FULL ARTICLE

ISSN Online 1678-4456

Sexual motivation and performance in sexual naïve and experienced rats treated with ivermectin: an ultrasonic vocalization study

Motivação e desempenho sexual em ratos com e sem experiência sexual tratados com ivermectina: um estudo da vocalização ultrassônica

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ABSTRACT

In mammals, ivermectin acts as a GABAA receptor agonist and stimulates GABA release. Previous studies showed that ivermectin (IVM) reduces sexual performance, impairing the latency to the first mount and intromission. These parameters are usually considered motivational parameters of sexual behavior. However, IVM increases GABAergic activity leading to motor incoordination. Thus, it is reasonable to propose that IVM affects sexual performance via motor incoordination pathways. The present study analyzed ultrasonic vocalization in rats to verify whether IVM impairs sexual behavior via motivational mechanisms or motor impairment. Because sexual experience attenuates the impairment of motor performance, rats with sexual experience were also studied. Sexually naive and experienced rats were administered a therapeutic IVM dose and saline. The rats were exposed to receptive females, and the latency to the first mount was evaluated, followed by the 50-kHz USV test. IVM treatment in naïve rats increased the latency to first to mount relative to Saline naïve rats, while no differences were observed between saline and experienced rats. In naïve-IVM rats, a reduced frequency and total calls and increased mean time of calls occur relative to SAL-naïve rats. Experienced IVM rats did not show differences in the frequency, mean, and maximal calls close to Saline experienced rats. However, an increase in the total calls and the dominant frequency of calls were observed in IVM-experienced rats compared to Saline experienced rats. A negative and positive correlation occurred between the latency to the first mount and USVs in groups with and without ivermectin exposure. Hence, we propose that ivermectin increased the sexual motivation of rats exposed to a female in estrous based in USVs despite an increased latency to the first mount that occurred. The increased latency to the first mount resulted from motor incoordination, as previously observed and proposed by our group.

Keywords: Sexual behavior. Ultrasonic vocalizations. Latency to the first mount. Motivation.

RESUMO

Em mamíferos, a ivermectina (IVM) atua como agonista do receptor GABAA e estimula a liberação de GABA. Estudos anteriores mostraram que a IVM reduz o desempenho sexual, prejudicando a latência para a primeira monta e intromissão. Esses parâmetros são geralmente considerados parâmetros motivacionais do comportamento sexual. Por outro lado, a IVM aumenta a atividade GABAérgica levando à incoordenação motora. Assim, é possível que a IVM afete o desempenho sexual devido a um impedimento motor. O presente estudo analisou a vocalização ultrassônica em ratos para verificar se a IVM prejudica o comportamento sexual via mecanismos motivacionais ou comprometimento motor. Uma vez que a experiência sexual atenua o comprometimento do desempenho motor, também foram estudados ratos com experiência sexual. Ratos sexualmente inexperientes e experientes foram administrados com uma dose terapêutica de IVM ou solução salina IVM. Os ratos foram expostos a fêmeas receptivas e foi avaliada a latência para a primeira monta, seguida do teste de vocalização ultrassônica (USV) de 50 kHz. O tratamento com IVM em ratos inexperientes aumentou a latência para a primeira monta em relação a ratos inexperientes tratados com solução salina, enquanto não foram observadas diferenças entre ratos experientes tratados com IVM e solução salina. Em ratos inexperientes tratados com IVM ocorreu redução da frequência e total de USVs, bem como aumento do tempo médio de USVs em relação aos ratos sem experiência. Ratos experientes tratados com IVM não mostraram diferenças na frequência, média e máxima das USVs em relação aos ratos experientes tratados com solução salina; no entanto, observou-se aumento no

total de USVs e na frequência dominante de USVS em ratos experientes tratados com IVM comparados aos experientes tratados com solução salina. Observou-se correlação negativa e positiva entre a latência para a primeira monta e USVs nos grupos sem e com experiência tratados com IVM, respectivamente. Assim, propomos que a IVM aumentou a motivação sexual de ratos expostos a uma fêmea em estro com base em USVs, apesar de apresentar aumento na latência para a primeira monta. O aumento da latência para a primeira monta foi atribuída à incoordenação motora, conforme observado anteriormente e proposto por nosso grupo.

Palavras-chave: Comportamento sexual. Vocalização ultrassônica. Latência para primeira monta. Motivação.

