial hypothalamus (VMN), or ventral tegmental area (VTA) were investigated. In the OB, BP101 was studied in combination with the high EB supplementation only; in the MPA, VMN, and VTA, BP101 was administered to female rats with the low and high EB supplementation (Table 1).

After the training, and 3 days before the sexual behavior tests, the batch of female rats was implanted with a cannula for drug infusion. Anesthesia was applied as described for ovariectomy; in addition, Novocain (Biokhimik, Saransk, Russia) was used as a local anesthetic. The head was shaved, the skin above the temporal and frontal bones was removed, and the skull was cleared of connective tissue. The rats were mounted onto a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA) in a flat skull position. After the skull was drilled with injection needles, a 23-gauge stainless steel guide cannula was advanced into the brain so that its tip was 1 mm above the target area. The

Table 1. Experiment 3 design and number of animals per group

Brain site	EB supplementation	BP101 doses studied*	n
OB	low	—	—
	high	0, 1, 5, 25 μg	13
MPA	low	0, 1, 5, 25 μg	17
	high		14
VMN	low	0, 1, 5 μg/kg	5
	high		11
VTA	low	0, 1, 5 μg/kg	10
	high		13

EB = estradiol benzoate; MPA = medial preoptic area; OB = olfactory bulb; VMN = ventromedial hypothalamic nucleus; VTA = ventral tegmental area.
*BP101 0 μg refers to placebo (artificial cerebrospinal fluid).

coordinates for guide cannula implantation were anteroposterior (AP) +8.0, mediolateral (ML) ± 1.5, and dorsoventral (DV) −3.0 in the OB; AP −0.2, ML ± 0.8, and DV −7.4 in the MPA; AP −3.0, ML ± 1.0, and DV −8.6 in the VMN; and AP −5.2, ML ± 1.4, and DV −7.4 in the VTA.[14] The guide cannulas were fixed to the skull using acrylic and stainless steel screws; a 29-gauge stainless steel plug was used to secure the patency of the cannula.

Sexual behavior was tested on three consecutive hormonal supplementation cycles, with two different BP101 doses and a placebo administered in a randomized order. Randomization was performed using GraphPad software (http://www.graphpad.com/quickcalcs). For brain infusion, BP101 was dissolved in artificial cerebrospinal fluid (sodium 150 mmol/L, potassium 3.0 mmol/L, calcium 1.4 mmol/L, magnesium 0.8 mmol/L, phosphorus 1.0 mmol/L, chlorine 155 mmol/L), which was used as a placebo. To reach the desired brain location, the infusion cannula was 1 mm longer than the guide cannula. Drugs were infused 10 minutes before the behavioral tests with a 10-μL syringe driven by a syringe pump at a rate of 250 nL/min for 2 minutes (total volume infused = 500 nL). Female rats were allowed to calm down for 5 minutes after the infusion and were placed in the arena for the sexual behavior test.

To verify implantation accuracy, female rats were anaesthetized with urethane (1.5 g/kg), India ink 500 nL was infused through the guide cannula to mark the application site, and the brain was excised after a transcardial perfusion with saline 150 mL and phosphate buffered 4% formaldehyde 150 mL. After an additional 48-hour fixation, histologic sections were prepared and stained with hematoxylin and eosin. Results from rats with a cannula positioned outside the target area were discarded.

Statistical Analysis

Statistical analysis was performed with GraphPad Prism using analysis of variance (ANOVA) followed by Sidak post-tests. Specifically, experiment 1 was analyzed with a simple one-way ANOVA, experiment 2 was analyzed with repeated measures two-way ANOVA (factors group and time), and experiment 3 was analyzed with repeated measures ANOVA (factors EB regimen and BP101 dose). In addition, the χ^2 test was used to compare differences in the incidence of female rats with a parameter value higher or lower than the control group in experiment 1. Differences were considered significant at a P value less than 0.05. Data are presented as mean ± standard error.

