Dopamine receptor activation elicits a possible stress-related coping behavior in a songbird

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Animals experience stress throughout their lives and exhibit both physiological and behavioral responses to cope with it. The stress response can become harmful when prolonged and increasing evidence suggests that dopamine plays a critical role in extinguishing the stress response. In particular, activation of the D2 dopamine receptor reduces glucocorticoids and increases coping behavior, which are behavioral responses to adverse stimuli that reduce the harmful effects of stress. However, few studies have examined the effects of dopamine on the stress responses of wild species. We therefore tested the hypothesis that activation of the D2 dopamine receptor influences stress-related coping behavior in a wild-caught species. We recorded behavior of house sparrows (*Passer domesticus*) before and after they received injections of D2 dopamine agonists, D2 dopamine antagonists, or saline. We found that the birds significantly increased biting of inanimate objects after the agonist but there was no change following the antagonist or saline. The biting may be a mechanism of behavioral coping. This change in behavior was not correlated with general movement. This study supports the hypothesis that D2 dopamine receptor activation is involved in the regulation of the stress response in a wild bird.

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Abstract

Animals experience stress throughout their lives and exhibit both physiological and behavioral responses to cope with it. The stress response can become harmful when prolonged and increasing evidence suggests that dopamine plays a critical role in extinguishing the stress response. In particular, activation of the D2 dopamine receptor reduces glucocorticoids and increases coping behavior, which are behavioral responses to adverse stimuli that reduce the harmful effects of stress. However, few studies have examined the effects of dopamine on the stress responses of wild species. We therefore tested the hypothesis that activation of the D2 dopamine receptor influences stress-related coping behavior in a wild-caught species. We recorded behavior of house sparrows (*Passer domesticus*) before and after they received injections of D2 dopamine agonists, D2 dopamine antagonists, or saline. We found that the birds significantly increased biting of inanimate objects after the agonist but there was no change following the antagonist or saline. The biting may be a mechanism of behavioral coping. This change in behavior was not correlated with general movement. This study supports the hypothesis that D2 dopamine receptor activation is involved in the regulation of the stress response in a wild bird.

Introduction

Animals experience stress – a real or perceived threat to homeostasis – throughout their lives. This stress has the potential to negatively impact their survival and reproduction (Chrousos, 2009). Stressors such as predator encounters, conflicts with social competitors, and harsh weather can threaten survival through a reduction in foraging success and a weakening of the immune system which can result in death (Brown & Kotler, 2004; Lange & Leimar, 2004; Pravosudov et al., 2001; Cirule et al., 2012). The presence of stressors can also suppress reproduction and result in the abandonment of dependent offspring (Love et al., 2004). Because stress can significantly reduce fitness, mechanisms have evolved to physiologically and behaviorally cope with it.

When a stressor is perceived, it triggers the activation of the hypothalamic-pituitary-adrenal (HPA) axis, beginning a hormonal cascade that ultimately releases glucocorticoids into the bloodstream (Sapolsky et al., 1986). Elevated glucocorticoids, such as cortisol and corticosterone, disrupt normal functions and shifts the individual into an 'emergency life history stage' (Wingfield et al., 1998). This stage can temporarily suppress the immune system (Shini et al., 2010), mobilize energy stores, and modify behavior to prioritize survival. Behavioral modifications in this stage can include increasing anti-predator behaviors (Thaker et al., 2009), decreasing parental care, and increasing group coordination (Raulo & Dantzer, 2018).

Although physiological and behavioral stress responses are often beneficial during stress, they can become harmful when prolonged. This can occur when either the stressor is chronic or because the stress response persists after the stressor is gone. Prolonged activation of the stress response can cause physiological problems (e.g. metabolic dysfunction and impaired reproduction (Wingfield & Sapolsky, 2003; López et al., 2018)) as well as cognitive issues (e.g. impaired memory and decision making (Voellmy et al., 2014; Aisa et al., 2007)). The ability to efficiently extinguish the stress response is important for resilience against the negative physiological and behavioral effects of prolonged stress (Romero et al., 2010; Vitousek et al., 2019; Zimmer et al., 2019).

