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INFLUENCE OF BIOLOGICAL SEX ON SOCIAL BEHAVIOR, INDIVIDUAL RECOGNITION, AND NON-ASSOCIATIVE LEARNING IN THE ADULT GRAY SHORT-TAILED OPOSSUM (*MONODELPHIS DOMESTICA*)

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Abstract

Social behavior is critical for relationship formation and is influenced by myriad environmental and individual factors. Basic and preclinical research typically relies on rodent models to identify the mechanisms that underlie behavior; however, it is important to use non-rodent models as well. A major objective of the present study was to test the hypothesis that biological sex and social experience modulate the expression of social behavior in the adult gray short-tailed opossum (*Monodelphis domestica*), a non-traditional model. We also investigated the non-associative learning abilities of these animals. Following a period of social isolation, animals of both sexes were paired with a non-familiar, same-sex partner for 10 minutes on three different occasions, with 24-hour inter-trial intervals. We are the first research group to find significant sex differences in submissive and nonsocial behaviors in *Monodelphis*. Females displayed significantly higher

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Conflicts of Interest

The authors declare no competing financial interests.

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durations of nonsocial behavior that increased over trials. Males were more aggressive; their latencies to the first attack and submissive behavior decreased over trials whereas these latencies increased for females; males' duration of submissive behavior increased over trials whereas it decreased for females. A different group of subjects habituated in response to repeated presentations to neutral odors and dishabituated in response to novel odors. In addition, both males and females demonstrated the ability to form social memories in a standard individual (social) recognition test. Our results contribute to the characterization of this marsupial species, an important first step in developing it as a model of complex social behaviors.

Keywords

Aggression; social dominance; social subordination; animal models; learning; social memory

1. Introduction

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Mammals are driven to seek social contact and there are multiple interactive (behavioral and reward) brain systems [1] that regulate and facilitate the expression of parent-offspring interactions [2], play behavior among juveniles [3], sociosexual [4] and affiliative [5] behaviors in opposite-sex partners, as well as social interactions between individuals of the same sex [6]. The study of social behavior in laboratory animals is important for identifying the proximate mechanisms that regulate behavior and informing research on the fundamental factors that underlie changes in behavior at the population level [7]. Moreover, behavioral animal research is crucial for developing preclinical models of neuropsychiatric disorders that are characterized by varying degrees of social dysfunction (e.g., Autism Spectrum Disorder, Schizophrenia) [8, 9]. Most animal research is conducted using rodent models; however, scientific progress benefits from the use of non-traditional laboratory animals and multiple research approaches (see, e.g., Kabelik and Hofmann [10]). A major aim of the present study is to provide social and learning behavioral data for the gray short-tailed opossum (*Monodelphis domestica*), a marsupial species that is ideal for laboratory research, which will serve as a foundation for investigators that use this animal model in their basic and preclinical research.

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Monodelphis is a powerful animal model for a number of reasons, including but not limited to: detailed information regarding care and management in a laboratory setting, small size (typically 60–100g for females; 90–150g for males), continuous availability for experimental use, non-seasonal reproductive function, and relative immaturity of neonates compared to other mammalian species [11]. Birth occurs approximately 13.5 days after ovulation and fertilization; therefore, at the time of parturition, the readily accessible *Monodelphis* neonate is comparable to a mouse embryo, making the *Monodelphis* a powerful resource for neurodevelopmental research [11, 12]. In terms of behavior, based on the pioneering work by Fadem and Corbett [12], it is known that *Monodelphis* social behavior changes as a function of age. Fadem and colleagues provided data on aggression, threat behavior, scent marking, and reproductive behavior, which are salient behaviors that are relatively easy to quantify. To our knowledge, there are no reports on other aspects of *Monodelphis* social behavior, such as submissive behavior. The present study was designed to address this gap in

knowledge, and to explore other factors that influence *Monodelphis* behavior, including sex and prior social experience.

Fadem (1989) reported that males display higher levels of aggression than females [13]. Inclusion of female subjects is commendable because inter-male aggression is the most common paradigm in aggression research, which has led to the exclusion of females from studies on aggression and has fueled the misguided assumption that aggression falls solely in the domain of male social behaviors [14]. In fact, both males and females display offensive aggression that can be modulated by environmental, neuroendocrine, and/or experiential factors [15, 16]. Sex differences in the expression of social behavior can be crucial for the species-specific organization of social structures [17]. Even more, the social defeat stress model, which relies on aggressive behavior, has long been used to study dimorphic brain mechanisms that govern certain psychiatric disorders such as depression and anxiety [18], possibly shedding light on the sex-specific prevalence of psychiatric conditions.

Social experiences influence an individual's response to subsequent social stimuli. For example, social dominance-related behaviors, which include social communication and aggression, are modulated by an individual's housing status and recent exposure to a same-sex partner [19, 20]. We have previously reported that social experience influences the expression of social behaviors in dominant and subordinate male hamsters [6]; a major objective of the present study was to determine how social experience influences *Monodelphis* social behavior. Social behavior is also dependent on the ability of an individual to recognize others. Individual (social) recognition is a type of learning and memory that influences various aspects of social behavior and underlies relationship formation between conspecifics [21, 22]. Although there is some evidence that *Monodelphis* males are able to distinguish between unfamiliar and familiar social odors [23], less is known about individual (social) recognition in *Monodelphis* males and females using standard social memory paradigms that have been validated in laboratory rodents.