RESULTS

Acute Intranasal BP101 Administration

Acute administration of BP101 25 to 300 μg moderately increased female sexual activity, whereas social interactions were not affected (Table 2). Specifically, solicitation frequency varied with the group ($F_{5,59} = 2.91$; $P = .0205$); compared with the "placebo" group, it was 65 ± 17% higher in PC rats that received

Table 2. Female rat behavior after single intranasal administration of BP101*

Parameter	BP101 (μg/kg)					PC (n = 11)
	0 (placebo; n = 9)	25 (n = 11)	75 (n = 12)	200 (n = 11)	300 (n = 11)	
Solicitations frequency	49.4 ± 5.7	62.2 ± 10.3† (+26%)	54.0 ± 7.0† (+9%)	51.7 ± 8.0 (+5%)	78.4 ± 7.6† (+59%)	81.4 ± 8.5†‡ (+65%)
Lordosis frequency	10.8 ± 2.4	15.6 ± 3.5 (+44%)	12.9 ± 3.1 (+19%)	14.2 ± 2.6 (+31%)	18.5 ± 2.3 (+71%)	20.2 ± 3.1† (+87%)
Lordosis ratio	90.6 ± 8.6	98.8 ± 0.9 (+9%)	84.3 ± 4.9 (−7%)	84.4 ± 6.8 (−7%)	89.0 ± 6.2 (−2%)	97.7 ± 1.5 (+8%)
Social approach frequency	34.7 ± 3.8	33.1 ± 3.7 (−5%)	33.4 ± 4.0 (−4%)	27.6 ± 2.6 (−20%)	36.3 ± 3.2 (+5%)	35.7 ± 3.6 (+3%)
Anogenital sniffing frequency	10.4 ± 1.4	10.1 ± 2.0 (−3%)	11.1 ± 1.7 (+7%)	10.6 ± 1.5 (+2%)	11.9 ± 2.6 (+14%)	9.5 ± 1.8 (−9%)

PC = positive control.
*Values in parentheses represent differences from placebo-treated female rats.
†$P < .05$ vs 0 (placebo) by the Dunnett post-test; ‡$P < .05$ vs 0 (placebo) by χ^2 test; see text for details.