Growing evidence indicates that dopamine plays an important role in extinguishing the stress response (Cabib & Puglisi-Allegra, 2012; Sullivan & Dufresne, 2006). Dopamine concentration increased in the nucleus accumbens, striatum, and medial frontal cortex of rodents exposed to a physiological stressor (Abercrombie et al., 1989). Activation of one dopamine receptor in particular, the D2 receptor, has been shown to increase with stress and may mediate coping behaviors (Cabib & Puglisi-Allegra, 2012; Lattin et al., 2019), which are behavioral responses to adverse stimuli that reduce the harmful physiological effects of stress (Schouten & Wiepkema, 1991). Examples of behaviors in some mammals and birds that can contribute to stress coping include grooming, freezing, and biting at inanimate objects (Giorgi et al., 2003; Henson et al., 2012; Reis-Silva et al., 2019; Koolhaas et al., 1999; Hori et al., 2004; Savory et al. 1992). Some of these behaviors can appear to be purposeless during stressful situations and have been described as stereotypies (Dantzer, 1991). Stereotypy can be a sign of behavioral pathology and welfare problems; however, there is some evidence that stereotypy is a form of coping with inescapable stress (Mellor et al., 2018). Behaviors in response to stress can be crucial to regulating arousal levels and maintaining homeostasis by regulating glucocorticoids following a disturbance such as a stressor. Some animals that exhibit stereotypic coping behaviors have reduced glucocorticoids when they are exposed to a stressor (Sato et al., 2010; Kostal et al., 1992). In addition to altering stress related hormones, coping behaviors also impact other markers of stress including reducing the occurrence of stomach lesions (Koolhaas et al., 1999). Some of these coping behaviors are dependent on dopamine in the mesostriatal dopamine region in the brain (Jones et al., 1989) and blocking D2 dopamine receptors during stress results in elevated post-stress glucocorticoids and increased stress-related health problems (Sullivan & Dufresne, 2006; Puri et al., 1994; Sullivan & Szechtman, 1995).

Our current understanding of dopamine's role in animal's behavioral response to stress largely comes from studies on domesticated and laboratory animals (Baik, 2020). The effects of stress have been shown to vary between wild and domestic animals, even in the same species, therefore it may not be possible to generalize these findings (Cabezas et al., 2013). There have so far been few studies investigating dopamine's effect on behavioral responses to stress in wild species. In wild house sparrows

(*Passer domesticus*), increasing dopamine receptor activation increased preening, a possible stress-related coping behavior (Lattin et al., 2019; Henson et al., 2012). In contrast, increasing dopamine receptor activation in crayfish had no effect on behavior after exposure to a stressor (Fosset et al., 2015). Further research is therefore required to understand dopamine's role in stress coping in wild animals.

The aim of this study was therefore to test the hypothesis that activation of the D2 dopamine receptor influences stress-related coping behaviors in a wild species. We tested this hypothesis using wild-caught house sparrows, a songbird species that is a model system for studying bird behavior and physiology (Hanson et al., 2020). Similar to the impact of D2 activation on the coping behavior of laboratory and domesticated animals (Sullivan & Dufresne, 2006; Puri et al., 1994; Cheng et al., 2003; Dennis et al., 2006), we predicted that increasing D2 receptor activation would increase coping behavior and blocking D2 receptor activation would decrease coping behavior. To test these predictions, we peripherally administered selective D2 dopamine agonists and antagonists to manipulate the activation of dopamine receptors in the brains of house sparrows.

