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Chronic sildenafil (Viagra) administration reduces anxiety in intact and castrated male rats

Abdel A. Solís, José A. Bethancourt* and Gabrielle B. Britton*
Universidad de Salamanca and * Instituto de Investigaciones Científicas y Servicios de Alta Tecnología

Epidemiological research indicates that sildenafil (Viagra) abuse is associated with increased risk behaviors. The present study employs the open field, a standard animal model used in the field of anxiety research, to examine whether chronic exposure to sildenafil affects anxiety and risk-taking behaviors in gonadally intact and castrated male Wistar rats. Sildenafil (10 mg/kg) or saline were administered three times a week for three weeks. Animals were tested once a week in the open field during and after drug treatment. Sildenafil treatment increased the number of center entries and time spent in the center in intact and castrated animals during and after treatment, suggesting that repeated drug use decreases anxiety. Sildenafil also restored the deficits in exploration and locomotion produced by castration, indicating that sildenafil effects on open field behaviors are independent of endogenous androgens. We caution against generalizing from this study to human behaviors, but propose that the behavioral effects produced by a chronic high dose of sildenafil warrant further studies into its abuse potential.

La administración crónica de sildenafil (Viagra) reduce la ansiedad en ratas machos intactas y castradas. Estudios epidemiológicos indican que el abuso de sildenafil (Viagra) está asociado con comportamientos de riesgo. En el presente estudio utilizamos el campo abierto, un modelo animal estándar en investigaciones sobre la ansiedad, para examinar los efectos de la administración crónica de sildenafil sobre la ansiedad y comportamientos de riesgo en ratas machos Wistar intactas y castradas. Sildenafil (10 mg/kg) o suero salino fueron administrados tres veces semanalmente durante tres semanas. Se midió el comportamiento en el campo abierto una vez por semana durante y posteriormente al tratamiento. El tratamiento con sildenafil incrementó las entradas al centro del campo y el tiempo en el centro en animales intactos y castrados, lo que sugiere que la administración crónica disminuye la ansiedad. Sildenafil también restauró los déficit asociados con la castración, lo que indica que los efectos de sildenafil sobre comportamientos en el campo abierto son independientes de la presencia de andrógenos endógenos. Alertamos en contra de generalizar estos resultados a los comportamientos humanos, pero proponemos que los efectos conductuales que produce la administración crónica de una dosis alta de sildenafil justifican el estudio del potencial de abuso de esta sustancia.

Sildenafil (Viagra), a powerful treatment for male erectile dysfunction (Goldstein et al., 1998), has become one of the most commonly prescribed and abused drugs available (Swearingen & Klausner, 2005). The drug, a selective inhibitor of phosphodiesterase type 5 (PDE-5), facilitates penile erection by producing an accumulation in cyclic guanosine monophosphate (cGMP) in the corpus cavernosum, causing smooth muscle relaxation and increased blood flow to the penis (Langtry & Markham, 1999). Because sildenafil prolongs erection and ejaculation latency in men without erectile dysfunction, it is a popular drug of abuse (Swearingen & Klausner, 2005). Epidemiological data indicate that sildenafil abuse is associated with increased risk behaviors, including unprotected sex (Paul, Pollack, Osmond, & Catania, 2005; Swearingen & Klausner, 2005) and illicit substance abuse (Crosby & DiClemente, 2004; Paul et al., 2005).

In addition to the peripheral effects of sildenafil, the localization of PDE-5 to the brain enables sildenafil to affect central nervous system (CNS) functions such as cognitive, motivational and emotional processes (Devan et al., 2006; Kurt et al., 2004; Prickaerts et al., 2002; Tahsili-Fahadan et al., 2006). A recent animal behavior study provided evidence that sildenafil has rewarding properties, which may be related to its abuse potential (Tahsili-Fahadan et al., 2006). Similarly, observations that sildenafil modifies anxiety-related behaviors (Kurt et al., 2004; Volke, Wegener, & Vasar, 2003) may also be relevant to its abuse potential in light of the relationship between anxiety and risk-taking behavior in humans (Kashdan, Collins, & Elhai, 2006; Mitte, 2007) and animals (Laviola, Macri, Morley-Fletcher, & Adriani, 2003). To our knowledge, the effects of repeated sildenafil use on anxiety have not been explored, but may offer insight into the relationship between sildenafil abuse and risk-taking behaviors. A recent study showed that chronic sildenafil (10 mg/kg) exposure over three weeks produced an increase in