We point out that under natural conditions, adult *Monodelphis* have a low population density and they are solitary animals, except for short periods of time when animals of the two sexes come into close proximity, inducing ovulation in the female within approximately one week [24]. After mating, the animals disperse. Consequently, *Monodelphis* are usually singly housed in the laboratory except when they are paired for the purpose of reproduction. In contrast, mice are communal animals that may have high population densities under natural conditions, and are generally pair-housed or group-housed in the laboratory. Obviously, these distinctions between the two species might be expected to be reflected in major behavioral differences. The present study tested the hypothesis that sex and social experience influence the expression of *Monodelphis* social behavior. In addition, we investigated the non-associative learning abilities of *Monodelphis* in social and non-social contexts.

2. Materials and Methods

2.1 Animals

Forty-eight adult *Monodelphis* were obtained from a colony of animals housed in the URTGV School of Medicine South Texas Diabetes and Obesity Institute. The genetic stock used in this study is designated LL2; this stock is descended from four males and five females that were captured in Brazil in 1977 (see VandeBerg and Williams-Blangero, 2010). Animals were single-housed in direct exhaust ventilated Optimice polycarbonate cages (484 sq. cm. of floor space, AnimalCare Systems) in a climate-controlled room, with food and water available *ad libitum*. The light cycle was 14:10 h, with lights on at 7:00 A.M. and off at 9:00 P.M., and testing occurred during the light phase of the cycle. Males ($\bar{X} = 267$ days old, $SD = 41.80$) were on average slightly older than females ($\bar{X} = 258$ days old, $SD = 42.84$), but the ages of the two sexes were not statistically different. All procedures were conducted in accordance with the National Institutes of Health Guidelines for the Use of Animals and were approved by the University of Texas Rio Grande Valley Animal Care and Use Committee.

2.2 Experimental design

We carried out three experiments using a factorial repeated measures design, with trials (time) as the within-subjects factor, and sex (of the participant) as the between-subjects factor. Both males and females were sexually experienced and had been used as breeders. During the study period, subjects were isolated and not housed with mating partners. For all experiments, half of the research subjects were males and half were females. Experiment 1 tested the hypothesis that social experience and sex influence the expression of social behavior. First, twenty-four animals were socially isolated (i.e., individually housed) for four weeks prior to the start of experiment 1. Each animal was then randomly paired with a same-sex partner that was similar in age and size (i.e., 12 same-sex dyads: six male and six female) for three social interaction trials on consecutive days. Experiments 2 and 3 were conducted to determine whether *Monodelphis* demonstrate the same type of non-associative learning abilities that are displayed by rodents. Sixteen animals were used in the olfactory habituation-dishabituation task to test the hypothesis that *Monodelphis* habituate to neutral odors and dishabituate in response to a novel odor. Eight animals were used in the individual (social) recognition task to test the hypothesis that *Monodelphis* habituate to social stimuli and dishabituate in response to a novel social stimulus.

2.3 Social interaction test

Following a four-week social isolation period, the behaviors of twelve same-sex dyads were recorded using AnyMaze video tracking software and camera (Stoelting, Wood Dale, Illinois). The testing apparatus was $19.1 \times 8.6 \times 14$ in, and there were three 10-minute social interaction trials with 24-hour inter-trial intervals. Anymaze video files were converted to mpeg files and scored using Jwatcher software (<http://www.jwatcher.ucla.edu/index.htm>). Table 1 provides an ethogram that was developed for the present study and is also intended to serve as a resource for future investigations of *Monodelphis* social behavior (video examples of each operationalized behavior are provided as Supplementary material and can be accessed at <http://bit.ly/MonoBehav>). It should be noted that this ethogram was based on

Fadem and Corbett (1997) and Fadem (1989) [12, 13], who provided detailed descriptions of aggression and open-mouth posture in *Monodelphis*, as well as our previous work with male hamsters [6] and published reports of social behavior in rodents [25]. The focal-animal sampling method was used to quantify all behaviors [26]. The latencies to the first non-aggressive social behavior, aggressive behavior, attack, submissive behavior, flee, and open mouth posture were quantified by observers blinded to the sex of the animal. The frequencies and durations of the same behaviors were also quantified. Gutzler et al. (2009, 2010) made a distinction between low- and high-intensity aggression [15, 27]. Informed by these previous reports, we quantified different intensities of aggression by assigning one key (in the JWatcher program) to the behaviors listed in the low-intensity aggression row, and another key was assigned to the behaviors in the high-intensity aggression row (see Table 1). Total aggression was calculated by taking the sum of low- and high-intensity aggression durations for the entire 10-min test.