Materials & Methods

Animals

Twenty adult male house sparrows were captured with baited traps between February and May of 2019 in College Station, TX. Sample size was similar to those in a previous study (Balthazart et al. 1997). The birds were housed in randomly-assigned pairs in cages (0.6 x 0.33 x 0.3 m) at Texas A&M University (30°36'N, 96°21'W) in an indoor room ('housing room': 5 x 6.3 m). They were kept on a 13h:11h light:dark cycle at 24.0 ± 0.5 °C (mean ± SD). They were individually marked with metal and colored leg bands. The pairs were given at least seven days to acclimate to captivity and their cagemate before being tested. Water and food were available *ad libitum*. The study was approved by Texas A&M University's Animal Care and Use Committee (IACUC#2019-0219).

Experimental procedure

During each trial, a pair of birds (consisting of a focal bird and non-focal bird; the initial designation of birds as the focal or non-focal bird was randomly assigned within each pair) was transported within its

cage from the 'housing room' to an adjacent room (2.2 x 2.6 m) that contained a sound attenuation chamber (1.12 x 0.67 x 0.57 m). The pair, within its cage, was placed within the middle of this chamber, which was visually and acoustically isolated from the other captive birds in the 'housing room'. For 30 minutes, the birds' behavior was recorded using two video cameras (VIXIA HF R70; Canon Inc.) positioned on each side of the chamber. After the 30 minutes, the experimenter (MRF) briefly removed the focal bird from his cage and intramuscularly injected 0.05 mL of a drug treatment or control into his breast. An experimenter monitored the birds throughout their time in the chamber to ensure there were no signs of pain or adverse reactions from injection. The drug treatment consisted of a D2 agonist (PPHT; 1 mg/kg) or a D2 antagonist (raclopride; 10 mg/kg; Santa Cruz Biotechnology, Dallas, TX); the control consisted of 0.9% saline. Both drugs were dissolved in 0.9% saline. The doses were chosen to be high enough to insure they produced a behavioral effect and were the same as those used in previous avian studies (Balthazart et al., 1997; Zawilska et al., 1996); similar to these previous studies, all of the birds also received the same amounts of the drug treatment or control (the doses were calculated using 28g as the average weight of the birds). Immediately following the injection, the focal bird was returned to his cage for another 30 minutes and their behavior was recorded. Once injected, the drugs were likely taken up into the brain rapidly (within 2 minutes in Sprague-Dawley rats (Mukherjee et al., 2004)).

Each focal bird was tested in three separate trials in which he was administered either the D2 agonist, D2 antagonist, or saline (the order of the drug treatments and control was randomized across birds using a random number generator). Two days after the focal bird's trials were completed, the above experimental procedure was repeated except that the designation of the birds reversed: the previously non-focal bird in the pair was designated as the focal bird while the previously focal bird became the non-focal bird. Because the half-life of raclopride and PPHT are both estimated to be 30 minutes (Mukherjee et al., 2004; Köhler et al., 1985), two days between trials ensured that the drugs were eliminated from the body between trials. All six trials of a given pair were conducted within 95 days of the birds' capture from the wild (mean ± SD: 31 ± 25 days) and between the hours of 0800 and 1300. No data from the trials were excluded. Birds were released to their capture site at the conclusion of the study.

Behavioral analysis

The behavior of the focal birds was scored from the video recordings (QuickTime; version 7; Apple Inc.). During a 10-minute period preceding the injection as well as a 10-minute period following the injection, we recorded the amount of time the focal birds spent biting inanimate non-food objects (including the cage, perches, or their own leg bands) with their bill (henceforth 'biting'). In some species, biting is a coping behavior that can reduce stress associated with physical restraint, food restriction, or unfavorable environmental conditions (Cabezas et al., 2013; Fossat et al., 2015; Hanson et al., 2020). During the 10-minute period following the injection, we also scored the amount of time the focal birds engaged in intraspecific aggression (pecking or biting the non-focal bird), preening (cleaning feathers with the bill or feet), bill wiping (rubbing bill on perch), feather ruffling (fluffing up and shaking feathers) and general movement (hopping or flying around the cage). Behaviors were scored by recording the frames at which the behaviors began and ended, and then calculating the number of seconds they spent engaging in those behaviors. One experimenter (MRF) scored all of the videos and was blinded to the treatment.