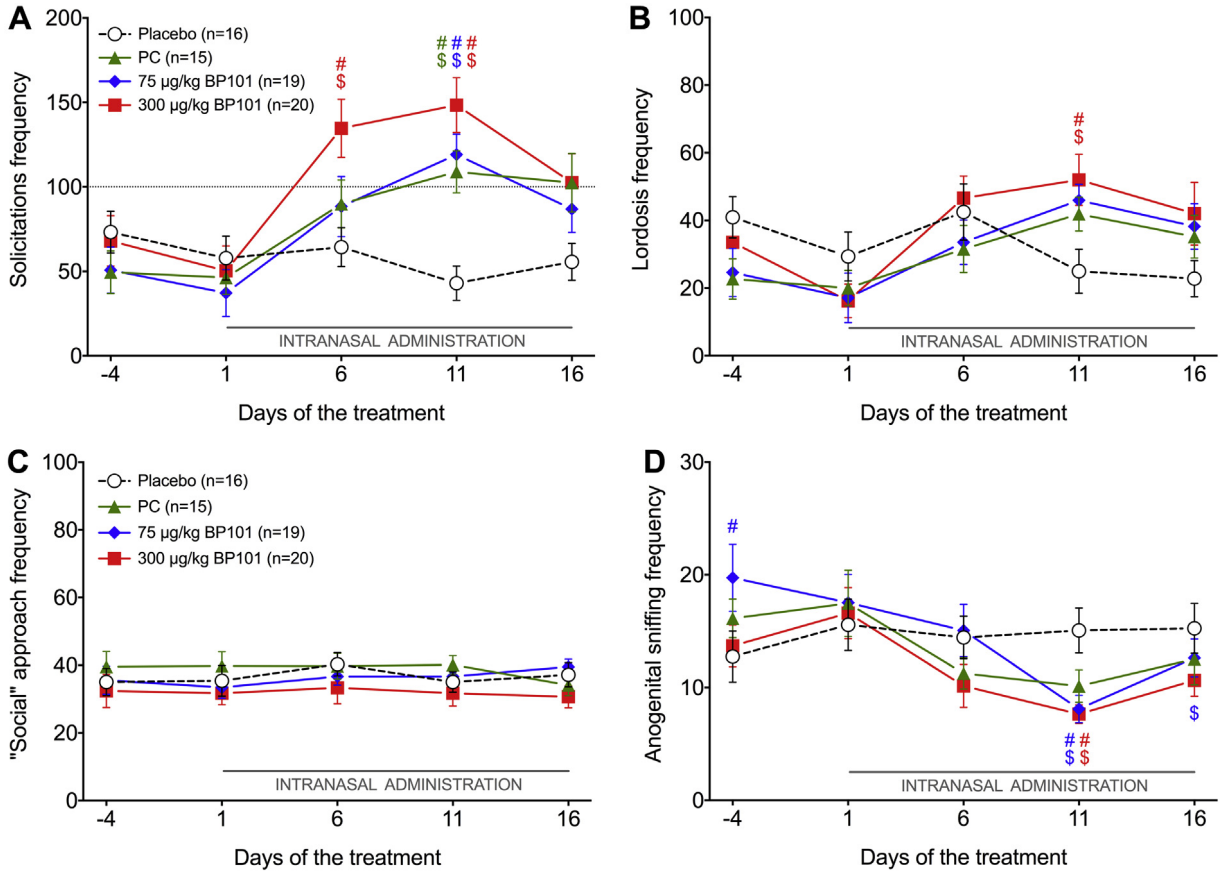

Figure 1. Panel A shows that solicitations were increased after 6 to 16 days of intranasal BP101 administration or optimal progesterone priming (PC group) compared with the placebo group. Panel B shows that lordosis frequency was increased after 6 to 16 days of intranasal BP101 administration or optimal progesterone priming (PC group) compared with the placebo group. Panel C shows that the frequency of "social" approaches was unaffected by the treatments. Panel D shows that anogenital sniffing frequency was decreased in sexually active groups. $^{\#}P < 0.05$ vs placebo; $^{\$}P < 0.05$ vs baseline values by Dunnett test. PC = positive control. Figure 1 is available in color at www.jsm.jsexmed.org.

the optimal P dose and 59 ± 15% higher in rats treated with BP101 300 µg/kg (marginally significant at $P = .07$). The incidence of female rats displaying more solicitations than placebo-treated rats increased after BP101 25, 75, and 300 µg/kg intranasal administrations ($P < .05$ by χ^2 test).

Concomitantly with increase in solicitations, lordosis frequency was 87 ± 29% higher in PC rats and 71 ± 21% higher in rats treated with BP101 300 µg/kg compared with placebo. However, the variance did not depend on the group ($F_{5,59} = 1.40$; $P = .2360$), whereas the incidence of female rats displaying more lordosis was higher in the PC group ($P < .05$ by χ^2 test). Lordosis ratio ($F_{5,59} = 1.59$; $P = .1762$), frequency of female rats' approaches to the studs, and anogenital sniffing frequency were similar in all groups.

Long-Term Intranasal BP101 Administration

Female rats administered long term with BP101 75 or 300 µg/kg displayed increased sexual behavior and somewhat decreased social interactions (eFigure 1). Solicitations varied with group ($F_{3,66} = 10.47$; $P < .0001$) and time ($F_{4,264} = 11.96$; $P < .0001$), and the dynamics of solicitation frequency were different between groups (group × time interaction, $F_{12,264} = 2.22$; $P = .0114$). Thus, BP101 75 µg/kg enhanced female solicitations on treatment days 6 to 16 compared with placebo-treated rats and on day 11 compared with baseline values (Figure 1A). Higher BP101 dose (300 µg/kg) was similarly effective in increasing solicitations compared with the placebo group or baseline values on days 6 and 11, followed by a decrease on day 16. PC rats displayed a higher solicitation frequency on days 6 to 16 compared with the placebo group and on days 11 and 16 compared with baseline activity (Figure 1A).