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Received: May 31, 2022
Approved: January 13, 2023

How to cite: Silva-Rodrigues P, Silva-Sampaio AC, Silva ÉP, Suffredini IB, Bondan EF, Kirsten TB, Bernardi MM. Sexual motivation and performance in sexual naïve and experienced rats treated with ivermectin: an ultrasonic vocalization study. Braz J Vet Res Anim Sci. 2023;60:e198441. https://doi.org/10.11606/issn.1678-4456.bjvras.2023.198441.

Introduction

Avermectins are antiparasitic agents extensively used in agricultural and domestic animals (Campbell, 2012; Hotson, 1982). Ivermectin (IVM) is the first agent of this group and belongs to the macrocyclic lactones class of endectocides. IVM is a mixture of two homologous compounds, 22, 23-dihydro avermectin B1a (H2B1a; not > 80%) and 22,23-dihydro-avermectin B1b (H2B1b; not > 20%) (Albers-Schoenberg et al., 1981). IVM is the first macrocyclic lactone-synthesized avermectin (Elgart & Meinking, 2003).

As anthelmintic, avermectins act by binding to glutamatergic chloride channels in nematode neurons and pharyngeal muscle cells, leading to a deep and long-lasting hyperpolarization or depolarization of neurons and muscle cells and blockade of other functions (Wolstenholme & Rogers, 2005). In vertebrates, these agents produce γ-aminobutyric acid (GABA)-mimetic effects acting as GABAA receptor agonists and stimulating GABA release (Dawson et al., 2000; Shoop et al., 1995; Spinosa et al., 2000; Yang, 2012). Because avermectins do not readily cross the blood-brain barrier (Campbell et al., 1983; Lee et al., 2019; Yang, 2012), and in mammals, the GABA-mediated nerves are found mainly in the central nervous system (Lee et al., 2019), mammals are less susceptible to the toxic effects of avermectins.

Several studies showed that GABAergic agonists inhibit sexual behavior. Administration of low doses of baclofen, a GABA agonist, produced almost complete inhibition of sexual behavior at a low dose (Agmo & Paredes, 1985). In addition, Zarrindast & Farahvash (1994) showed that GABAergic agonists inhibited the penile erection induced by apomorphine. Also, administration of the GABA transaminase inhibitors γ-acetylene GABA and sodium valproate reduced motor execution and sexual behavior in a dose-dependent way (Agmo et al., 1987). After the sexual experience, motor impairment is diminished after the sexual experience (López et al., 1999).

Our previous studies revealed that therapeutic doses of avermectins induced several impairments in sexual behavior. In this respect, IVM impaired sexual behavior in male rats (Bernardi et al., 2011) and reduced the female rats' sexual behavior (Moreira et al., 2014). Also, doramectin impaired sexual behavior and penile erection (Ferri et al., 2013). Otherwise, Bernardi et al. (2011) showed that therapeutic IVM doses degraded the latencies to first mount in inexperienced male rats. This parameter reflects the sexual motivation of male sexual behavior (Agmo, 1997).

However, the male rat's sexual behavior is a highly ordered sequence of motor acts involving both striate and smooth muscles. It occurs spontaneously displayed by most adult male rats in the presence of a sexually receptive female. The male mounts typically from the rear, sometimes posing his forelegs over the female's back, and makes rapid anteroposterior pelvic thrusts for about 300 ms (Agmo, 1997). Thus, the latency to the first mount involves motivational and motor aspects.

To clarify whether IVM reduces motor execution or sexual motivation, Moreira et al. (2017) investigated in male rats the effects of a therapeutic IVM dose on motor incoordination, penile erection, and sexual stimulation. The results showed increased motor in coordination with a decreased striatal dopaminergic system activity but no effects on sexual stimulation or penile erection. Thus, these authors suggest that IVM may activate the GABAergic system, reducing motor coordination due to the inhibition of striatal dopamine release.

Ultrasonic vocalization (USV) has a vital role in rats' social interactions (Burgdorf et al., 2008; Peters et al., 2017; Riaz et al., 2015; Scattoni et al., 2009). Ultrasonic vocalization is considered a simple and exciting model to explain the function of calls in expressing emotions. The 50 kHz USVs reflect the animals' positive or hedonic emotional state, and the 22 kHz USVs reflect a negative

one. The 50-kHz USVs were emitted during appetitive experiences (Burgdorf et al., 2005). These USVs occur during ludic behavior (Himmler et al., 2014; Kisko et al., 2017), alimentary behavior (Galef, 1990; Mayeaux et al., 2014), and sexual behavior (Barfield et al., 1979; McIntosh & Barfield, 1980). Male rats, before the first time of contact with an unfamiliar estrous female, emit vocalizations that contain calls types from both the 22 kHz USVs and 50 kHz USVs categories (Barfield et al., 1979; Harding & McGinnis, 2003; Snoeren & Agmo, 2014; Willadsen et al., 2014). The emission of 50 kHz USVs occurs during mating and other types of pleasant social interactions or the expectation of reward (Brudzynski, 2021; Burgdorf et al., 2008).