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Correspondencia: Gabrielle B. Britton
Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP)
0843-01103 Panamá
E-mail: gbritton@indicasat.org.pa

aggressive behavior in mice following but not during drug administration (Hotchkiss et al., 2005), but did not report other behavioral data. Thus, the purpose of the present study was to characterize the effects of repeated sildenafil use and withdrawal on anxiety-related and risk-taking behaviors using the rat open field (OF) paradigm, a standard procedure employed in the field of anxiety research (for a review see Prut & Belzung, 2003). Novel open environments such as the OF create conflict situations in rodents by simultaneously evoking exploration and anxiety-related behaviors. In OF testing, rodents naturally prefer the periphery over the central parts due to an innate aversion towards open areas. Therefore, entries into the center areas and the frequency of rearing behavior can be interpreted as measures of anxiolysis and risk-taking, respectively (Prut & Belzung, 2003). Although there are limitations to the applicability of rodent models to human behaviors, the neural and psychological processes related to anxiety have revealed remarkable consistency across human and animal species (Rosen & Donley, 2006). As such, much can be gained from examining drug effects on basic animal behaviors.

Our first aim was to examine how sildenafil affects anxiety and risk-taking behaviors in the OF, which permits the assessment of three different kinds of behavioral responses: anxiety-like behaviors (central area exploration), risk assessment (investigating aversive areas in a stretched posture known as rearing), and locomotion (a simple control for drug-induced impairment of locomotor capacity). Chronic dosing with sildenafil (10 mg/kg; Hotchkiss et al., 2005) was conducted over three weeks in order to characterize the effects of repeated drug use and withdrawal on anxiety and risk-taking behaviors. Moreover, because a number of pharmacological studies have established that baseline behavior in tests such as the OF can vary widely between sessions (Prut & Belzung, 2003), repeated testing was conducted in order to assess changes in behavior that may not emerge in a single session.

A second aim was to investigate sildenafil effects on OF behaviors in the absence of the drug's sexually stimulating effects. Sildenafil has been shown to facilitate sexual behavior in gonadally intact but not in castrated male rats (Ottani, Giuliani, & Ferrari, 2002). It is well known that sexual behavior is totally disrupted by castration (Beach & Holz-Tucker, 1949), and that castration significantly reduces aggression (Albert, Walsh, Gorzalka, Siemens, & Louie, 1986). Thus, in order to control for the non-specific influence of testosterone-dependent behaviors on OF performance, we examined sildenafil effects in castrated as well as intact male rats. Castration has been shown to reduce locomotion, exploration, and entries into the central area of an OF (Adler, Vescovo, Robinson, & Kritzer, 1999; Edinger & Frye, 2005, 2006). Thus, by including castrated animals in our study, we examined whether sildenafil and testosterone interact to produce changes in anxiety and risk-taking behaviors.

Method

Subjects

Forty-five adult male Wistar rats (Harlan, Mexico) weighing 200-250 g at the outset of the experiment were used. Animals were single-housed in polyurethane tubs within a colony maintained at 25° C on a 12 h light/dark cycle, and were provided free access to food and water throughout the experiment. Except for the one-week period following surgery, animals were handled every other day. Behavioral sessions were conducted in an isolated, air-conditioned room. Experiments were conducted in accordance with the National Institutes of Health regulations relating to the care and use of laboratory animals (Publication No. 85-23).

Surgery

Subjects were randomly assigned to castrated (n= 23) and intact (n= 22) groups. Animals were castrated under anesthesia with pentobarbital (40 mg/kg, i.p.). A midline scrotal incision was made, and the testes and surrounding fat tissue were tied off and removed. Following surgery, animals were left undisturbed in their home cages for seven days.

Drug treatment

Animals were randomly assigned to one of two drug treatment conditions, and received either saline or sildenafil (10 mg/kg; Pfizer) injections in a volume of 1 ml/kg (i.p.) three times weekly over the course of three weeks with 48 h between injections. We selected a dose of sildenafil that would effectively mimic drug abuse patterns, but that would not impair basic OF behaviors. The dose of 10 mg/kg and pattern of administration were obtained from a report that suggested possible withdrawal effects following chronic drug administration (Hotchkiss et al., 2005); in contrast, acute drug administration with the same dose has been shown to have no effect on locomotion or exploration (Prickaerts et al., 2005; Tahsili-Fahadan et al., 2006; Volke et al., 2003). Sildenafil tablets (50 mg) were ground into powder, mixed with saline, and filtered through 40 μm filter paper. The solution was refrigerated at 4° C and brought to room temperature prior to injections. All animals were weighed on injection days to ensure appropriate drug dosing and equal handling. Treatment groups were as follows: castrated animals treated with sildenafil (n= 8); intact animals treated with sildenafil (n= 7); castrated control group (n= 15); intact control group (n= 15).