Statistical analysis

We performed linear models with pair identity as a random effect using the package 'stats' in R, Version 3.6.2 (R Core Team, 2019). The dependent variable was the number of seconds each behavior was performed after injection with the treatment/control. The independent variables were the treatment/control (D2 agonist, D2 antagonist, or saline), percentage of the time that the bird engaged in each behavior before the treatment/control, percentage of time the focal bird engaged in general movement after the treatment/control (log transformed to meet underlying assumptions of normality), and trial order (order in which the D2 agonist, D2 antagonist, or saline were administered). Feather ruffling and bill wiping were both rare behaviors, occurring on average less than 2% and 1% of the time each trial respectively and therefore were not included in the analysis. For any model in which treatment/control type was significant we then performed three pairwise comparisons to evaluate differences among the

drug treatments and control using the R package 'emmeans'; we used a Bonferroni correction to evaluate statistical significance. Raw data is available in the supplemental information.

Results

The effect of the drug treatment significantly increased the time the birds spent engaged in biting behavior (n=20, F=53.27, p = 2.07e-13; Table 1; Fig. 1). The D2 agonist increased the amount of time the birds spent biting compared to the control (n=20, F = -9.42, n = 20, $p < 0.001$; Table 2) and D2 antagonist (n=20, t = 9.67, n = 20, $p < 0.001$; Table 2). The amount of time the birds spent biting was similar when the birds were injected with the D2 antagonist and control (n=20, t = -0.45, n = 20, p = 0.99; Table 2). Biting behavior was also unrelated to amount of general movement (n=20, F = 0.01 p = 0.96; Table 1; Fig. 2). Treatment had no effect on time spent on movement, preening, or aggression (n=20, F = 2.09, p = 0.13; n=20, F = 1.88, p = 0.16; n=20, F = 1.31, p = 0.28; Table 1).

Discussion

We found possible support for the hypothesis that dopamine influences stress-related coping behavior in a wild-caught species. In particular, the house sparrows spent more time biting at inanimate objects when they were administered the D2 agonist. Biting at inanimate objects may be a coping mechanism in some bird species (Savory & Kostal, 1993; Nicol, 1987; Zarrindast et al., 1992). The amount of time house sparrows spent biting did not decrease when they were administered the D2 antagonist, although this is not surprising as they rarely exhibited biting prior to the drug administrations. Biting behavior also did not increase when administered a saline control, indicating that the behavior is unlikely to be a response to stress induced by handling and injection. Furthermore, increases in dopamine receptor activation are often associated with increases in general movement (Beninger, 1983). However, even after controlling for general movement, we still found that the house sparrows spent more time biting at inanimate objects when administered the D2 agonist. The D2 agonist and antagonist were likely binding to dopamine receptors in the mesolimbic and nigrostriatal pathways within the brain where there are high concentrations of dopamine receptors in this species (Lattin et al. 2019).

In rodent models as well as poultry, environmental stressors elevate dopamine levels in the mesolimbic system (Cabib & Puglisi-Allegra, 1996; Cheng et al 2003). It is therefore possible that the activation of D2 dopamine receptors by the agonist induced neurochemical changes consistent with stress in the house sparrows and the birds behaviorally coped with this perceived stress by biting at inanimate objects. Coping behaviors are de-arousal mechanisms that work to restore homeostasis after a disturbance (Savory & Kostal, 2006). Domesticated chickens (*Gallus gallus*) bite at inanimate objects when they experience stressful conditions such as high densities and low temperatures (Spinu, 2003). This suggests that the biting behavior we observed in the house sparrows could be similar to this coping behavior in chickens. Coping behaviors may restore homeostasis because of their effect on the stress-related hormone corticosterone. In chickens, individuals that engaged in more stereotypic pecking at inanimate objects had lower corticosterone levels (Kostal et al., 1992). In rodents, individuals that were allowed to bite at inanimate objects during stressful situations such as restraint and introduction into a new environment also have lower levels of cortisol and corticosterone, respectively (Sato et al., 2010; Hennessy & Foy, 1987). When dopamine neurons are destroyed in rodents, they stop biting at inanimate objects and have elevated corticosterone levels relative to controls during stress (Jones et al., 1989). Future studies are needed to determine whether the observed biting behavior also impacts corticosterone levels in wild songbirds.