Because it was proposed that IVM reduces sexual behavior by decreased motor coordination (Moreira et al., 2017) and ultrasonic vocalization, a parameter related to sexual motivation (Bogacki-Rychlik et al., 2021), occurs independently of motor execution, this study examined the IVM effects until the first mount of sexual behavior in naïve rats and the USVs at the same time in naïve rats. In the present study, we chose the latency of the first mount because it is related to an anticipatory state of positive motivation expressed by the 50kHz calls. The presence of a female rat in estrous is the positive incentive.

Sexual experience improves the motor performance of sexual behavior, and the facilitation of latency to first mount occurs after one exposure is attributed to a learning process (Bialy et al., 2000). Thus, the effects of IVM inexperienced rats were also observed to examine if the anticipatory USVs are associated with the level of motivation and not with motor impairment (Bogacki-Rychlik et al., 2021).

Material and Method

Animals

Thirty-three adult male Wistar rats (100-110 days of age, weighing 330-370 g) and 16 adults female Wistar rats (90-95 days of age, weighing 280-300 g) were acquired from the facilities of the Institute of Biomedical Sciences of the University of São Paulo (ICB-USP). The rats were maintained in the facilities of the University of Paulista for 10 days before the experiment to habituations. They were housed in groups of four (separated by sex) in micro isolator cages with controlled temperature (22-26°C) and humidity (50-65%) in artificially lit rooms with an inverted cycle of 12 h/12 h light/dark cycle (lights on at 7:00 PM) for three weeks before experiments. Free access to filtered water, irradiated food (BioBase, Águas Frias, Brazil), and sterilized residue-free wood shavings were used for animal bedding.

Treatments

Ivermectin (1% Ivomec® injectable, Merial Animal Health Ltda., Paulínia, SP, Brazil) dissolved in a saline solution (NaCl 0.9%) plus a drop of Tween 80 was administered subcutaneously (s.c.) at a 1.0 mg/kg dose. This dose is considered a therapeutical dose to rats (Soll, 1989), and its use is already standardized in studies in our laboratories, with known effects (Bernardi et al., 2011; Moreira et al., 2014). The control solution consisted of saline solution plus a drop of Tween 80. Both solutions were administered in a volume of 1.0 mL/kg. The tests and evaluations were made 24 h after IVM or saline administration due to IVM half-life: 24-72 h administered by s.c. route (Cordeiro et al., 2018).

Latency to the first mount

A sexually receptive female (incentive female) was used in the estrous phase. For this, the females' vaginal smears were evaluated in the morning (8:00 - 12:00 AM) under an optical microscope to determine the period of the reproductive cycle. In the afternoon (2:00 - 3:00 PM), male rats were allowed to mount the incentives female in a box with a glass front wall. (56 x 35 x 31 cm) with a removable cover. A layer of sawdust served as bedding, and a 40 W lamp with a red filter provided illumination (Bernardi et al., 2012). The latency to the first mount (mount without intromission in seconds) was recorded. Mounting was defined as when the male rat mounted the female from behind and grabbed her flanks with the front paws (Reynolds & Wayman, 2010).

Vocalization (USV)

The USVs were detected using Ultravox software (XT, version 3.1, Noldus Information Technology, Leesburg, USA) and an ultrasonic microphone (Noldus Information Technology, Leesburg, USA) in a test room acoustically isolated. All ultrasounds up to 125 kHz were detected and evaluated. The parameters automatically recorded during 5-minute sessions were the number of vocalizations, mean vocalization duration (ms), maximal vocalization duration (ms), total (ms) vocalization time, and dominant frequency of vocalizations. Supplementary Figure S1 shows an example of 50-kHz ultrasonic vocalizations (USVs) emitted by a rat after its exposure to a receptive female.