Open field activity

A circular OF (100 cm diameter and 25 cm walls) was constructed out of aluminum and painted gray. The floor was divided into four equivalent quadrants using a black permanent marker. The perimeter was marked along the 20-cm wide ring adjacent to the walls, and a 20-cm diameter circle marked the center. Two 100-W lamps focused bright illumination over the arena. A video camera recorded all behavioral sessions.

OF observations were conducted once a week for four consecutive weeks. During drug treatment, OF testing occurred on the third injection day, allowing 30-45 min between injection and placement in the OF. A fourth test was conducted one week after the last injection. The order in which animals were tested was randomized each week. On test days, animals were transported individually in clear plastic tubs, placed in the center of the OF, and allowed to explore freely for five min. At the conclusion of the test, animals were returned to their home cages and the arena was washed and dried before the next test.

The following variables were measured: entries into the center of the arena, time spent in the perimeter and central areas, quadrant crossings and rearing. An animal was considered within the perimeter of the arena when all four paws were within the marked area and in the center when both front paws crossed into

the center circle. These measures served as indices of anxious behavior. Quadrant crossings were recorded when all four paws crossed from one quadrant into another and served as a measure of overall motor activity. Rearing was scored when both front paws left the floor in a stretching posture and grooming did not occur, and served as a measure of exploration. Videotaped sessions were analyzed by three independent observers unaware of the subjects' treatment conditions. Inter-rater reliability checks revealed the observer correlations to be over .94 in each case (M= .97).

Data analysis

Three-factor analysis of variance (ANOVA) with surgical condition and drug treatment as between-subjects factors and test session as the within-subjects factor was applied to four measures: entries into the center, time spent in the center, frequency of quadrant crossings and rearing. Significant interactions involving test session were analyzed with *post hoc* one-way ANOVAs to examine effects on a week-by-week basis. Interactions between surgical condition and drug treatment were examined with one-way ANOVA comparing two means. Mean differences were considered statistically significant if p<.05. When significant differences involving anxiety-related measures were obtained, analysis of covariance (ANCOVA) was applied to these measures with locomotor activity as a covariate. Remaining significant differences between groups indicate independence between measures of locomotor activity and anxiety-related behaviors.

Results

Three-way ANOVA conducted on center entries revealed a significant main effect of drug treatment, F(1,41)= 7.41, p<.01, where sildenafil-treated animals performed more center entries than controls, and a test session × drug treatment interaction F(3,123)= 2.82, p<.05, indicating that behavior differed across test sessions between drug treatment groups. One-way ANOVAs revealed that sildenafil-treated rats exhibited a greater number of entries into the center than controls on the third [F(1,43)= 9.78, p<.01] and fourth [F(1,43)= 4.45, p<.05] sessions (figure 1A). Three-way ANOVA conducted on time spent in the center revealed a main effect of drug treatment, F(1,41)= 11.54, p<.01, where sildenafil-treated animals not only entered the center area more often than controls, but also spent more time in the center (figure 1B). As expected, parallel results were obtained for the time spent in the periphery; sildenafil treatment reduced thigmotaxis relative to control animals (data not shown).

Table 1 summarizes the number of grid crossings across test sessions. Three-way ANOVA revealed a significant main effect of surgical condition, F(1,41)= 4.05, p= .05, and two-way interactions between test session and surgical condition, F(3,123)= 3.82, p<.05, test session and drug treatment, F(3,123)= 3.92, p<.05, and surgical condition and drug treatment, F(1,41)= 8.34, p<.01. One-way ANOVA used to analyze the interactions involving test session confirmed that castration reduced locomotion on the first [F(1,43) = 17.89, p < .001] and third [F(1,43)= 10.84, p<.01] sessions, while sildenafil treatment restored locomotion on the second test session, F(1,43)= 4.32, p<.05. Figure 2A illustrates the interaction between surgical condition and drug treatment, confirming that castrated animals that did not receive drug treatment exhibited reduced motor activity relative to all other groups (p<.01).

