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Monitoring Differences in Vaginal Hemodynamic and Temperature Response for Sexual Arousal by Different Anesthetic Agents Using an Optical Probe

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The selection of anesthetic agent is important in preclinical studies, since each agent affects the systemic hemodynamics in different ways. For that reason, we hypothesized that different anesthetic agents will result in different vaginal hemodynamic response and temperature during sexual arousal, in an animal model. To validate the hypothesis, animal experiments were performed using female rats with two anesthetic agents widely used in preclinical studies: ketamine and isoflurane. Our previously developed near-infrared-spectroscopy-based probe was used to measure the changes of oxyhemoglobin (OHb), deoxyhemoglobin (RHb), and total hemoglobin (THb) concentrations along with temperature from the animal vaginal wall. As a control, saline was administered to both isoflurane- and ketamine-anesthetized animals, and did not show any significant changes in OHb, RHb, THb, or temperature. However, an administration of apomorphine (APO, 80 μg/kg) induced increases of OHb (63 ± 28 μM/DPF), RHb (35 ± 20 μM/DPF), and THb (98 ± 49 μM/DPF) in ketamine-anesthetized animals, while decreases of OHb (52 ± 76 μM/DPF) and THb (38 ± 30 μM/DPF) and an increase of RHb (28 ± 51 μM/DPF) were found in isoflurane-anesthetized animals. The vaginal temperature decreased from the baseline in both ketamine-(0.42°C) and isoflurane-(1.22°C)anesthetized animals. These results confirmed our hypothesis, and suggest that a preclinical study monitoring hemodynamic responses under anesthesia should employ an appropriate anesthetic agent for the study.

Keywords : Anesthetic agents, Sexual arousal, Vaginal temperature, Hemodynamics, Near-infrared spectroscopy (NIRS)
OCIS codes : (170.1610) Clinical application; (170.4580) Optical diagnostics for medicine; (170.6510) Spectroscopy, tissue diagnostics; (170.7230) Urology

I. INTRODUCTION

It is widely known that anesthetics may disturb physiological functions, for example by suppressing blood pressure and breathing [1]. However, anesthesia is commonly used in preclinical animal experiments because it offers several benefits, such as relief of pain or discomfort in the animals, ease of animal handling, and acquisition of stable signals from the animals. To minimize the unwanted effects of anesthetics on preclinical experiments, a great deal of consideration is required when it comes to choosing anesthetic agents and designing anesthetic protocols, since they may alter the outcome of experiments.

There has been a number of studies that aimed to monitor the altered responses caused by different anesthetic agents [2, 3]. One report showed that ketamine/xylazine/acepromazine cocktail and isoflurane resulted in different hearing sensitivity in the animal cochlea, which may be

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due to alternation of cochlear function [2]. Another study compared the differences in electrophysiological responses of the visual cortex during ketamine and isoflurane anesthesia [3]. The first study concluded that isoflurane suppresses hearing ability, while ketamine-based anesthetic agent did not; the second showed that isoflurane increased electrophysiology in the frequency range of 10~40 Hz, suppressing the response to visual stimulation, while ketamine increased electrophysiology in the frequency range of 2~4 Hz, enhancing the response to visual stimulation. Therefore, it is necessary to select a suitable anesthetic agent so that unwanted effects do not occur in animal experiments. In another aspect, the use of anesthetic agent minimally affecting the normal physiology will increase the translatability of preclinical studies into clinical studies.

While various kinds of anesthetic agent are available for animal studies, ketamine and isoflurane were tested here, since they are the two main, popular anesthetic agents in animal studies. Ketamine is a general, injectable anesthetic agent, while isoflurane is volatile. Both anesthetic agents have been used to assess female sexual response in preclinical animal studies [4, 5]. In spite of the importance of selecting the most suitable anesthetic agent, however, to the best of our knowledge no research has been conducted to compare the effects of different anesthetics in preclinical sexual arousal study.