Experimental design and groups

Sexually naïve male rats received saline (SAL) solution (n=12) or ivermectin (IVM) solution (n=7), and 24 h later, they were paired with a receptive female for the sexual first mount test and USVs tests. In the afternoon (2:00 - 3:00 PM), the males were transported inside a box protected from light to a test room to record the USVs. Each rat was introduced into the glass arena for 5-min habituation. The receptive females (n=9) were not present during habituation. Then, females were placed with the male to latency to the first mount evaluation. Immediately after the mount, the female was removed from the room, and the vocalization recording began.

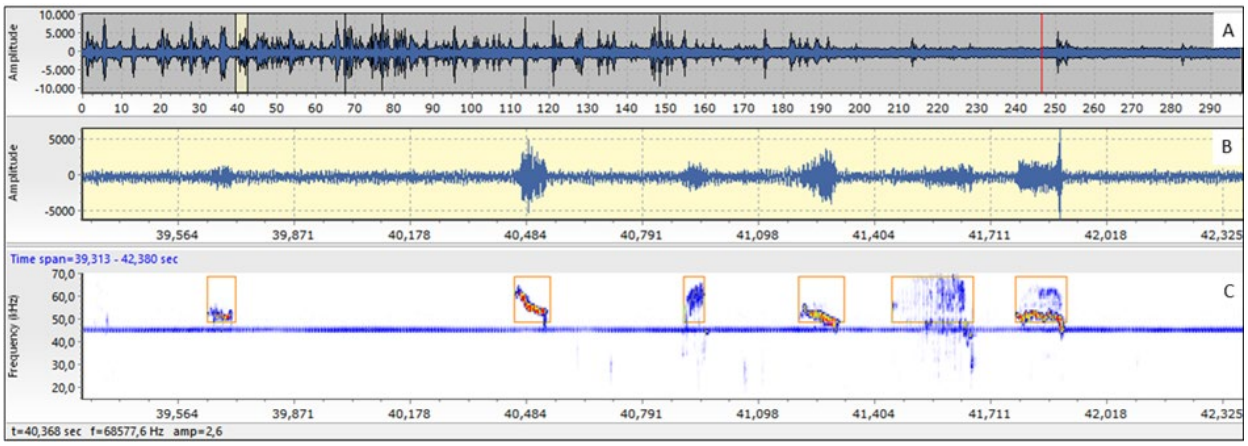

Figure S1. Example of 50-kHz USVs. 50-kHz ultrasonic vocalizations (USVs) emitted by a rat after their exposure to a receptive female. (A) the amplitude-time plot of the entire recording (300 s); (B) the amplitude-time plot of the selected interval (corresponds to the part of A marked in yellow); (C) the spectrogram of the chosen data, showing syllables emitted by a rat.

Another group of male rats was exposed to other receptive females (n=13) and were allowed to mount these females. One week after this procedure, these experienced rats were divided into two groups: receiving SAL solution (n=6) or IVM solution (n =7). Twenty-four hours after the treatments, both groups were again paired with other receptive females (n=7), and the latency to the first mount and USVs were evaluated.

The glass arena was washed with a 5% alcohol/water solution to obviate possible biasing effects from odor clues left by the earlier rats.

Statistical analysis

The Shapiro-Wilk test verified the normality of the data. Adjustments for normality were achieved by excluding outliers by the ROUT Method (considering Q=5%). The estimation sample size was based on the formula n=1+[2C*(s/d)2], C=(zα+zβ)2, considering a confidence interval of 0.95/2 (0.475), z=1.96, and test power of 90% (zβ=1.282), the maximum deviation of 0.2 (20%) and the difference between groups of 0.5 (50%; (Eng, 2003). Before statistical analysis, all data were submitted to the Rout test to identify outliers. After these initial statistics, two-way ANOVA followed by Sidak's multiple comparisons test was applied. Bartlett's test expressed homoscedasticity data and Levene and Welch's correction when needed. The Pearson test was used to establish correlations between latency to the first mount and USVs, and the linear regression formulas expressed for each pair of variables. The results were considered significant if $\alpha < 0.05$.