Table 1 summarizes the frequency of rearing across sessions. Three-way ANOVA revealed a significant main effect of test session, F(3,123)= 7.51, p<.001, and two-way interactions between test session and surgical condition, F(3,123)= 4.15, p<.01, test session and drug treatment, F(3,123)= 8.19, p<.001, and surgical condition and drug treatment, F(1,41)= 7.41, p<.05.

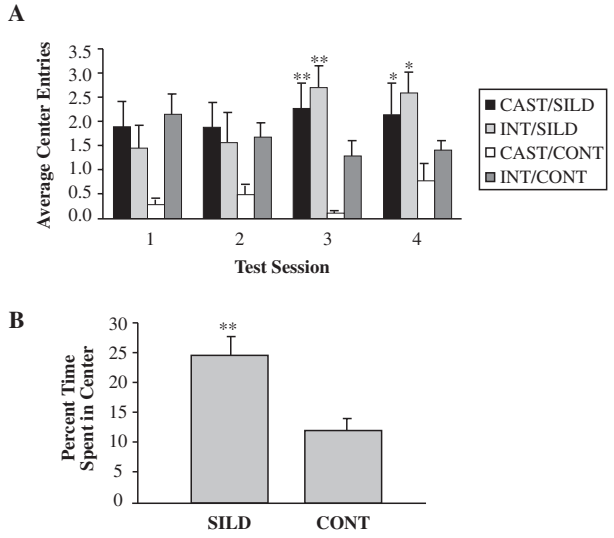

Figure 1. *Sildenafil treatment produced anxiolytic effects in OF testing. (A) Sildenafil treatment significantly increased the number of center entries performed by intact and castrated rats on the third and fourth test sessions. Values are group means + standard error of the mean (SEM) (* p<.05; ** p<.01 versus control groups on corresponding weeks). (B) Sildenafil treatment also increased the time spent in the center of the field. Values are drug treatment group averages (+ SEM) across test sessions (** p<.01). INT= intact; CAST= castrated; SILD= sildenafil (10 mg/kg); CONT= controls*

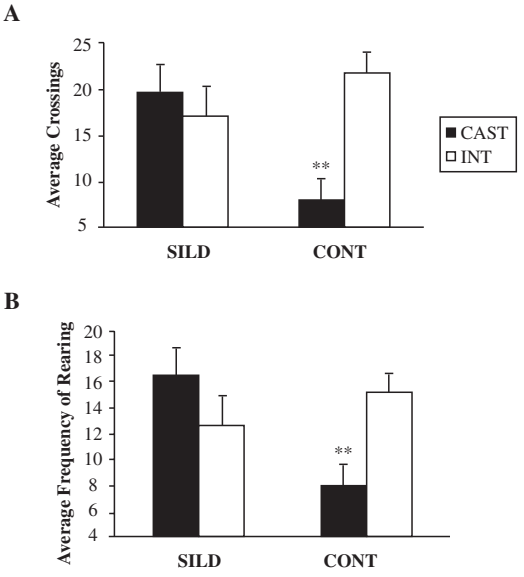

Figure 2. *Sildenafil treatment restored motor activity and exploration in castrated animals in OF testing. (A) Sildenafil treatment reversed the deficits in the number of grid crossings and (B) the frequency of rearing produced by castration. Values are drug treatment group averages (+ SEM) across test sessions (** p<.01)*

One-way ANOVAs indicated that castration reduced rearing behavior on the first [$F(1,43)= 6.37$, $p<.05$] and third [$F(1,43)= 7.30$, $p<.05$] sessions, whereas drug treatment restored rearing on the fourth test session [$F(1,43)= 14.63$, $p<.001$] after the cessation of drug treatment. Figure 2B illustrates the interaction between surgical condition and drug treatment, where castrated animals that did not receive drug treatment exhibited reduced exploration relative to all other groups ($p<.01$).