2.4 Olfactory habituation-dishabituation test

Sixteen animals were exposed to three neutral odor stimuli, following a modified version of a mouse protocol reported by Wrenn, Harris [28]. Stimuli included deionized water or 1:100 dilutions of lime or almond (dissolved in deionized water). The testing apparatus was 20 × 10 × 12 in, and contained a piece of filter paper affixed to a microscope slide that was mounted to the wall of the apparatus. Before the start of the experiment, each animal was placed in the apparatus (without an odor stimulus) for three minutes, to allow the animal to habituate to the new environment. For each trial, 50 µl of odorant solution was added to the slide-mounted filter paper, and each animal was then placed and left in the apparatus for three minutes. Animals underwent a total of nine 3-minute trials with 1-minute inter-trial intervals, including trials for water alone (3x), lime (3x), and almond (3x). Behaviors were recorded and quantified using AnyMaze video tracking software and camera (Stoelting, Wood Dale, Illinois). We counted the frequency and measured the duration of sniffing behavior toward the filter paper (i.e., nose touches).

2.5 Individual (social) recognition test

Eight animals were exposed to two social stimuli (familiar individual and novel individual), following a modified version of a mouse protocol reported by Ferguson, Young [29]. The testing apparatus was 20 × 10 × 12 in, and contained a small, plastic holding cage (with six holes drilled through the sides) mounted to the floor. Before the start of the experiment, each animal was placed in the apparatus (without a social stimulus) for three minutes, to allow the animal to habituate to the novel environment. For each trial, a same-sex conspecific (stimulus) animal of similar age and size as the experimental subject was placed first in the plastic holding cage, and then the experimental subject was added and left in the apparatus for three minutes. We completed a total of four 3-minute trials with 10-minute inter-trial intervals; three with the first stimulus animal, followed by one with a novel stimulus animal. Behaviors were recorded and quantified using AnyMaze video tracking software and camera (Stoelting, Wood Dale, Illinois). The frequency and duration of sniffing behavior toward the plastic holding cage (i.e., nose touches) were quantified.

2.6 Data analysis and statistics

A factorial repeated measures ANOVA, with trials (time) as the within-subjects factor and sex as the between-subjects factor, was used to determine: whether the expression of social behavior changed over the three social interaction trials and whether animals habituate/dishabituate to neutral odors and/or social stimuli. We also investigated whether sex of the subject influenced social behavior and/or habituation/dishabituation to the different stimuli. Statistical significance was defined as $p < 0.05$; *post-hoc* tests (with Bonferroni corrections) were conducted for significant trial (time) and interaction effects, to deconstruct significance between trials (time). Data were analyzed with SPSS 25 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.2 Sex influences the expression of social and non-social behaviors

Sex strongly determined the expression of behavior. Duration of nonsocial behavior was significantly higher for females compared to males ($F_{1,22} = 24.482$, $P < 0.01$, Fig 1A). Males expressed a significantly higher frequency of flees compared to females ($F_{1,22} = 4.117$, $P < 0.05$), and males also expressed higher durations for open mouth posture ($F_{1,22} = 5.989$, $P < 0.05$), flees ($F_{1,22} = 4.254$, $P < 0.05$), low-intensity aggressive behavior ($F_{1,22} = 7.153$, $P < 0.05$), and total aggressive behavior ($F_{1,22} = 4.498$, $P < 0.05$, Fig 1F). Trends, indicating higher levels in males compared to females, were observed for duration of total submissive behavior ($F_{1,22} = 3.571$, $P = 0.072$) and frequencies of flees ($F_{1,22} = 4.117$, $P = 0.055$), low-intensity aggression ($F_{1,22} = 3.649$, $P = 0.069$), total submissive behavior ($F_{1,22} = 3.766$, $P = 0.065$), and total aggressive behavior ($F_{1,22} = 3.084$, $P = 0.093$).

3.3 The expression of social and non-social behaviors changes across time

There was a significant effect of social interaction trials for three behavioral measures and trends toward significance for three others. Over time, the duration of nonsocial behavior significantly increased for all animals ($F_{2,44} = 8.702$, $P < 0.01$, Fig 1A); *post-hoc* comparisons revealed that nonsocial behavior durations for trials two and three were significantly higher compared to trial one ($p < 0.01$). Conversely, there was a significant decrease in two measures of social behavior over time: duration ($F_{2,44} = 6.303$, $P < 0.01$, Fig 1B) and frequency ($F_{2,44} = 7.287$, $P < 0.01$). *Post-hoc* comparisons revealed that, for duration of social behavior, trial one results were significantly higher compared to trials two and three ($P < 0.05$), and trial three results were significantly lower compared to both prior trials ($P < 0.05$); whereas, for social behavior frequency, trial one results were significantly higher compared to trial three ($P < 0.05$), but there was no difference between results of trials two and three. There was a trend toward an increase in the latency to engage in the first social behavior over time ($F_{2,28} = 2.791$, $P = 0.078$); conversely, trends toward decreasing over time were observed for frequency of a low-intensity aggressive behavior ($F_{2,44} = 2.332$, $P = 0.100$) and total aggressive behavior ($F_{2,44} = 2.594$, $P = 0.086$).