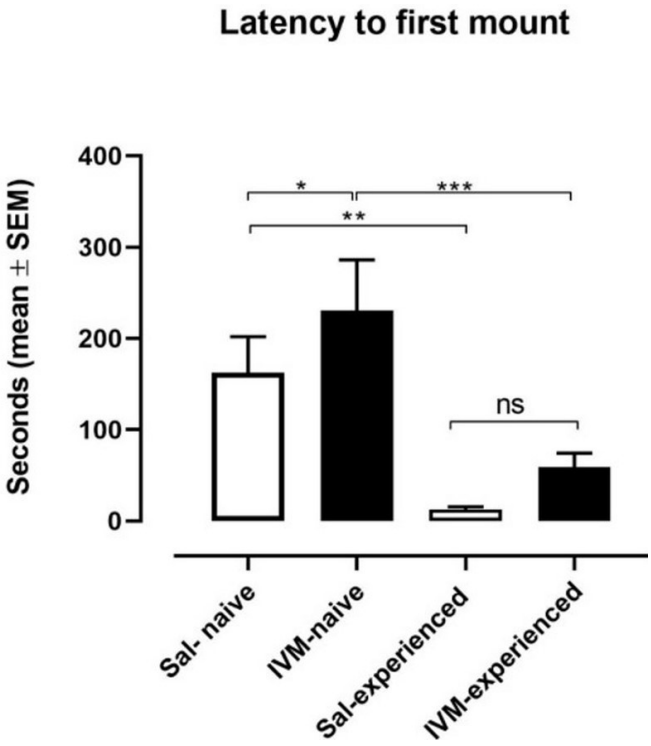

Figure 1 – Latency to the first mount of male rats treated with 1.0 mg/kg of ivermectin (IVM) or its vehicle (saline, SAL) 24 h before the exposure to receptive females. Saline-naïve rats- n=11; IVM-naïve rats- n=7; Saline-experience rats- n=6; IVM-experienced rats- n=7. Two-way ANOVA followed by Sidak's multiple comparisons test. *P< 0.05 relative to IVM-naïve rats; **P< 0.01 relative to saline-experienced rats; *** p< 0.001 relative to IVM-experienced rats.

Results

Latency to first mount evaluation

The Rout test identified one outlier in the saline-naïve rat. Figure 1 shows that treatment with IVM modifies the results (p=0.009) and the sexual experience (p < 0.0001) without interactions between factors(p=0.234). Then, Sidak's multiple comparisons tests showed that relative to saline-naïve rats, the latency to the first mount of IVM-naïve rats was increased (p=0,0463) and decreased relative to saline-experienced (0.002). However, no differences occur between saline-naïve and IVM-experienced rats

(p = 0.07). Sexual-experienced rats showed a reduced latency first to mount compared to IVM-naïve rats (p = 0.0002). No difference was observed between saline-experienced rats and IVM-experienced rats (p=0.86). Data of statistics are shown in Supplementary Table S1.

USVs evaluation

Figure 2 illustrates de USVs parameters of USVs naïve and experienced rats treated with saline or IVM. Data from two-way ANOVA were shown in Supplementary Table S1.

In the frequency of calls (Figure 2a), the IVM-naïve rats showed a reduced frequency of calls relative to saline-naïve rats (p =0.009); no differences occurred in the remaining groups.

The mean time of calls (Figure 2b) and the maximal time of calls (Figure 2c) was increased in IVM-naïve rats relative to saline-naïve, saline-experienced, and experienced-IVM rats.

The total calls (Figure 2d) were decreased in the IVM-naïve group relative to the saline-naïve group. Otherwise, the total calls of the IVM-experienced group were increased compared to IVM-naïve and saline-experienced rats.

The dominant frequency of USVs (Figure 2e) did not differ between saline-naïve and IVM-naïve groups. However, a decrease in this parameter in saline-experienced rats and an increase in the IVM- experienced rats compared to saline-naïve rats were observed.

Figure 3 shows the Pearson correlation test results between the latency to the first mount and the USVs.

A significant and negative correlation was observed between the frequencies (Figure 3a) and total calls (Figure 3b) relative to the latency to first mounts in saline-naïve rats. However, no correlation between the latency to the first mount and these USVs parameters were observed in saline-experienced rats (Figure 3c and Figure 3d). Also,

Table S1. Two-way ANOVA of the latency to first mount and USVs.