Lordosis frequency was affected by treatments ($F_{3,66} = 4.76$; $P = .0046$) and time ($F_{4,264} = 6.51$; $P < .0001$), but not by their interaction ($F_{12,264} = 1.36$; $P = .1858$). Female rats receiving 75 µg/kg had more lordosis reactions compared with placebo-treated rats on days 11 and 16, and on day 11 lordosis frequency surpassed baseline values. Lordosis frequency in PC rats was higher than in controls on days 11 and 16. The increase in lordosis frequency in rats treated with 300 µg/kg reached statistical significance on day 11 (Figure 1B). Similar results were retrieved from the lordosis ratio analysis (data not shown), which varied with group ($F_{3,66} = 4.91$; $P = .0039$) and time

($F_{4,264} = 6.84$; $P < .0001$), whereas the group × time interaction was insignificant ($F_{12,264} = 1.24$; $P = .2551$).

Female social approaches to the male rat (Figure 1C) were similar between groups and had similar dynamics in different groups. However, anogenital sniffing was affected by treatments ($F_{3,66} = 4.32$; $P = .0077$) and time ($F_{4,264} = 7.21$; $P < .0001$); the interaction of these factors was not significant ($F_{12,264} = 1.50$; $P = .1244$). Specifically, anogenital sniffing was decreased in all sexually active groups (Figure 1D).

Effects of BP101 Infusion Into Brain Centers

Solicitations were increased after BP101 infusion into the MPA (Figure 2C), but not into the OB (Figure 2A), VMN (Figure 2E), or VTA (Figure 3G). In the MPA, solicitation frequency varied with BP101 dose ($F_{3,74} = 8.17$; $P < .0001$); the most pronounced relative increase in solicitations was observed in the low EB primed female rats infused with BP101 1 and 5 μg per rat, whereas with high EB supplementation the BP101 effect was almost five times less robust. No change in solicitation frequency was observed after BP101 infusion into the OB ($F_{3,32} = 0.42$; $P = .7401$), VMN ($F_{2,32} = 0.23$; $P = .7957$), or VTA ($F_{2,60} = 0.17$; $P = .8457$). Higher compared with lower EB supplementation expectedly increased solicitation frequency (MPA, $F_{1,74} = 34.79$; $P < .0001$; VMN, $F_{1,32} = 23.50$; $P < .0001$; VTA, $F_{1,60} = 10.78$; $P = .0017$).

Lordosis frequency was not significantly affected by BP101 infusion into the OB ($F_{3,33} = 1.31$; $P = .2889$; Figure 2B), MPA ($F_{3,74} = 0.9757$; $P = .4089$; Figure 2D), VMN ($F_{2,32} = 0.95$; $P = .3961$; Figure 2F), or VTA ($F_{2,64} = 0.38$; $P = .6887$; Figure 2H). Higher EB supplementation increased lordosis frequency compared with low EB (MPA, $F_{1,74} = 14.07$; $P = .0003$; VMN, $F_{1,32} = 13.24$; $P = .0010$; VTA, $F_{1,64} = 11.04$; $P = .0015$).

Similarly, no effect of BP101 was found for the lordosis ratio after peptide infusion into the OB ($F_{3,32} = 2.16$; $P = .1118$), MPA ($F_{3,72} = 1.36$; $P = .2617$), VMN ($F_{2,32} = 0.47$; $P = .6271$), and VTA ($F_{2,64} = 0.01$; $P = .9939$). Priming female rats with the higher EB dose resulted in an increased lordosis ratio compared with low EB supplementation (MPA, $F_{1,72} = 10.52$; $P = .0018$; VMN, $F_{1,32} = 14.40$; $P = .0006$; VTA, $F_{1,64} = 11.12$; $P = .0014$). It should be noted that cannula implantation itself affected female sexual behavior, explaining different baseline activities in different groups. In these experiments, animals with cannulas implanted into the VTA had a particularly low baseline activity (Figure 2).