To monitor sexual arousal in animal models, laser Doppler flowmetry (LDF) has been used to monitor the change in blood flow in the vaginal wall. However, LDF primarily targets arteries and does not reflect hemodynamic changes in microvessels, where supplying oxygen to the tissue and exchanging nutrients take place [6]. Continuous-wave near-infrared spectroscopy (CW-NIRS) is a technique that can monitor the relative changes in oxy- (OHb), deoxy- (RHb), and total hemoglobin (THb) concentration using NIR light in the range of 650~900 nm. Due to the capability of miniaturization, a NIRS probe is suitable for monitoring hemodynamic changes in the vagina [7]. Moreover, NIRS is sensitive to both arteries and microvessels, which makes it a more suitable tool to monitor vaginal hemodynamics in preclinical studies of female sexual arousal. NIRS can also collect hemodynamic information from both the surface and subsurface of the vaginal wall, while LDF collects information primarily from the surface.

In this study we hypothesized that different anesthetic agents will result in different vaginal hemodynamic response and temperature during sexual arousal in an animal model. To prove this hypothesis, vaginal hemodynamics and temperature of the animals were monitored under either ketamine or isoflurane anesthesia during apomorphine (APO) administration, using an optical probe based on NIRS. Furthermore, we discussed which anesthetic agent is more suitable for studies of female sexual arousal using APO as a sexual stimulation, based on our results and the working mechanisms of APO, isoflurane, and ketamine.

II. MATERIALS AND METHODS

Apomorphine (APO, A4393, Sigma-Aldrich, USA) was administered to each animal through the subcutaneous route at a dose of 80 μg/kg, to induce sexual arousal (APO group). For the same animal, the same volume of saline as the one of APO was administered for comparison (control group). Animals could freely access food and water and were kept under a 12/12 h light/dark cycle. Measurements were performed on female Sprague-Dawley rats (180~220 g, $n = 6$) under either ketamine or isoflurane anesthesia. For ketamine anesthesia, each animal was placed in a chamber with 5% isoflurane for anesthesia induction for 3 minutes, and ketamine (50 mg/kg) was administered through the intraperitoneal route right after induction, to maintain the anesthesia. For isoflurane anesthesia, each animal underwent the same induction procedure for 3 min and 1.5~2% isoflurane was supplied later on, to maintain anesthesia during the measurement. To maintain arterial oxygen saturation above 98%, 50% of oxygen gas balanced by nitrogen was supplied during the measurement. The experiment was performed for 50 min, which includes 5 min of baseline measurement and 45 min post administration of either APO (80 μg/kg) or the same volume of saline. This work was conducted following the protocols approved the Institutional Animal Care and Use Committee of the Gwangju Institute of Science and Technology.

We used a previously developed NIRS based probe to monitor vaginal hemodynamics and temperature changes. The details of the probe can be found in our previous report [7]. Briefly, a cylindrical tube with three holes was utilized as a probe sheath. The probe size was determined considering diameters of 3 mm and 5 mm, depending on the body weight and cylindrical shape of the *ex vivo* rat vagina in our previous study [7]. Two optical fibers (105 μm core size, FG105UCA-CUSTOM, Thorlabs, USA) were placed on the side of the probe, to transmit broadband light from a tungsten-halogen light source (HL-2000-HP, Ocean Optics, USA) and to collect diffuse reflected light from vaginal wall using a commercial NIR spectrometer (USB4000, Ocean Optics, USA) (Fig. 1(b) left). The source-detector separation was 1.5 mm. A microthermistor (10 kΩ, TH10K, Thorlabs, USA) was placed on the opposite side from the two optical fibers, to monitor the temperature of vaginal wall (Fig. 1(b) right). For monitoring of vaginal temperature, the microthermistor signals were transferred to a data acquisition system (USB-6259 BNC, National Instruments, USA), and both NIRS and temperature signals were saved in a computer for data analysis.