	Interaction	Sex Experience	Treatments
Latency to the first mount	F (1, 28) = 1.48, P=0.23	F (1, 28) = 40.69, P = 0.0001	F (1, 28) = 7.79, P = 0.009
Frequency of USVs	F (1,27) =5.33, P = 0.03	F (1,27) = 0.37, p = 0.54	F (1,28) = 5.35, P = 0.03
Mean of calls	F (1,28) = 147.7, P< 0.0001	F (1,28) = 142.80, P< 0.0001	F (1,28) = 126,50, P< 0.0001
Maximal calls	F (1,28) = 28.42, P < 0.0001	F (1,28) = 49.63, P < 0.0001	F (1,28) = 49.95, P< 0.0001
Total calls	F (1,28) = 44.58, P < 0.0001	F (1,28) = 2.45, P 0.14	F (1,28) = 2.37, P 0.14
Dominant frequency	F (1,28) = 4.36, P = 0.04	F (1,28) = 5.41, P =0.03	F (1,28) = 7.64, P = 0.01

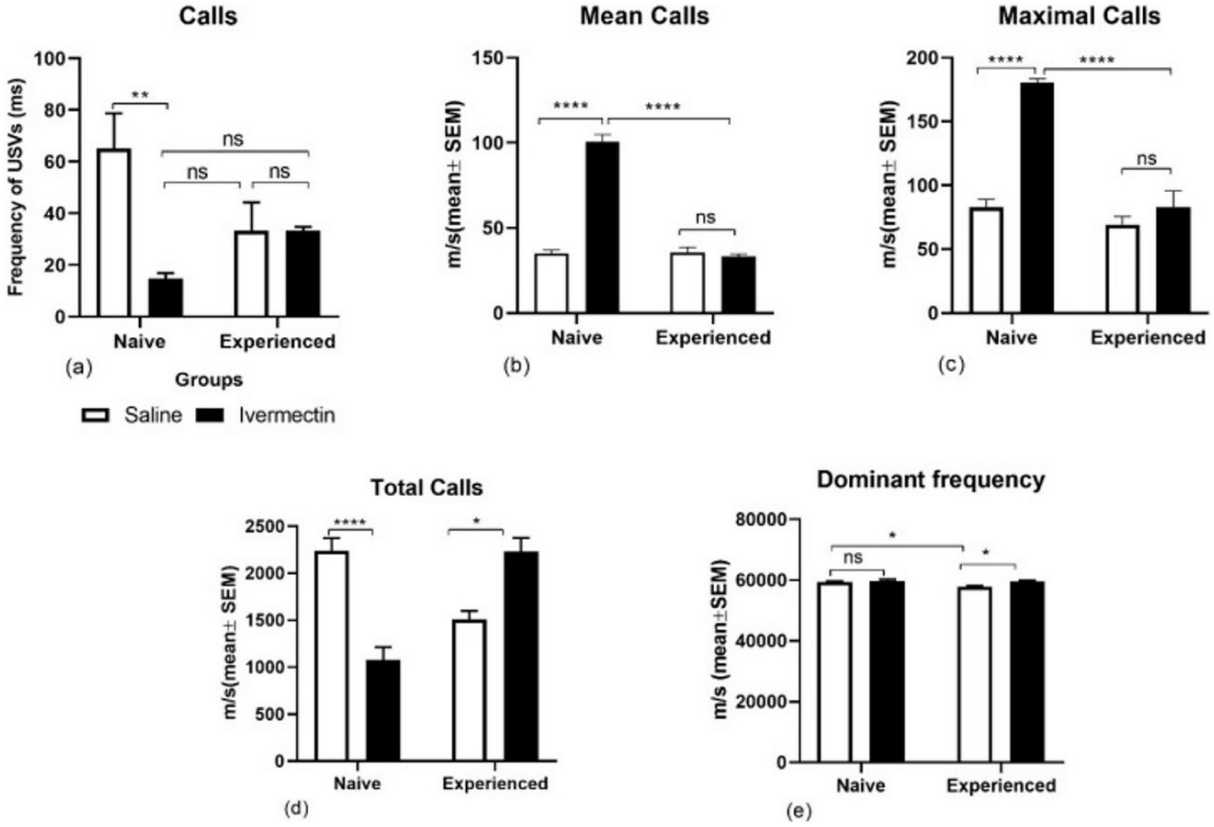

Figure 2 – 50-kHz ultrasonic vocalizations (USVs) emitted by rats after their exposure to receptive females. a- frequency of USVs; b- meantime of USVs, c- Maximal time of USVs, d- total calls; e- dominant frequency. Rats were treated with 1.0 mg/kg of ivermectin (IVM) or its vehicle (saline, SAL) 24 h before female exposure. Male rats were sexually naïve or sexually experienced. Saline-naïve rats- n=11; IVM-naïve rats- n=7; Saline-experience rats- n=6; IVM-experienced rats- n=7. Two-way ANOVA followed by Sidak's multiple comparisons test. *P< 0.05; **P< 0.01; ****P <0.0001.