Frequency of social approaches was not affected by BP101 or the EB supplementation regimen in the MPA and VMN; conversely, in the VTA, the EB priming dose had a significant effect on the frequency of social interactions ($F_{1,64} = 10.27$; $P = .0021$; Figure 3G).

BP101 had no effect on anogenital sniffing frequency when infused into the MPA (Figure 3D), VMN (Figure 3F), or VTA (Figure 3E). Conversely, in the OB, BP101 increased sniffing frequency (Figure 3B). The higher EB dose decreased sniffing frequency in female rats with a cannula implanted in the MPA ($F_{1,72} = 4.74$; $P = .0328$) and VMN ($F_{1,32} = 4.71$; $P = .0376$), but not in the VTA ($F_{1,64} = 2.23$; $P = .1405$).

DISCUSSION

We report for the first time the facilitatory effect of BP101, a synthetic peptide, on female sexual behavior in the rat. The peptide enhanced primarily solicitations after acute and more robustly after long-term intranasal administration, with a concomitant increase in lordosis frequency and decrease in some types of social interaction. Local infusion into the MPA, but not into the OB, VMN, or VTA, replicated the effects of BP101 after intranasal administration.

After long-term intranasal treatment with BP101, we observed increased solicitations in the ovariectomized female rats primed with a combination of EB and P. Solicitation frequency in BP101-treated female rats approximated the level of PC rats that received the higher P dose, which induces maximal receptivity in this model.[15,16] An increase in lordosis frequency correlates with an increase in the frequency of male mounts, which in turn might arise from higher sexual activity of the stud male rat in contact with a highly proceptive female rat.[17–19] A decrease in anogenital sniffing frequency, observed in the highly sexually active rats that received the high P dose or BP101, resulted in extremely active copulatory behavior in our tests that left virtually little time for any other interaction. In contrast, our observations indicated that female rats sniffed sluggish male rats more often, trying to start copulation or for social interest (investigation of a conspecific).

The intranasal effect of BP101 increased over the duration of the experiment and peaked on day 11 of the treatment, indicating the influence of BP101 on protein expression. Contrary to the results of experiment 1, we did not observe any increase in sexual activity after the first administration of BP101 in experiment 2. Surprisingly, the PC group showed no increase in response to the increased P dose. Despite efforts to standardize the experimental conditions and to habituate the rats to stressful experimental manipulations, the discrepancy in our data might arise from some unaccounted variation in the experimental conditions or the physiologic condition of the animals. This also indicates that the experimental conditions or the physiologic or psychological condition of the animals that prohibited increased sexual activity in response to high P also prohibited increased sexual activity in response to BP101. This means that effect of BP101 is not unconditional, or it is not strong enough to overcome influence of certain external or internal conditions.

Because the OB is the central site for analysis of olfactory cues, the MPA plays a key role in female sexual motivation, the VMN integrates hormonal levels with behavior and regulates the lordosis reaction, and the VTA is a key dopaminergic region in the brain in the regulation of female sexual behavior (reviewed in