The changes in oxyhemoglobin (OHb), deoxyhemoglobin (RHb), and total hemoglobin (THb) concentration induced by APO or saline administration were obtained using the modified Beer-Lambert Law (MBLL). Eq. (1) shows the MBLL for 5 wavelengths (730, 750, 800, 830, and 850 nm), assuming that OHb and RHb are the main chromophores in the wavelength range of 650~900 nm [7]:

$$\begin{bmatrix} \triangle OHb \\ \triangle RHb \end{bmatrix} = \frac{1}{dx\,DPF} \begin{bmatrix} \epsilon_{OHb}^{730} & \epsilon_{RHb}^{730} \\ \epsilon_{OHb}^{750} & \epsilon_{OHb}^{800} \\ \epsilon_{OHb}^{830} & \epsilon_{OHb}^{850} \\ \epsilon_{RHb}^{750} & \epsilon_{RHb}^{800} \\ \epsilon_{RHb}^{830} & \epsilon_{RHb}^{850} \end{bmatrix}^{-1} \begin{bmatrix} \triangle OD^{730} \\ \triangle OD^{750} \\ \triangle OD^{800} \\ \triangle OD^{830} \\ \triangle OD^{850} \end{bmatrix}, \quad (1)$$

where ΔOD is the change in optical density ($OD_{Transient}$ - $OD_{Baseline}$), ε is the extinction coefficient for OHb or RHb at the given wavelengths, d is source-detector separation, and DPF is a differential path length factor that is wavelength-dependent. The change of total hemoglobin concentration (ΔTHb) can be calculated by the sum of ΔOHb and ΔRHb. Since we do not measure DPF in CW-NIRS, DPF was included within the unit of hemoglobin concentration as mM/DPF.

III. RESULTS

Figures 2(a) and 2(b) show the averaged changes of OHb, RHb, and THb concentrations and temperature from

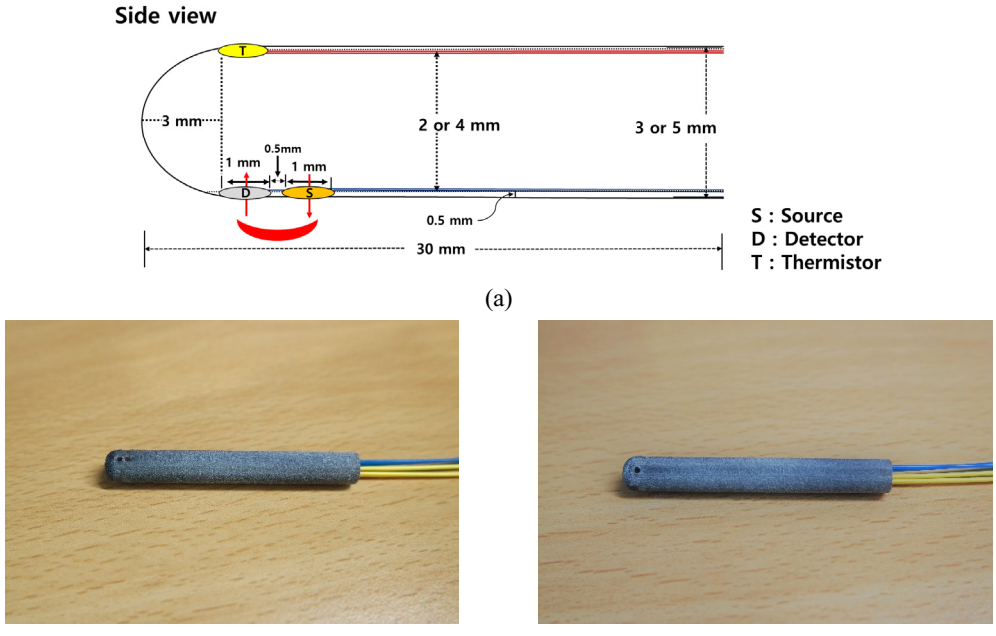

FIG. 1. (a) Schematic diagram of the probe, and (b) photograph of the probe used in the study: NIRS (left), temperature (right).

