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Effects of PDE5 Inhibitors Sildenafil, Vardenafil and Tadalafil on Radial Maze Acquisition in
Male and Female Rats

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Abstract

Sildenafil (Viagra™), Vardenafil (Levitra™) and Tadalafil (Cialis™) increases the release of nitric oxide by inhibiting the phosphodiesterase type five (PDE5) receptors. Nitric Oxide (NO) has been shown to regulate the release of various neurotransmitters, which play an important role in learning and memory in humans and animals (Noda, Yamada, Nabeshima, 1997). The aim of our study is to evaluate the effects of Sildenafil, Vardenafil and Tadalafil on learning and memory, using an eight-arm radial maze acquisition test. The main hypothesis is that the inhibition of the PDE5 receptors by Sildenafil, Vardenafil and Tadalafil will increase the nitric oxide levels, which will improve the performance of the rat in the eight-arm radial maze spatial memory task. **Results and Conclusions:** The results for our study revealed that Sildenafil (Viagra™), Vardenafil (Levitra™) and Tadalafil (Cialis™) did not enhance spatial learning and memory in the eight-arm radial maze acquisition test in male or female rats when compared to saline control groups.

Effects of PDE5 Inhibitors Sildenafil, Vardenafil and Tadalafil on Radial Maze Acquisition in Male and Female Rats

Sildenafil (Viagra™), Vardenafil (Levitra™) and Tadalafil (Cialis™) are commonly used to treat male erectile dysfunction. The 1993 National Institute of Health Consensus Statement defines male erectile dysfunction as the inability to achieve or sustain a penile erection adequate for sexual satisfaction (Moreland, 2001). A normal erection is achieved by the following mechanisms: upon stimulation of the penis, nitric oxide is released into the corpus cavernosum, which activates the guanylate cyclase enzyme. The guanylate cyclase enzyme increases the cyclic guanosine monophosphate (cGMP) levels causing smooth muscle relaxation in the corpus cavernosum, allowing the inflow of blood, which causes an erection. However, Male Erectile Dysfunction (MED) is caused when a receptor known as phosphodiesterase type five (PDE5) inhibits the nitric oxide released into the corpus cavernosum, and breaks down the cGMP level that is responsible for smooth muscle relaxation.

Sildenafil, Vardenafil and Tadalafil inhibit the PDE5 receptor, which allows the release of nitric oxide into the corpus cavernosum. The nitric oxide increases the cGMP levels, and allows smooth muscle relaxation, which leads to an erection. However, these drugs will not produce an erection in the absence of sexual stimulation. Nevertheless, nitric oxide will still be produced in the absence of sexual stimulation.

There are eleven known phosphodiesterase receptors (Corbin & Francis, 2003). Caffeine was the first drug known, which inhibited most of the PDE receptors. However, Sildenafil, Vardenafil and Tadalafil were developed as more selective and potent inhibitors of PDE receptors, targeting the PDE5 receptor. Sildenafil and Vardenafil also cross-react with the PDE6 receptor, which affects vision (a reported side effect of Sildenafil), and Tadalafil affects the PDE

11 receptor (Corbin & Francis, 2003). The PDE5 inhibitors (Sildenafil, Vardenafil and Tadalafil) play a role in learning and memory by allowing the production of nitric oxide.

Nitric Oxide (NO) has been shown to regulate the release of various neurotransmitters, which play an important role in learning and memory in humans and animals (Noda, Yamada, Nabeshima, 1997). Nitric Oxide has been implicated in hippocampal long-term potentiation, a mechanism that underlies certain forms of learning and memory. Nitric Oxide also plays a role in the acquisition, of spatial learning in rats (Noda, Yamada, Nabeshima, 1997).

The aim of our study is to evaluate the effects of Sildenafil, Vardenafil and Tadalafil on learning and memory, using an eight-arm radial maze acquisition test. The main hypothesis is that the inhibition of the PDE5 receptors by Sildenafil, Vardenafil and Tadalafil will increase the nitric oxide levels, which will improve the performance of the rat in the eight-arm radial maze spatial memory task.

Methods

Subjects

Sixteen male Long-Evans and sixteen female Long Evans rats were used in this study. The rats were housed two to a cage in a sound attenuated room inside the laboratory. The rats were kept on a light-dark cycle (12 hours on and 12 hours off), and were fed Lab Diet (PMI Nutrition International, MO), and water *ad Libitum*. During Habituation Training (HT) procedures, the rats were weighed daily and reduced to 85% of their normal body weight.