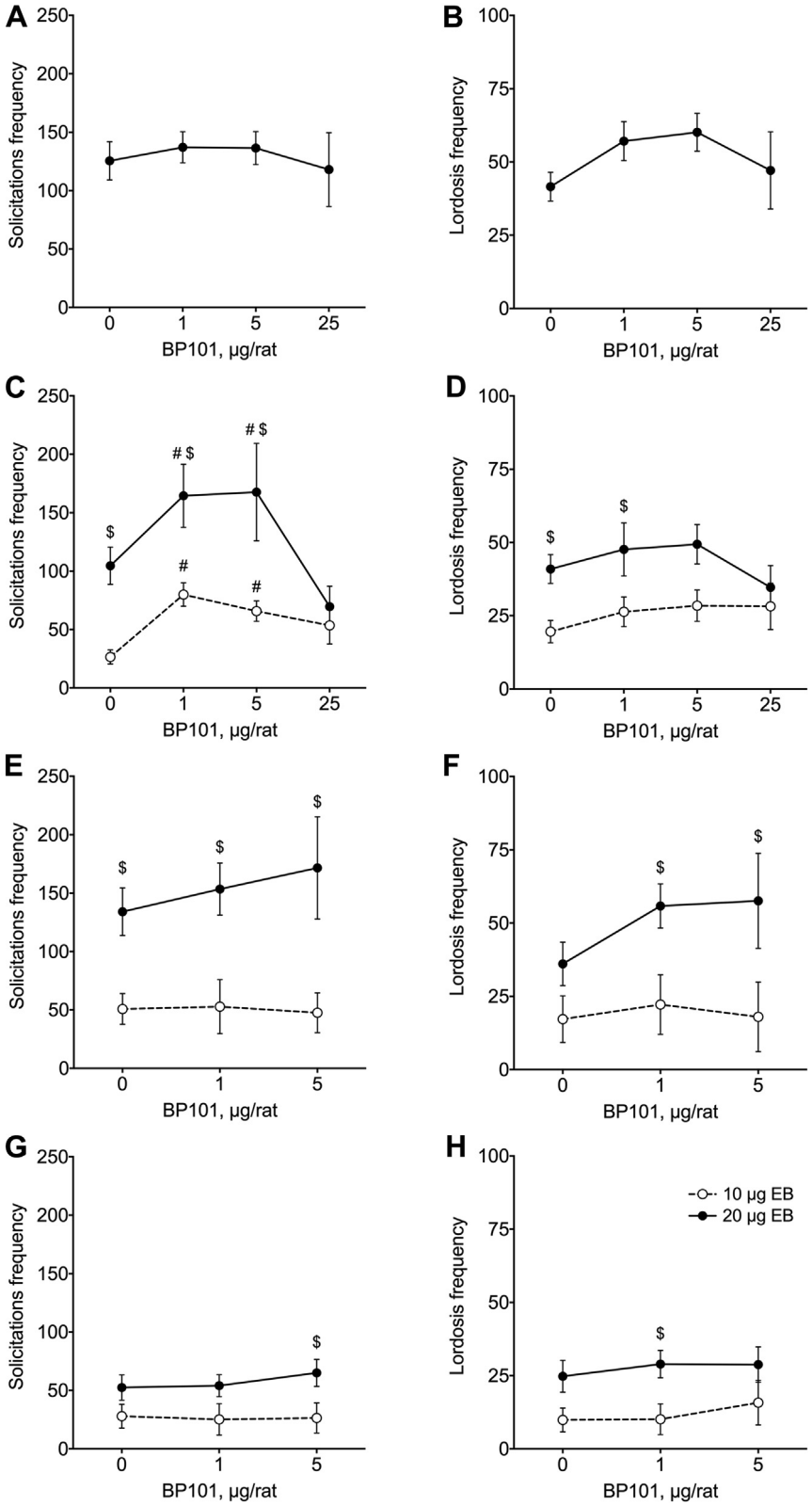

Figure 2. BP101 1 and 5 μg per rat increased solicitation frequency when infused into the medial preoptic area (C), but not into the olfactory bulb (A), ventromedial hypothalamic nucleus (E), or ventral tegmental area (G). The stimulatory action of EB is clearly depicted. BP101 did not have a significant effect on lordosis frequency after infusion into the olfactory bulb (B), medial preoptic area (D), ventromedial hypothalamic nucleus (F), or ventral tegmental area (H). Lordosis frequency was higher with higher EB supplementation. $^{\#}P < 0.05$ vs artificial cerebrospinal fluid; $^{\$}P < 0.05$ vs low EB dose by the Sidak test. EB = estradiol benzoate.