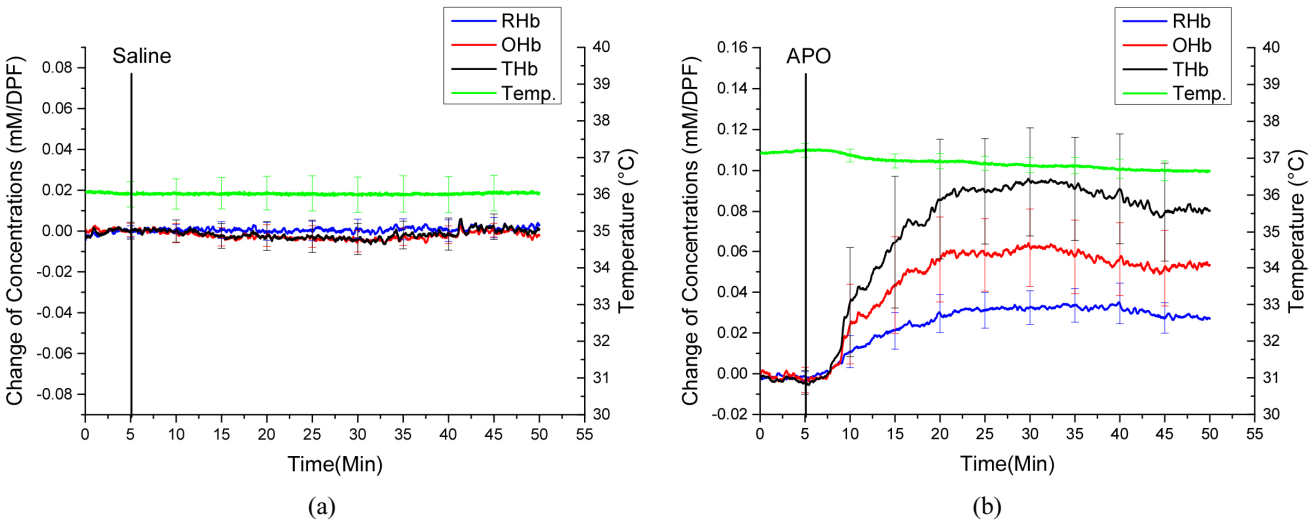

FIG. 2. The averaged hemoglobin parameters and temperature changes from all rats with ketamine anesthesia, in (a) the control group and (b) the APO group. RHb: deoxyhemoglobin, OHb: oxyhemoglobin, THb: total hemoglobin, mM: millimolar, DPF: differential path length factor.

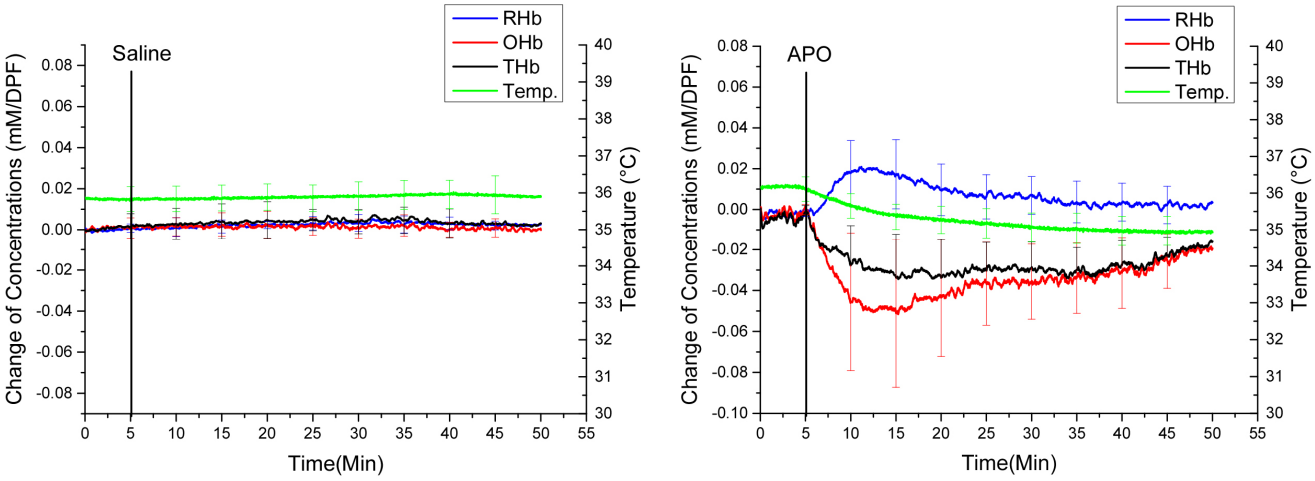

FIG. 3. The averaged hemoglobin parameters and temperature changes from all rats with isoflurane anesthesia, in (a) the control group and (b) the APO group. RHb: deoxyhemoglobin, OHb: oxyhemoglobin, THb: total hemoglobin, mM: millimolar, DPF: differential path length factor.