3.4 Sex and time interact to modify social, aggressive and submissive behaviors

Figure 2 illustrates sex differences in the changes of behaviors over the three social interaction trials, and table 2 provides information about significant interaction effects. There were significant sex by time interactions for two behavioral measures and trends for four others. For duration of social behavior, males were highly social in trial 1 compared to females, and the males' trial one duration was significantly higher compared to the durations for trials two and three, whereas females showed consistently lower social behavior durations across trials ($F_{2,44} = 3.820$, $P < 0.05$, Fig 1B). The change over time in the latency to the first attack showed an interesting sex difference: the latency for males decreased over the three trials, whereas it increased over trials in females ($F_{2,16} = 4.254$, $P < 0.05$, Fig 1E). A similar trend was observed for latency to the first submissive behavior, as the latency decreased over time in males, whereas it increased in females ($F_{2,14} = 2.895$, $P = 0.089$, Fig 1C). Duration of submissive behavior ($F_{2,44} = 2.344$, $P = 0.10$, Fig 1D) and frequency of submissive behavior ($F_{2,44} = 2.993$, $P = 0.060$) also showed trends toward a sex difference in the change of these measures over time: males' submissive behavior increased over time, whereas it decreased in females. There was also a trend detected for frequency of social behavior: males' social behavior appeared to decrease over time, whereas the frequency remained generally the same across trials in females ($F_{2,44} = 2.354$, $P = 0.10$).

3.5 Performance in olfactory habituation-dishabituation test

There was a significant main effect of stimulus presentation (over multiple trials) on the frequency of nose touches ($F_{8, 112} = 9.151$, $P < 0.00$, Fig 3A). *Post-hoc* comparisons revealed that the frequency for trial one (water) was significantly higher compared to the two subsequent water trials ($P < 0.01$). Frequency of nose touches was significantly higher for lime 1 (i.e., trial 4) compared to the previous trial (water 3; $P < 0.01$) and the two subsequent lime trials ($P < 0.05$). Frequency of nose touches was also higher for almond 1 (i.e., trial 7) compared to the previous trial (lime 3), although this difference only approached statistical significance ($P = 0.10$); and almond 1 was significantly higher compared to the two subsequent almond trials ($P < 0.05$). There was a significant main effect of stimulus presentation (over multiple trials) on the duration of nose touches ($F_{8, 112} = 6.392$, $P < 0.00$, Fig 3B). *Post-hoc* comparisons revealed that the duration for trial one (water) was significantly higher compared to the two subsequent water trials ($P < 0.01$). Duration of nose touches was significantly higher for lime 1 (i.e., trial 4) compared to the previous trial (water 3; $P < 0.05$) and to the subsequent lime trial (trial 5; $P < 0.01$). There was a trend toward a significant difference between lime 1 and lime 3 results (trial 6; $P = 0.09$). Duration of nose touches was also higher for almond 1 (i.e., trial 7) compared to the previous trial (lime 3) and lower than the subsequent almond trial (i.e., trial 8), although these differences only approached statistical significance ($P = 0.08$ and $P = 0.06$, respectively). The duration of nose touches for almond 1 was significantly higher compared to almond 3, the final task trial (i.e., trial 9; $P < 0.05$). There were no significant main effects of sex on the frequency ($F_{1,14} = 1.476$, $P = 0.245$) or duration ($F_{1,14} = 2.605$, $P = 0.129$) of nose touches, and there were no sex by stimulus presentation interactions for nose touch frequency ($F_{8,112} = 0.619$, $P = 0.761$) or duration ($F_{8,112} = 0.646$, $P = 0.737$).

3.6 Performance on individual (social) recognition test

There was a significant main effect of social stimulus presentation (over multiple trials) on the frequency of nose touches ($F_{3,18} = 12.685$, $P < 0.00$, Fig 3C). *Post-hoc* comparisons revealed that the frequency for trial four (novel stimulus) was significantly higher compared to the previous three social stimulus trials ($P < 0.05$); there were no significant differences among trials one, two, and three. There was a significant main effect of social stimulus presentation (over multiple trials) on the duration of nose touches ($F_{3,18} = 9.155$, $P < 0.001$, 3D). *Post-hoc* comparisons revealed that the duration of nose touch behavior for trial one was significantly higher compared to the duration for trial three ($P < 0.01$), whereas there was a trend toward a difference in results between trial one and trial two ($P = 0.10$). The duration for trial four (i.e., novel social stimulus) was significantly higher compared to the trial three duration ($P < 0.05$). There was no difference between trials two and three, nor was there a difference between trials one and four (novel social stimulus). There were no significant main effects of sex on the frequency ($F_{1,6} = 0.273$, $P = 0.620$) or duration ($F_{1,6} = 0.190$, $P = 0.896$) of nose touches, and there were no sex by stimulus presentation interactions for nose touch frequency ($F_{3,18} = 0.766$, $P = 0.528$) or duration ($F_{3,18} = 0.622$, $P = 0.610$).