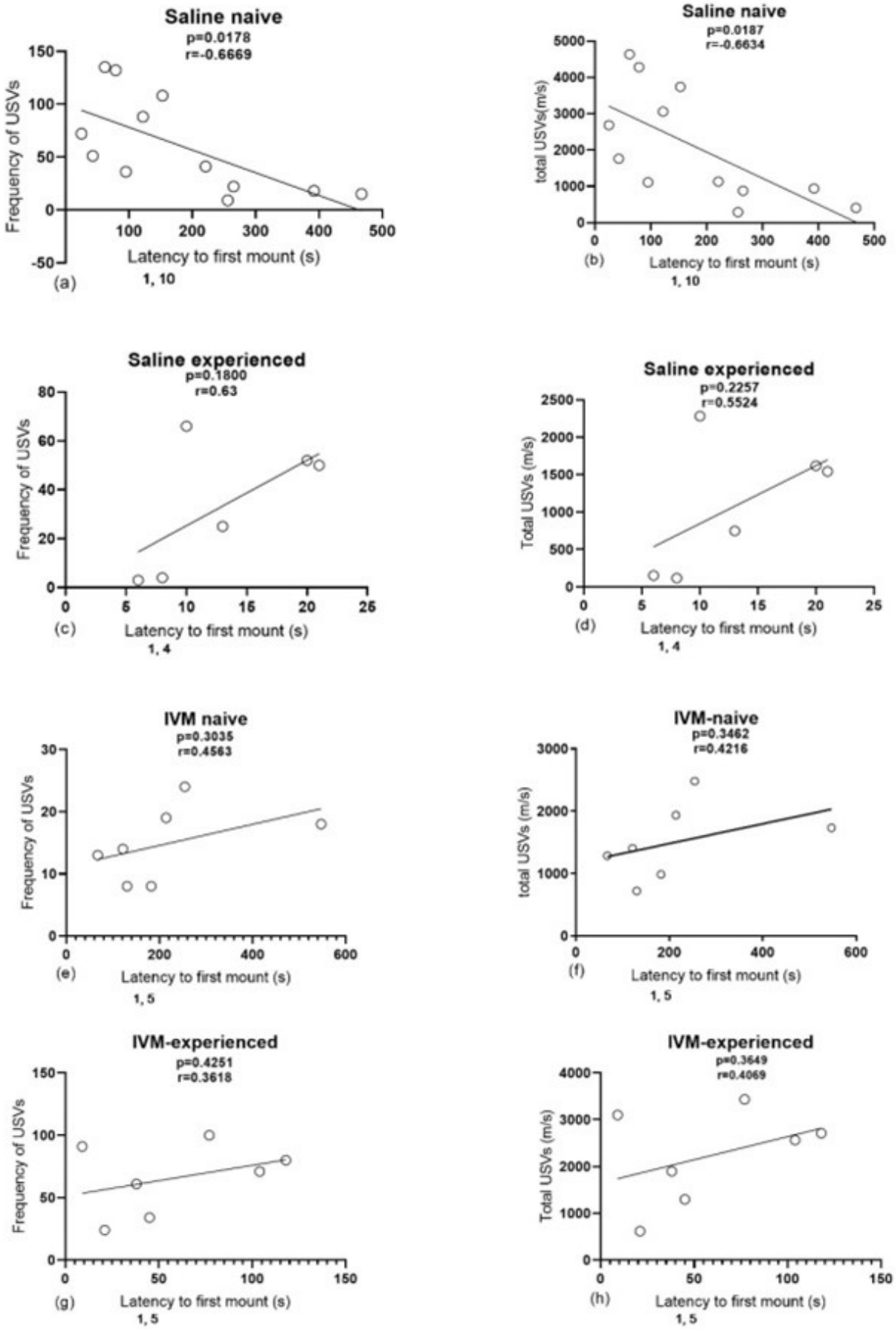

Figure 3 – Correlations between the latency for the first mount and the frequency of ultrasonic vocalizations (USVs) of rats after exposure to receptive females. Rats were treated with 1.0 mg/kg of ivermectin (IVM) or its vehicle (saline, SAL) 24 h before female exposure. Male rats were sexually naïve or sexually experienced. Saline-naïve rats- n=11; IVM-naïve rats- n=7; Saline-experience rats- n=6; IVM-experienced rats- n=7. Pearson correlation test, significance if $\alpha<0.05$.

the IVM- naïve (Figure 3e and Figure 3d) and the IVM-experienced (Figure 3g and Figure 3h) rats did not show a significant correlation between the latency to first mount and both USVs parameters. The remained correlations did not show significant differences (data do not show).