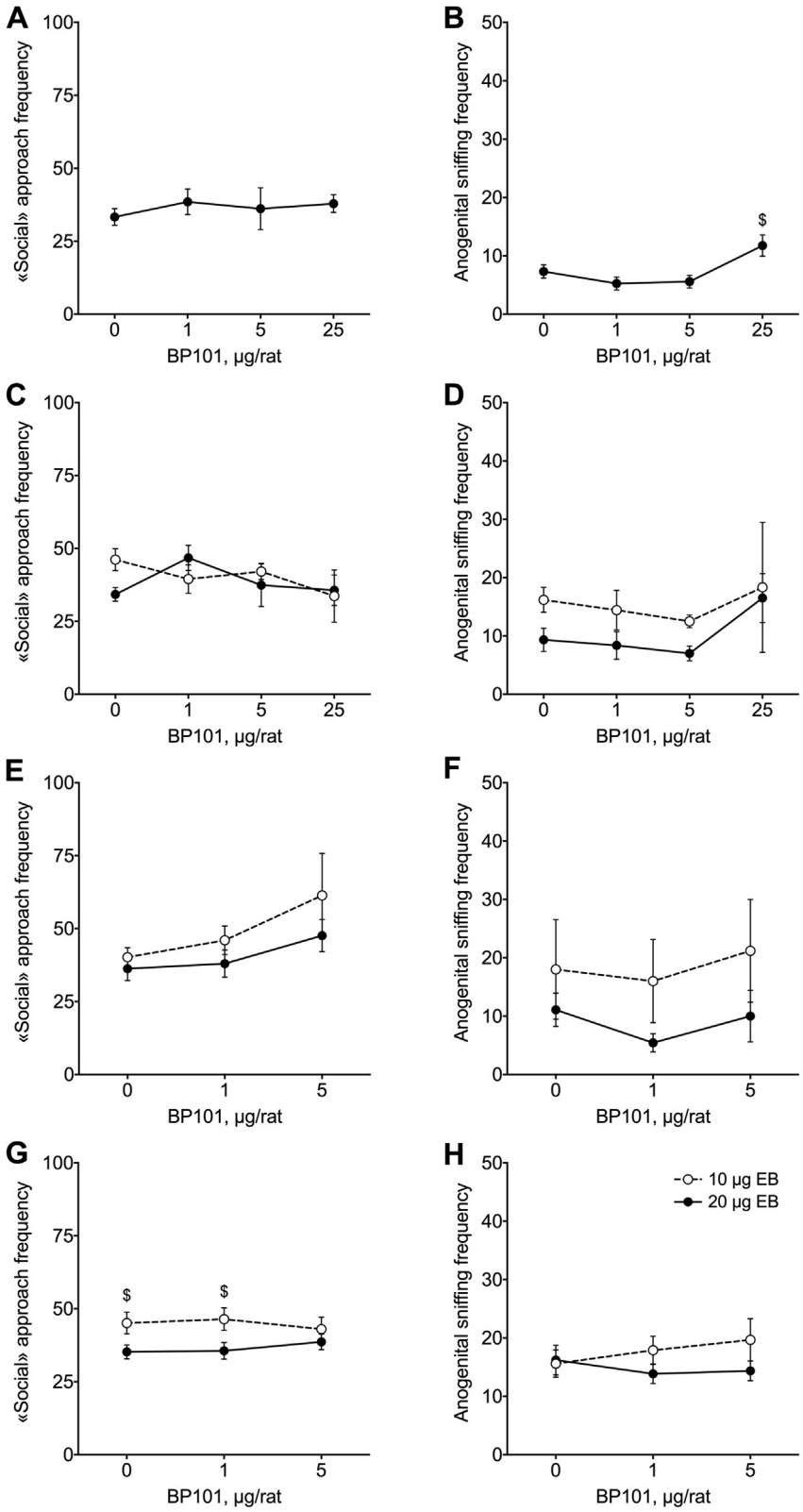

Figure 3. Frequency of "social" approaches was not affected by BP101 infusion into the olfactory bulb (A), medial preoptic area (C), ventromedial hypothalamic nucleus (E), or ventral tegmental area (G). Anogenital sniffing frequency did not change with BP101 administration in the olfactory bulb (B), medial preoptic area (D), ventromedial hypothalamic nucleus (F), and ventral tegmental area (H). $^{\$}P < 0.05$ vs low EB does by the Sidak test. EB = estradiol benzoate.

Pfaus[20] and Pfaus et al[21]), these brain centers were chosen for this study. Among these centers, the MPA proved to be a likely candidate for the site of action of BP101.