4.0 Discussion

The pattern of expression of *Monodelphis* social behavior was, not surprisingly, different for each sex. In particular, submissive behaviors (i.e., defensive postures and flees) were more common in males, whereas females spent more time on nonsocial behaviors (i.e., investigating or exploring the cage, inactivity) compared to males. As reported by others [12, 13], male-male pairs showed higher levels of aggression than female-female pairs. As animals gained social experience, the expression of social behavior decreased whereas nonsocial behavior increased. Aggressive behavior also appeared to decrease over the course of the trials. Based on our previous work, we found that social status modulates the impact of social experience on social behavior in hamsters [6]. However, more research is needed to determine whether these factors also influence *Monodelphis* behavior.

As previously stated, sex modulated the effects of social experience on the expression of social behavior. Males engaged in higher levels of social behavior than females. As males gained social experience, their latency to attack significantly decreased, whereas the latency to attack increased in females; a similar trend was observed for submissive behavior. To our knowledge, this is the first report of a significant interaction between biological sex and social experience in a marsupial species, supporting the hypothesis that social experience modulates the expression of social behavior in a sex-dependent manner in *Monodelphis*.

Although most previous studies of *Monodelphis* social behavior have focused on aggression [12, 13], we found that both male and female *Monodelphis* also express submissive behaviors. However, an important caveat is needed on our findings. Given the experimental design we used, repeated exposure of the same animals to each other (the social aspect of the experiment) is invariably associated with repeated exposure to the same testing apparatus, such that the impact of the testing environment on aggression or other social behaviors cannot be independently tested or excluded. This is an important point to consider for future

investigations. Nonetheless, based on Fadem and colleagues' previous reports and our current findings, the social behavior of the *Monodelphis* has now been well characterized.

Social behavior has a long evolutionary history and is expressed by the vast majority of extant species, including microorganisms [30], and these behaviors confer reproductive and survival benefits [31]. Our findings are well aligned with descriptions of the social behaviors of the brush-tail possum (*Trichosurus Vulpecula*), a marsupial that is native to Australia, in the wild [32] and in captivity [33]. Indeed, *Trichosurus* display aggression that is similar to our findings and to those reported for *Monodelphis* by Fadem and colleagues [12, 13]. Likewise, *Monodelphis* avoidance behaviors, fleeing, and defensive postures were also similar with behaviors expressed by subordinate *Trichosurus* [32, 33]. Interestingly, the patterns of behavior expressed by marsupials of different social ranks [32, 33] are remarkably similar to the social behaviors of mice and rats [25], as well as hamsters [6, 15], suggesting a common regulatory mechanism for mammalian social behavior.

Our data show that *Monodelphis* detected changes in the environment, indicating that elemental learning took place [34], which supports the notion that non-associative learning is one of the simplest forms of learning that is present across species [35]. Non-associative learning processes such as habituation and dishabituation are vital in order to detect changes in the environment. By habituating to persistent stimuli, attention can be allocated to new or more relevant stimuli at that moment in time [36], while dishabituation allows an organism to refocus on a stimulus if needed. Documentation of non-associative learning in the opossum is important, as differences with other laboratory species have been pointed out for other common behaviors (e.g., locomotor activity in an open field) [37]. Associative learning has been described in the Virginia opossum, *Didelphis virginiana* [38], but this marsupial is quite different from *Monodelphis* in size and natural habitat. Learning and behavioral research about *Monodelphis* are relevant since the use of this model keeps increasing, mainly in biomedical research.

Laboratory rodents are able to detect conspecific chemosensory cues [39] and to develop olfactory-based memories of individuals of the same species [40]. In the present study, *Monodelphis* males and females demonstrated the same ability to recognize individuals, as evidenced by a significant decrease in nose touch duration following repeated exposures to the same individual (i.e., habituation) and dishabituation when a novel individual was presented. Interestingly, the nose touch frequency data suggest that repeated exposures to the same individual significantly enhance nose touch frequency when subjects are exposed to a novel individual (i.e., sensitization). A previous study reported that *Monodelphis* males and females spend more time investigating opposite-sex, conspecific odors relative to neutral (control) odors [41]. In addition, another study reported that *Monodelphis* males spend more time investigating the odor of an unfamiliar same-sex conspecific compared to the odor of a familiar stimulus male, but females were not tested [23]. Our findings are consistent with the results reported by Ferguson, Young [29]. Specifically, the authors reported that mice are able to hold a social memory for ten minutes, and our data suggest that *Monodelphis* males and females are also able to hold a social memory for at least that long.

In summary, we identified sex differences in social behavior in *Monodelphis* and found that males and females demonstrated non-associative learning abilities in social and non-social contexts. Social behavior, olfactory-based learning, and individual (social) recognition are critical for intraspecific communication and relationship formation in animals [1], and identifying the factors that influence these behaviors may lead to the development of treatments for psychopathologies that involve social dysfunction. Characterizing the behavior of the *Monodelphis* is an important first step in developing this marsupial species as a (preclinical) model of complex neuropsychiatric disorders. Furthermore, in addition to rodents, the laboratory opossum may be an effective model for studies on social dominance and subordination.