Because levels of overall motor activity can affect anxiety-related measures, we applied ANCOVA to center entries and time spent in the center, with quadrant crossings as a covariate, to assess whether differences between drug treatment groups in anxiety-related behaviors and motor activity were independent. ANCOVA revealed that the increases in center entries [$F(1,39)= 5.71$, $p<.05$] and time spent in the center [$F(1,39)= 9.34$, $p<.01$] in sildenafil-treated animals remained significant, even after accounting for the variance associated with overall levels of motor activity. Thus, sildenafil treatment decreased anxiety in OF testing. We also tested the assumption that anxiety-related measures were related to exploration on corresponding weeks. Bivariate correlations revealed significant positive associations between center entries and rearing on the first [$r(43)= .53$, $p<.001$], third [$r(43)= .39$, $p<.01$] and fourth [$r(43)= .77$, $p<.001$] test sessions, and a trend toward significance on the second test session [$r(43)= .27$, $p= .07$]. However, rearing was not correlated with time spent in the center on any test session. Thus, increases in exploration were associated with a greater number of entries into the center area, but not with a tendency to remain in the center.

Discussion

The purpose of the present study was to determine whether chronic exposure to sildenafil modifies basic behaviors related to anxiety and risk-taking. Our results show that sildenafil treatment in castrated and intact male rats produced an increase in the number of center entries and time spent in the center of the OF, as well as a decrease in the time spent in the periphery of the OF, on the third and fourth weeks of testing. These effects were shown to be independent of overall motor activity, and indicate that chronic treatment with a selective inhibitor of PDE-5 modifies OF behavior in a manner consistent with anxiolytic compounds (Prut & Belzung, 2003). The observation that sildenafil-treated rats were more likely to enter the center of the arena and remain in the center on the third and fourth weeks of testing suggests that changes in cGMP breakdown produced by chronic drug treatment were inadequate to produce behavioral changes after two weeks of treatment, but were revealed during the third week and after the cessation of drug treatment. To our knowledge, the present study and that of Hotchkiss et al. (2005) are the reports of the effects of chronic sildenafil treatment on innate behaviors. Taken together, these observations provide evidence that repeated sildenafil exposure produces alterations in innate behaviors that emerge after repeated testing, which supports possible withdrawal and abuse effects of sildenafil.

Table 1
Frequency or rearing and quadrant crossings recorded during four open field sessions in intact (INT) and castrated (CAST) rats treated with sildenafil (SILD) or not treated (CONT)[a]. Drug treatment ceased after the third test session

		Week 1	Week 2	Week 3	Week 4
Crossings	INT/CONT	29.7 ± 2.6	15.8 ± 2.2	21.9 ± 1.6	19.8 ± 2.1
	CAST/CONT	8.1 ± 1.2^{+++}	9.7 ± 1.8^{+}	8.0 ± 1.7^{+++}	6.3 ± 1.4^{+++}
	INT/SILD	17.0 ± 5.6	17.4 ± 7.7	16.7 ± 7.1	17.1 ± 8.5
	CAST/SILD	18.1 ± 4.4*	22.4 ± 3.8**	14.9 ± 3.7*	22.8 ± 5.1**
Rearing	INT/CONT	21.0 ± 1.8	10.5 ± 1.7	17.8 ± 2.2	11.3 ± 1.2
	CAST/CONT	8.0 ± 1.2^{+++}	10.4 ± 2.1	7.5 ± 2.0^{+++}	6.5 ± 1.7^{+}
	INT/SILD	13.6 ± 4.6	5.0 ± 2.0	15.6 ± 4.0	16.4 ± 5.3
	CAST/SILD	17.9 ± 4.7*	10.6 ± 1.7	14.6 ± 3.0*	22.8 ± 4.4**

[a] Data are means ± SEM
* $p<.05$; ** $p<.01$ versus castrated control (CAST/CONT) group
+ $p<.05$; +++ $p<.001$ versus intact control (INT/CONT) group