Lesion[22–24] electrophysiologic,[25,26] and pharmacologic[27,28] studies have shown that the MPA plays a central role in the mechanisms of female sexual motivation and participates in sexual behavior hormonal control.[29] Mating,[30] genitalia stimulation,[31,32] exposition to male odor,[33] or odor associated with sexually rewarding paced mating[34] activate MPA neurons in female rats as measured by c-fos immunoreactivity. Recordings of neural activity have shown a subpopulation of neurons actively firing before female proceptive behavior.[25] Importantly for this study, prior sexual experience determines MPA reactivity to sexual stimuli,[33] underscoring the importance of the extensive training period used in this study.

Several neurotransmitters have been implicated in proceptive behavior control within the MPA that can be grouped as promoting (dopamine, noradrenalin, melanocortin) or inhibiting (opioid peptides, serotonin, γ-aminobutyric acid) sexual behavior[20]; thus, several possible mechanisms of a direct activating influence of BP101 or BP101-induced disinhibition could be proposed.

To elucidate the mechanism of action, BP101 was screened on a panel of 98 G-protein—coupled receptor characteristic for the brain. Screening was conducted by the DiscoveRx Corporation (Fremont, CA, USA). Among others, the panel contained serotonin, dopamine, and melanocortin receptors. BP101 was tested as an agonist, antagonist, and positive allosteric modulator. No change in activity was observed for any combination of the receptor and experimental mode.

Juxtaposition of the apparent pro-sexual BP101 effect in the MPA and lack of activity upon infusion into the other brain areas can provide some cues about the neurotransmitter system mediating the BP101 effect. It seems likely that the dopaminergic system is involved in the action of BP101, although it does not act directly on dopamine receptors. Dopamine has been attributed a central role in sexual motivation control, similarly to other motivated behaviors.[20] Dopamine levels are increased in the striatum and nucleus accumbens of female rats during copulation[35]; in addition, the dopamine increase during sexually rewarding paced mating is higher than during non-paced copulation. Enhancement of dopamine transmission in the MPA facilitates appetitive sexual behavior.[27] High doses of the non-selective D1 and D2 agonist and the D1 antagonist have been shown to inhibit solicitations, whereas D2 antagonist has been shown to facilitate paced mating in female rats.[28] However, reliable reports to the contrary, indicating no influence, also exist.[36] Increased dopamine levels have been found after flibanserin administration, providing further evidence for its importance in appetitive sexual behavior control.[37] By combining the negative findings in vitro during BP101 screening with the observed BP101 effects, we can speculate that the dopamine transporter and/or synthesis or metabolism enzyme activity or their brain levels might be the targets of BP101 action.

At the same time, subjectively, changes of BP101-treated females sexual behavior resemble the description from Uphouse and Caldarola-Pastuszka[38] who reported "frenzied" hopping and darting behavior in female rats infused with the serotonin receptor 1A agonist 8-hydroxy-2-(di-n-propyl-amino)tetralin. Thus, further experiments are needed to elucidate the mechanisms of BP101 on sexual motivation.

In summary, BP101 displays a potent stimulatory effect on sexual motivation in the female rat. The MPA seems to be the probable site of its action. The effect of BP101, when infused directly into the brain, was relatively more pronounced in female rats primed with lower estrogen doses, although its action was still pronounced in "high" estrogen primed rats, making the data relevant to pre- and postmenopausal women with various hormonal statuses.

BP101 has successfully passed phase 1 clinical studies, where it demonstrated high safety, and currently undergoing a randomized, doubled-blinded, placebo-controlled phase 2 study to measure its efficacy.

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The BP101 peptide has a A-Thr-Lys-Pro-B-C-D sequence, where A, B, C, and D are amino acids, described in US patent US9409947B2. Currently, the complete structure cannot be disclosed in full due to patent prosecution worldwide. In the United States, the structure of BP101 is covered by US patent US9409947B2 granted to Ivix Ltd.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jsxm.2017.01.008.