the vaginal wall in the control and APO groups respectively, during ketamine anesthesia. All data from each rat induced by APO were compared to that for the control group. There were no significant changes in OHb, RHb, THb, or temperature in the control group, while all animals in the APO group showed increases in OHb (63 ± 28 μM/DPF), RHb (35 ± 20 μM/DPF), and THb (98 ± 49 μM/DPF). Also, the vaginal temperature dropped gradually from 37.19°C to 36.77°C, post APO administration. These results were presented in our previous report [7].

Figures 3(a) and 3(b) show the averaged changes in OHb, RHb, and THb concentrations and temperature from the vaginal wall in the control and APO groups respectively, during isoflurane anesthesia. There were no significant changes in OHb, RHb, THb, or temperature in the control group with isoflurane anesthesia, as in the control group with ketamine anesthesia. However, different responses of hemodynamics and vaginal temperature were observed in the isoflurane-anesthetized APO group. Specifically, OHb significantly decreased by 52 ± 76 μM/DPF and gradually returned to the baseline level in the isoflurane-anesthetized APO group, while OHb increased and reached to a plateau in the ketamine-anesthetized APO group. APO administration also caused an increase of RHb by 28 ± 51 μM/DPF and a decrease of THb by 38 ± 30 μM/DPF from the baseline in the isoflurane-anesthetized animals. The vaginal temperature had dropped from 36.14°C to 34.92°C by the end of the experiment in the isoflurane-anesthetized APO group.

IV. DISCUSSIONS AND CONCLUSION

In this study we monitored vaginal hemodynamics and temperature changes caused by APO administration as a means for sexual stimulation in an animal model, under either ketamine or isoflurane anesthesia. To elicit sexual arousal, two main methods are available: nerve stimulation and medication. Nerve stimulation targets the pelvic nerve, sacral nerve, and tibial nerve to induce sexual arousal, but it requires a surgical procedure, which can be a drawback when performed in a rodent model [8]. For the medication, although flibanserin (ADDYI, Sprout Pharmaceuticals) was approved by the FDA for the treatment of pre-menopausal women with hypoactive sexual desire disorder, a great deal of consideration is needed, because it can cause adverse effects such as nausea, dizziness, and drowsiness [9]. Meanwhile, APO, a dopamine agonist that acts in a short time, has shown little adverse effect [10] and has been used in both preclinical and clinical studies to induce sexual arousal [11]. For instance, APO with pelvic nerve stimulation could enhance clitorial and vaginal blood flow in a rabbit model [12], and APO was also used to improve libido in women suffering from sexual desire disorder [13].

It has been reported that vasocongestion (an increase of blood flow and a localized increased blood volume) occurs in the genitalia as a sexual response [14-17]. The results in this study also showed increases of both blood volume (represented by THb increase) and blood flow (OHb increase along with THb increase) during APO administration in the ketamine-anesthetized animals, which agrees with vasocongestion in the genitalia (Fig. 2(b)). However, opposite trends of both OHb and THb were observed in the isoflurane-anesthetized APO group (Fig. 3(b)). This can be explained by interaction between APO-induced vasodilation and blood-pressure changes, depending on anesthetic agent. The side effects of isoflurane include respiratory depression and low blood pressure, which are not found with ketamine [18]. Therefore, when vasodilation

occurs by APO administration, a ketamine-anesthetized animal is capable of supplying blood, so that vasocongestion happens in the vaginal wall, shown as increases in OHb and THb. However, APO-induced vasodilation cannot cause vasocongestion in the vaginal wall, due to low blood pressure from the effects of isoflurane anesthesia. As a result, OHb and THb decreased after APO administration in isoflurane-anesthetized animals. These results emphasize the importance of choosing the right anesthetic agent for a given physiological experiment, including this study. Compared to APO-administered groups, saline administration in both isoflurane- and ketamine-anesthetized animals did not show any significant changes in either vaginal hemodynamics or temperature (Figs. 2(a) and 3(a)), which proves that the anesthesia level was well controlled; also, the animals were kept warm using a warm-water pad.