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Highlights

- Biological sex and social experience modulated the expression of social behavior in the adult gray short-tailed opossum (*Monodelphis domestica*)
- Males displayed higher levels of aggression, submissive behavior, and open-mouth posture compared to females; females spent more time displaying nonsocial behaviors.
- Social isolation stimulated social interest/investigation and delayed the first attack in males, whereas social experience decreased attack latency in males and increased attack latency in females.
- Both males and females habituated and dishabituated to neutral odors and conspecific social stimuli, confirming non-associative learning abilities that are similar to rodents.
- *Monodelphis*, a marsupial species, has great potential to serve as a (preclinical) model of complex neuropsychiatric disorders that are associated with social dysfunction.

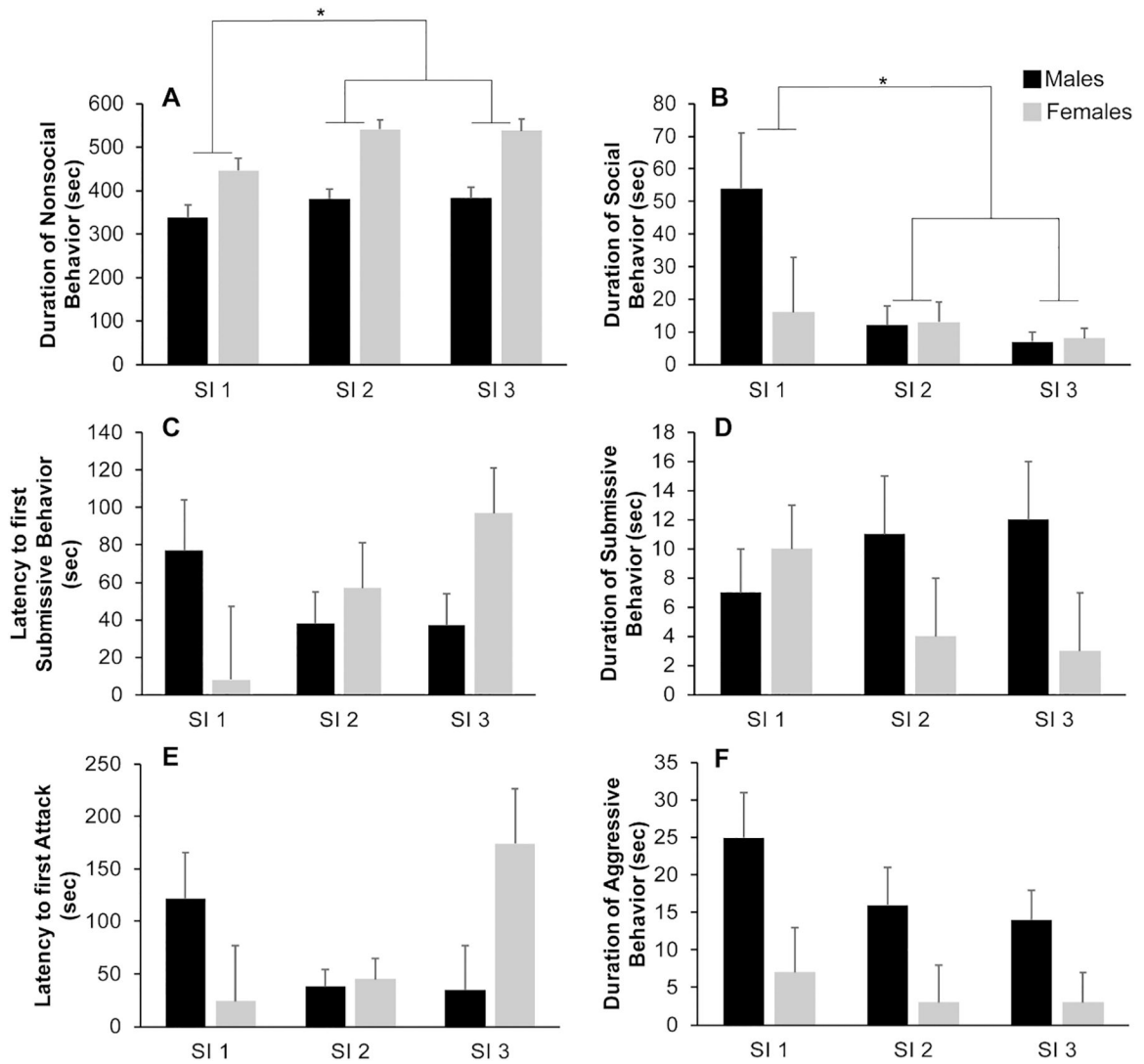


Fig 1. Changes in behavior in male and female opossums over three 10-minute social interaction trials. Duration of nonsocial behavior significantly increased over trials and females demonstrated significantly more nonsocial behavior compared to males (A). Duration of social behavior significantly decreased over trials, an effect that was driven by the males' high levels of social behavior in trial one compared to other trials (B). Males' submissive behavior latency decreased over trials, whereas the females' latency increased (C). Males' duration of submissive behavior increased over trials, whereas the females' duration decreased (D). Males' attack latency decreased over trials, whereas the females' latency increased; there was a significant sex difference (E). Males' duration of aggressive behavior was significantly higher compared to females (F). SI, social interaction trial. Table 2 provides information about significant interaction effects. Values are given as mean (+SEM). * $P < 0.05$.