Discussion

In our study, we observed the 50-kHz vocalization in the anticipatory phase of copulation. In IVM- naïve rats, statistical data show increased latencies of first mount relative to saline-naïve rats, suggesting an impairment of the anticipatory state of positive motivation. Despite the decreased latency to first mount in IVM-naïve rats, a reduced frequency and total calls with increased means of calls and maximal calls were observed. Thus, these rats presented increased motivation as measured with USVs.

In this respect, in sexual behavior, the 50-kHz vocalization can be observed during anticipation, at the initiation of copulation, during the copulatory performance, and in the late phase of the postejaculatory interval (Barfield et al., 1979; Bialy et al., 2019). Thus, these rats presented increased motivation as measured with USVs and reduced if we consider the latency to the first mount.

Several studies propose GABA agonists' role in impairing sexual behavior. Agmo & Paredes (1985) showed that baclofen, a GABAB agonist reduced sexual behavior independent from those on locomotor activity. In addition, it was suggested that GABA transaminase inhibitors affect sexual behavior only indirectly via impairment of motor execution (Agmo et al., 1987). Also, moxidectin, a macrocyclic lactone, reduced sexual behavior and penile erection by an action in the hypothalamic GABA system without interference with sexual motivation. The authors concluded that the increased mount and intromission latencies and decreased total mounts could explain as a consequence of the reduced male rat erection process (Rodrigues-Alves et al., 2008). Also, doramectin reduced male sexual behavior and penile erection in male rats (Ferri et al., 2013). Finally, Moreira et al. (2017) attributed the reduced sexual behavior after IVM exposure to motor incoordination and not to decreased sexual motivation.

The present results showing increased USVs in IVM-naïve rats suggest an increased motivation in these rats because the means of calls and the maximal calls were higher than in the saline-naïve rats. The reduced frequencies and the total calls could result from the increased means and maximal calls.

Thus, the motor impairment could be responsible for the increased USVs of these rats leading to the rats treated with IVM emitting longer calls to perform mounts. Moreover, a negative correlation occurred between the latency of the first mount, the frequency of calls, and the total calls.

Sexual experience of the saline rats reduced the meantime and the maximal time of USVs but slightly diminished the frequency of USVs ($p = 0.52$) relative to the saline-naïve group. A positive, no significant correlation between the latency to the first mount and the USVs parameters was detected in this group.

In addition, sexual experience in IVM rats showed a similar level of latency to mount and in the profile to USVs like saline-naïve rats when examining the frequency, means, and maximal calls. However, the total calls and dominant frequency were higher in experienced IVM rats than in experienced saline rats. Thus, the sexual experience revealed an increased motivation as measured by the USV after IVM exposure.

A more detailed analysis of the correlation data between USVs and the latency to the first mount could prove the ivermectin effects on sexual motivation. It was observed that the angular coefficient for the Sal-experienced group was higher than the ones observed for the IVM-naive and IVM-experienced groups, which means that the animals of the saline-experienced group promptly responded to the female and expressed the sex call through the vocalization. However, despite both ivermectin groups showing a low correlation between USVs and the latency to the first mount, the correlation after sexual experience increased. Thus, sexual experience improves sexual motivation.

Conclusions

We propose that ivermectin increased the sexual motivation of rats exposed to a female in estrous based in USVs despite an increased latency to the first mount observed in naïve rats. As previously observed and proposed by our group, the increased latency to the first mount resulted from motor incoordination.

Conflict of Interest

There is no conflict of interest.

Ethics Statement

The procedures were approved by CEUA-UNIP (permit No. 339/15) and followed the Committee on Care and Use of Laboratory Animal Resources (National Research Council) standards.

Acknowledgements

This study is part of the master dissertation of Paula da Silva Rodrigues, Environmental and Experimental Pathology. It was supported by grants from the National Council for Scientific and Technological Development (CNPq) and the Coordination of Improvement of Higher Education Personnel-Brazil (CAPES).

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Financial Support: This study was supported by grants from the National Council for Scientific and Technological Development (CNPq-Grant no. 303701/2014-5 and 441580/2014-9) and the Coordination of Improvement of Higher Education Personnel-Brazil (CAPES/PRO SUP, Grant 1678564- Code 001).