Other than blood volume and flow, the ketamine-anesthetized and isoflurane-anesthetized APO groups also showed a large difference in the change of vaginal temperature from the baseline: a 0.42°C drop in the ketamine group versus a 1.22°C drop in the isoflurane group. It has been shown that sexual arousal causes an increase of genital temperature [19], but the results in this study showed a *decrease* of temperature in the vaginal wall, for both ketamine- and isoflurane-anesthetized groups. This can be due to the vasodilation effect by APO, which is dose-dependent [20]. When vasodilation occurs, more blood goes to the peripheral area, heat is redistributed, and in turn the core temperature drops [21]. Likewise, isoflurane also leads to peripheral vasodilation and blood-pressure reduction [22, 23], while ketamine does not act as a vasodilator [24]. Thus, the greater temperature drop in the isoflurane-anesthetized APO group compared to the ketamine-anesthetized APO group could be explained by the compounding vasodilation effects of APO and isoflurane, which may complicate interpretation of the effect of APO. Therefore, in future study it is required to employ another method, such as nerve stimulation, to induce sexual arousal without a decrease in vaginal temperature, to better represent the sexual arousal in an animal study.

One limitation of this study is the lack of an experiment with unanesthetized animals. As the results of this study show, it is very important to minimize the number of factors that can affect the hemodynamics of body. In this case, this includes the anesthesia, since the anesthesia itself causes a change in body hemodynamics. Therefore, employment of an awake-animal model would be best for monitoring vaginal hemodynamics during APO administration; it may be possible, using a rodent restrainer with habituation. However, the adaptation of a restrainer can hardly satisfy the purpose of our study, since the measurement has to be from the vaginal wall, which will be very susceptible to any motion during the measurement. There are many reports using an awake-animal model for brain study, because the head can be held well by using a fixture on it [25-27]. However, it seems infeasible to hold animals for the measurement of vaginal hemodynamics using the probe developed in our previous study.

In summary, we compared vaginal hemodynamics and temperature changes in an APO-administered animal model under either isoflurane or ketamine anesthesia, and found that the vaginal hemodynamics showed a completely different response to APO administration depending on which anesthetic agent was used. These results suggest that the design of a preclinical study to monitor hemodynamic responses under anesthesia should be careful to choose an appropriate anesthetic agent.

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REFERENCES

1. L. J. Teppema and S. Baby, "Anesthetics and control of breathing," Respir. Physiol. Neurobiol. **177**, 80-92 (2011).
2. J. M. Cederholm, K. E. Froud, A. C. Y. Wong, M. Ko, A. F. Ryan, and G. D. Housley, "Differential actions of isoflurane and ketamine-based anaesthetics on cochlear function in the mouse," Hear. Res. **292**, 71-79 (2012).
3. N. J. Michelson and T. D. Y. Kozai, "Isoflurane and ketamine differentially influence spontaneous and evoked laminar electrophysiology in mouse V1," J. Neurophysiol. **120**, 2232-2245 (2018).
4. F. A. Guarraci, C. M. F. Gonzalez, D. Lucero, P. D. Womble, H. Abdel-Rahim, J. DeVore, M. N. Kunkel, E. Quadlander, M. Stinnett, and J. Boyette-Davis, "The effects of ketamine on sexual behavior, anxiety, and locomotion in female rats," Pharmacol. Biochem. Behav. **165**, 36-44 (2018).
5. F. Giuliano, J. Allard, S. Compagnie, L. Alexandre, S. Droupy, and J. Bernabe, "Vaginal physiological changes in a model of sexual arousal in anesthetized rats," Am. J. Physiol. Regul. Integr. Comp. Physiol. **281**, R140-R149 (2001).
6. M. Intaglietta, P. C. Johnson, and R. M. Winslow, "Microvascular and tissue oxygen distribution," Cardiovasc. Res. **32**, 632-643 (1996).
7. H. Jeong, M. Seong, H.-S. Lee, K. Park, S. Moon, and J. G. Kim, "Design of an optical probe to monitor vaginal hemodynamics during sexual arousal," Sensors **19**, 2129 (2019).
8. T. F. Al-Shaiji, M. Banakhar, and M. M. Hassouna, "Pelvic electrical neuromodulation for the treatment of overactive bladder symptoms," Adv. Urol. **2011**, 757454 (2011).
9. R. Baid and R. Agarwal, "Flibanserin: A controversial drug for female hypoactive sexual desire disorder," Ind. Psychiatry J. **27**, 154-157 (2018).
10. J. E. Altwein and F. U. Keuler, "Oral treatment of erectile dysfunction with apomorphine SL," Urol. Int. **67**, 257-263 (2001).