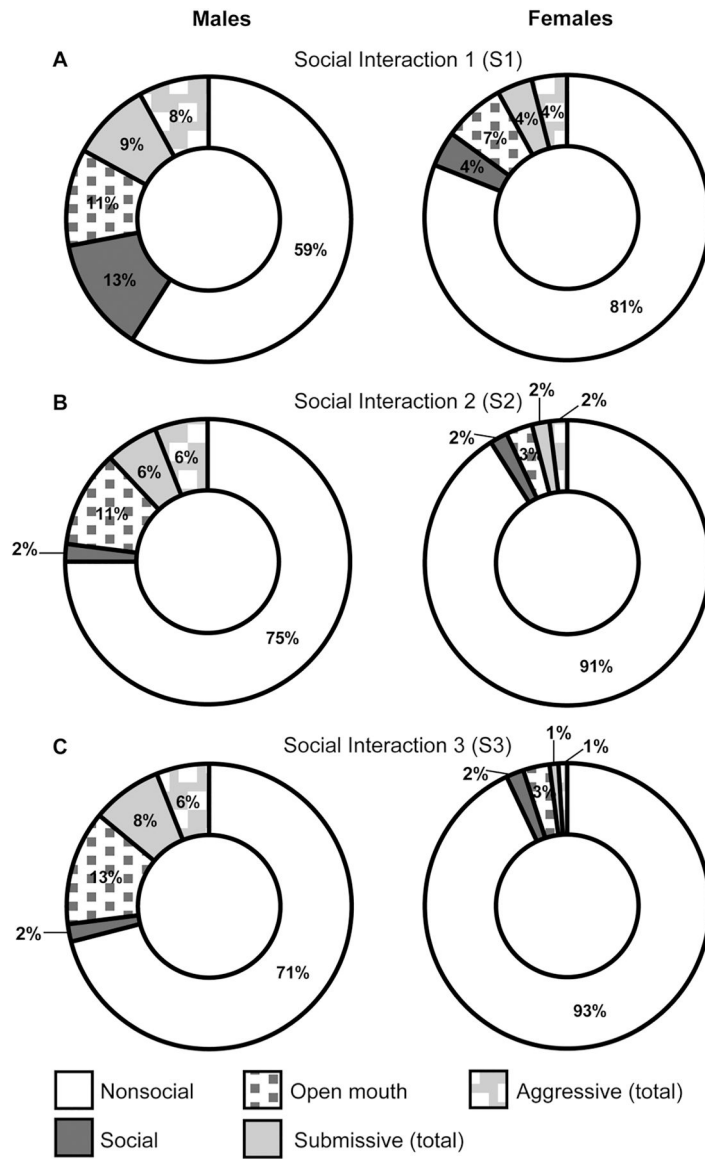


Fig 2. Behavioral time allocation for the following behaviors: nonsocial, social, open mouth posture, submissive behavior, and aggressive behavior. Data presented as percentages for males and females tested over three consecutive days. S, social interaction trial.