Whether the effects of chronic sildenafil treatment on innate behaviors are mediated by cGMP is unclear. It is possible that the accumulation of cGMP as a consequence of chronic drug treatment produces changes in the expression of various receptors associated with cGMP or in the responsiveness of these receptors in the brain. Alternatively, the inhibition of cGMP breakdown may produce changes in nitric oxide (NO) production via negative feedback mechanisms (Canteros et al., 1996). PDE-5 inhibitors are believed to affect anxiety-related behaviors by acting on the nitric oxide (NO)-cGMP signaling pathway, but this assertion is complicated by an abundance of contradictory results. To cite one example, a single injection of sildenafil at low doses has been reported to produce both increases (Kurt et al., 2004) and no effects (Volke et al., 2003) on anxious behaviors using the elevated plus-maze test in mice. As discrepancies among behavioral studies indicate, further investigation into whether sildenafil treatment differentially affects performance according to species, drug treatment, and testing protocols in animal models of anxiety is merited. Similarly, studies investigating the effects of NO levels on anxiety-related responses have also reported contradictory results; both increases (Czech, Jacobson, LeSueur-Reed, & Kazel, 2003; Vale, Green, Montgomery, & Shafi, 1998) and decreases (Faria et al., 1997; Forestiero, Manfrim, Guimarães, & de Oliveria, 2006) in anxiety have been linked to inhibition of NO by nitric oxide synthase (NOS) inhibitors. Sildenafil and other PDE-5 inhibitors have been shown to increase cGMP in the hippocampus in vitro (Prickaerts et al., 2002), but it remains to be determined whether the anxiolytic effects of sildenafil observed in the present study are related to enhanced cGMP levels or to its indirect effects on NO. One problem is the lack of information regarding the specific effects of sildenafil on the NO-cGMP pathway and the corresponding behavioral consequences of modifying activity within the pathway. Changes in NO levels may either facilitate or inhibit anxiety depending on the magnitude of NOS inhibition (Li, Chung, & Quock, 2004). Further, since NOS is not evenly expressed throughout the brain (Singh, Pervin, Shryne, Gorski, & Chaudhuri, 2000), NO may be able to affect neurotransmitter activity differentially across brain systems.

The observed effects of sildenafil on the behavior of castrated animals offer further evidence regarding the relationship between NO-cGMP signaling and anxious behaviors.

As predicted, castrated animals without treatment exhibited more time in the periphery of the field, less center entries, less rearing and locomotion than all other groups (Adler et al., 1999; Edinger & Frye, 2005, 2006). Sildenafil administration reversed

the decline in center entries, and restored locomotion during and after treatment, which suggests that drug effects were independent of the influence of endogenous androgens. Research has shown that castration significantly increases NOS activity in various brain regions, particularly in the hypothalamus and amygdala (Singh et al., 2000), two areas that participate in stress and defensive responses. PDE-5 expression and functional activity are also significantly reduced by castration (Morelli et al., 2004; Traish et al., 1999), which suggests that in the absence of androgens, changes in NOS levels might be accompanied by lower cGMP degradation (Morelli et al., 2004). It remains unclear how PDE-5 inhibitors and a reduction in PDE-5 expression interact to affect anxiety-related behaviors in castrated animals. PDE-5 inhibition may differentially activate and inhibit receptors and enzymes associated with cGMP, or may produce changes in receptor sensitivity at target sites. Thus, we cannot exclude the possibility that other centrally- or peripherally-mediated actions influenced the observed behaviors.

In our study, the use of a high dose of sildenafil may limit the generalizability of our results because of the possibility of drug-produced side effects. This is particularly relevant in light of the reported vasodilatory effects of sildenafil (Zusman, Morales, Glasser, & Osterloh, 1999). The 10 mg/kg dose was chosen in order to examine possible withdrawal effects reported in a previous study (Hotchkiss et al., 2005), but also because fewer administrations of the same dose produced no effects on locomotor activity (Tahsili-Fahadan et al., 2006; Volke et al., 2003) or exploratory behavior (Prickaerts et al., 2005), which suggests that at this dose the drug does not produce a generalized increase in arousal related to changes in blood pressure. However, in castrated animals, the effects of a high dose of sildenafil on blood pressure have not been examined previously, although castration alone has been shown to have no effect on blood pressure in at least two animal models (Blanco-Rivero, Balfagón, & Ferrer, 2005; Traish et al., 1999). Thus, we believe it is unlikely that sildenafil treatment in castrated animals produced adverse effects that interfered with OF behaviors, but we cannot rule out this possibility.

In conclusion, administration of sildenafil decreased anxious behavior in male rats, and restored the deficits in locomotion and exploration produced by castration. These results suggest that repeated sildenafil use over prolonged periods modifies anxiety-related and risk-taking behaviors in addition to producing its effects on peripheral tissues. However, it is important to note that the applicability of spontaneous behaviors of rodents in the OF to human anxiety-related and risk-taking behaviors remains unclear. Moreover, additional testing using other animal models of anxiety, such as the elevated plus-maze and light-dark box, should be completed in order to gain a better understanding of how sildenafil use modifies anxiety-related behaviors. These studies are warranted to determine whether the reported effects of chronically administered sildenafil on anxious behaviors are related to its potential for abuse.

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