Apparatus

The radial maze is framed with clear Plexiglas and consists of eight arms (70 cm long and 10 cm wide) radiating from an octagonal platform (62 cm in diameter). At the end of each arm is a sunken food cup, which contained a 94 mg chocolate flavored food pellet, the food reward

during the experimental procedures. Removable doors made of clear Plexiglas were used to randomly block the entrance of arms in the pre-delay sessions of the delayed non-match-to-sample (DNMTS) procedures. The maze is elevated 110 cm above the floor, and is located in an adjoin room inside the laboratory, equipped with overhead florescent lightening. Distinctive laboratory cues are placed throughout the room.

Procedure

The eight-arm radial maze acquisition test will follow the delayed non-matching-to-sample (DNMTS) procedure as outlined in Parasram et al. (2005). The DNMTS procedure consists of two trails, the pre-delay session and the post-delay session. For the pre-delay session, four of the eight arms in the radial maze were randomly blocked using removable doors, and the remaining four were baited with chocolate flavored food pellets. The rats were placed in the center of the maze to explore the four open arms. After visiting all open arms, the rats were returned to its cage for a 5-min delay. Subsequently, the rats were returned to the maze for the post-delay session, where all eight arms of the maze were opened, with only the arms blocked in the pre-delay session baited with food pellets. Task acquisition performance involved the rats associating information from the pre-delay session to the post-delay session in reference to the laboratory cues.

Habituation Training Procedures

Rats were handled for five days before the Habituation Training (HT) procedures. During the HT procedures, the rats were allowed to explore the maze for 5 minutes, for five consecutive days. Using operant shaping techniques, chocolate flavored food pellets were strategically placed in the maze, from the center of the platform, near the entrance of the arm, leading into the arm, to the food cups. The pellets were placed closer to the food cups as daily

sessions of HT proceed. During HT, the rats were randomly assigned to one of three experimental groups: Sildenafil, Vardenafil or Tadalafil, or the control group. After Habituation Training, the rats were tested using the DNMTS procedures for twenty consecutive days.

Drug Treatment

The rats were randomly assigned to one of eight groups. The experimental and control groups consisted of two drug administration conditions, where the drugs were administered orally either 20 minutes before the DNMTS procedures or immediately after. The drugs were grounded and mixed in saline (NaCl), then filtered with a .22- μ m filter unit before administration (Hotchkiss et al., 2005; Behn & Potter, 2001). The eight groups, consisting of two females and two males per group is listed in table 1.

Table 1 Drugs and Dosages for the Eight Groups

Groups	Drug & Dosage	Condition
1	3.0 mg/kg Sildenafil	20 mins before DNMTS procedure
2	3.0 mg/kg Sildenafil	Immediately after DNMTS procedure
3	0.3 mg/kg Vardenafil	20 mins before DNMTS procedure
4	0.3 mg/kg Vardenafil	Immediately after DNMTS procedure
5	1.5 mg/kg Tadalafil	20 mins before DNMTS procedure
6	1.5 mg/kg Tadalafil	Immediately after DNMTS procedure
7	Saline	20 mins before DNMTS procedure
8	Saline	Immediately after DNMTS procedure

Data Collection and Analysis

Data were collected using the Spontaneous Motor Activity Recording & Tracking (SMART) system. SMART is a computer hardware and software system, which tracks the rat traversing on the maze, via a video camera located in the ceiling above the maze. The SMART system records the distance of the rat, the speed, time started, time ended, and the entrances of

the rat into the arms and center platform of the maze. Data were also collected manually using data sheets, with templates of the radial maze. Odd behaviors and errors were noted. Data were analyzed using Analysis of Variance (ANOVA) to compare the performance of the rat in the experimental conditions with saline.

Results and Conclusions

The experimental groups and the control groups were analyzed using a one-way ANOVA. The results of our study indicates that no significant differences were found in the total number of errors between the experimental groups and the control groups ($F(3,255) = 2.33$, $p > .05$). In addition, no significant differences were found in the total number of proactive errors between the experimental and control groups ($F(3,255) = 2.59$, $p > .05$), and in the total number of retroactive errors between the experimental and control groups ($F(3,255) = 1.76$, $p > .05$). The data were also analyzed using a Tukey's Post Hoc test, to look for minute differences in the data however no significant differences were observed between the experimental groups and the control groups.

The results for our study revealed that Sildenafil (Viagra™), Vardenafil (Levitra™) and Tadalafil (Cialis™) did not enhance spatial learning and memory in the eight-arm radial maze acquisition test in male or female rats when compared to saline control groups. Some possibilities for our negative results could be that housing male and female rats simultaneously during the study may have confounded our data. Another possibility is that there were too many experimental groups in the study, with small numbers of animals in each group. In addition, we were not able to acquire pure compound from the major manufacturers of these male erectile dysfunction treatment drugs. Furthermore, compounding the drugs in a suspension and oral

administration may have confounded our findings. Current research in learning and memory continues to evaluate these drugs in a more systematic fashion, with positive results.

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