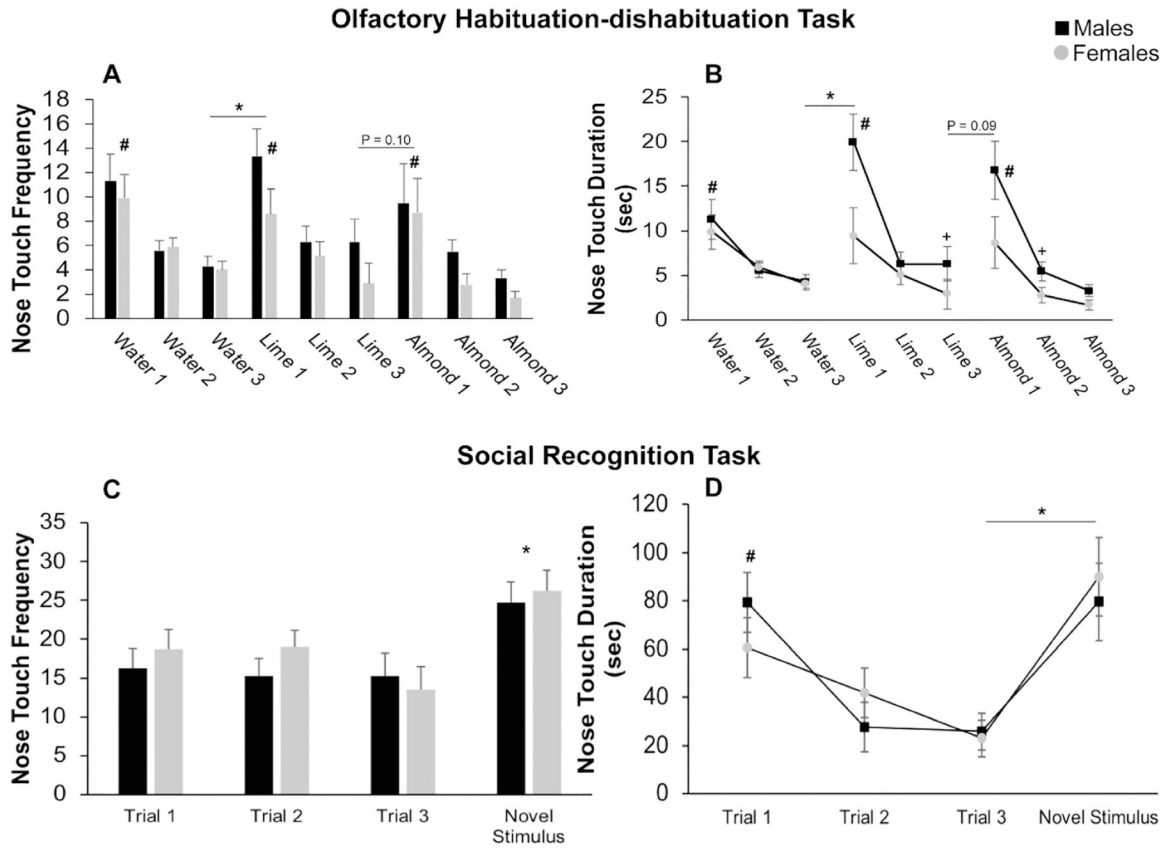


Fig 3. Male and female opossums demonstrated non-associative learning abilities, similarly to rodents. Animals habituated to three different olfactory stimuli (water, lime, and almond) and showed dishabituation when a new stimulus was presented (A, B). Frequency of nose touches remained unchanged when animals were presented with the same social stimulus over three consecutive 3-minute trials; however, animals showed a sensitized response when presented with a novel social stimulus on the fourth trial (C). Social stimulus nose touch duration followed the same pattern that was observed for nonsocial olfactory stimuli: animals habituated to the same social stimulus over three trials and dishabituated when a novel social stimulus was presented on the fourth and final trial (D). Values are given as mean (+/- SEM). * $P < 0.05$; # denotes significant difference between trial 1 and subsequent trials within each stimulus category; + denotes a trend toward a difference between either trial 2 or 3 and the first trial within each stimulus category ($P < 0.10$).

Table 1.*Monodelphis domestica* ethogram

Behavior	Definition
Non-social	Locomotor and exploratory activity (investigating the environment, often while sniffing; moving from one area to another)
	Vertical movements (climbing)
	Digging and manipulating objects (bedding material)
	Stationary (sitting, grooming, standing in one place)
Social	Investigating, often while sniffing, of the stimulus animal
	Locomotor activity and attention focused on the stimulus animal (following and/or circling behavior while in close contact)
	Physical contact (touching)
Open mouth posture	Opening mouth while stationary, usually oriented toward stimulus animal
Submissive	Rearing upright with forepaws extended (defensive posture, usually in response to stimulus animal)
	Locomotor activity and running away from stimulus animal (avoiding, fleeing)
	Assuming a supine posture (sometimes pinned to the ground by stimulus animal)
Low-intensity aggression	Rearing upright and offensive "pushing" or grabbing the fur of the stimulus animal
	Running behind and chasing the stimulus animal
	Pinning stimulus animal to the ground or against the side of the apparatus
High-intensity aggression	Chasing that leads to a rolling/tumbling fight or lunging toward stimulus animal and biting (attack)

An ethogram is a list of species-typical behaviors. This table describes the social behaviors of the *Monodelphis* in the context of an agonistic encounter between same-sex partners.

Table 2.

Effects of biological sex and social experience on individual social behaviors.

Parameter	Effect of biological sex	Effect of social experience (trials)	Interaction
(A) Nonsocial behavior duration	$F_{1,22} = 24.482$, P < 0.01	$F_{2,44} = 8.702$, P < 0.01	$F_{2,44} = 1.34$, n.s.
(B) Social behavior duration	$F_{1,22} = 1.093$, n.s.	$F_{2,44} = 6.303$, P < 0.01	$F_{2,44} = 3.820$, P < 0.01
(C) Submissive behavior latency	$F_{1,7} = 0.029$, n.s.	$F_{2,14} = 0.447$, n.s.	$F_{2,14} = 2.895$, P = 0.08
(D) Submissive behavior duration	$F_{1,22} = 0.319$, n.s.	$F_{2,44} = 0.030$, n.s.	$F_{2,44} = 2.344$, P = 0.10
(E) Attack latency	$F_{1,8} = 0.238$, n.s.	$F_{2,16} = 1.215$, n.s.	$F_{2,16} = 4.254$, P < 0.01
(F) Low-intensity aggressive behavior duration	$F_{1,22} = 7.153$, P < 0.01	$F_{2,44} = 2.004$, n.s.	$F_{2,44} = 0.427$, n.s.

This table provides a summary of results of the statistical analyses. F values are provided for each main effect and for the sex x social experience interaction.