11. K. J. Allahdadi, R. C. A. Tostes, and R. C. Webb, "Female sexual dysfunction: therapeutic options and experimental challenges," Cardiovasc. Hematol. Agents Med. Chem. **7**, 260-269 (2009).
12. T. Tarcan, M. B. Siroky, K. Park, I. Goldstein, and K. M. Azadzoi, "Systemic administration of apomorphine improves the hemodynamic mechanism of clitoral and vaginal engorgement in the rabbit," Int. J. Impot. Res. **12**, 235-240 (2000).
13. S. Caruso, C. Agnello, G. Intelisano, M. Farina, L. D. Mari, and A. Cianci, "Placebo-controlled study on efficacy and safety of daily apomorphine SL intake in premenopausal women affected by hypoactive sexual desire disorder and sexual arousal disorder," Urology **63**, 955-959 (2004).
14. N. Prause, J. Barela, V. Roberts, and C. Graham, "Instructions to rate genital vasocongestion increases genital and self-reported sexual arousal but not coherence between genital and self-reported sexual arousal," J. Sex. Med. **10**, 2219-2231 (2013).
15. C. Henson, H. B. Rubin, and D. E. Henson, "Women's sexual arousal concurrently assessed by three genital measures," Arch. Sex. Behav. **8**, 459-469 (1979).
16. P. Lavoisier, R. Aloui, M. H. Schmidt, and A. Watrelot, "Clitoral blood flow increases following vaginal pressure stimulation," Arch. Sex. Behav. **24**, 37-45 (1995).
17. R. J. Levin, "The physiology of sexual arousal in the human female: a recreational and procreational synthesis," Arch. Sex. Behav. **3**, 405-411 (2002).
18. C.-F. Yang, M. Y.-C. Chen, T.-I. Chen, and C.-F. Cheng, "Dose-dependent effects of isoflurane on cardiovascular function in rats," Tzu-Chi Med. J. **26**, 119-122 (2014).
19. T. M. Kukkonen, Y. M. Binik, R. Amsel, and S. Carrier, "Physiology: Thermography as a physiological measure of sexual arousal in both men and women," J. Sex. Med. **4**, 93-105 (2007).
20. R. Matika, M. Ibrahim, and A. Patwardhan, "The importance of body temperature: an anesthesiologist's perspective," Temperature **4**, 9-12 (2017).
21. R. E. Chipkin, "Effects of D1 and D2 antagonists on basal and apomorphine decreased body temperature in mice and rats," Pharmacol. Biochem. Behav. **30**, 683-686 (1988).
22. A. Kurz, "Effects of anaesthesia on thermoregulation," Curr. Anaesth. Crit. Care. **12**, 72-78 (2001).
23. E. I. Eger II, "The pharmacology of isoflurane," Br. J. Anaesth. **56**, 71S-99S (1984).
24. M. Johnstone, "The cardiovascular effects of ketamine in man," Anaesthesia **31**, 873-882 (1976).
25. L. Tian, S. A. Hires, T. Mao, D. Huber, M. E. Chiappe, S. H. Chalasani, L. Petreanu, J. Akerboom, S. A. McKinney, E. R. Schreiter, C. I. Bargmann, V. Jayaraman, K. Svoboda, and L. L. Looger, "Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators," Nat. Methods. **6**, 875-881 (2009).
26. W. Peng, X. Xia, M. Yi, G. Huang, Z. Zhang, G. Iannetti, and L. Hu, "Brain oscillations reflecting pain-related behavior in freely moving rats," Pain **159**, 106-118 (2018).
27. J. S. Taube, R. U. Muller, and J. B. Ranck Jr., "Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis," J. Neurosci. **10**, 420-435 (1990).