Not all behaviors in response to stress are a way to cope, some can simply be an outward expression of anxiety and have no effect at restoring homeostasis. Despite the potential benefit of biting during stress in birds and rodents the behavior observed in this study may be an expression of anxiety or drug induced stereotypy (Mellor et al., 2018; Sato et al., 2010; Hennessy & Foy, 1987; Zarrindast et al, 1992). Intense stereotypic pecking was observed in Japanese quail (*Coturnix japonica*) injected with PPHT, a D2 dopamine agonist, which may be a drug induced compulsion (Balthazart et al., 1997). Studies in rodents have also recognized the link between dopamine agonists and stereotyped behavior with no obvious function (Costall et al., 1977; Arnt et al., 1988) or that stereotyped behavior can be a result of neurological abnormalities (Shafiq-ur-Rehman, 1991).

It is unlikely that the house sparrows exhibited increased biting because they were hungry. Although the birds did not have access to food during any of the trials, they only increased their biting after administration of the D2 agonist but not the D2 antagonist or saline. Rodents and Japanese quail also increased their biting behavior after they were given a D2 agonist, but they did not increase their food consumption even though food was available (Nicol, 1987; Beninger, 1983). This suggests that biting induced by D2 receptor activation is not related to an increased motivation to feed.

We also did not find support for the possibility that the birds increased biting because they became more aggressive. There was no relationship between biting inanimate objects and intraspecific aggression. Furthermore, the house sparrows exhibited very low levels of aggression across all trials. Similarly, manipulating dopamine receptor activation in group-housed chickens did not influence aggression towards conspecifics (Dennis et al., 2006). These results indicate that the D2 receptors are not involved in aggression among familiar conspecifics, but further experiments could examine if this is also the case among unfamiliar conspecifics that are more likely to engage in aggression (Hegner & Wingfield, 1987).

We found that the D2 antagonist did not influence biting in house sparrows. Because the birds rarely exhibited biting before the D2 antagonist treatment, there was little opportunity for the behavior to become even less frequent. In fact, only two birds exhibited biting before the D2 antagonist injection and only one bird exhibited biting after the D2 antagonist injection. D2 antagonists may have greater influence on individuals that are actively stressed. A different dopamine antagonist administered to rats only have an impact on behavior after multiple treatments (Ikemoto & Panksepp, 1996). When chickens are chronically stressed, D2 antagonists reduced the amount of time they spent biting at inanimate objects (Savory & Kostal, 1993). Additional studies could examine whether D2 antagonists likewise decrease stress in songbirds when their stress levels are high.

Conclusions

We found that an increase in D2 dopamine receptor activation in house sparrows leads to increased biting of inanimate objects. Because biting behavior may be a coping behavior to reduce stress (Savory & Kostal, 1993; Nicol, 1987), our results could indicate that dopamine is involved in songbirds' stress response. However, the observed behavior may instead be an expression of stereotypic behavior. D2 dopamine receptor activation alters biting behavior in domesticated birds (Dennis et al., 2006; Savory & Kostal, 1993; Zarrindast et al., 1992), indicating that the dopamine pathways between these domestic species and the wild songbird in this study are likely similar. While many studies have examined the influence of hormones on the stress response (Schoech et al., 2011; Creel et al., 2013), our understanding of how neurotransmitters impact stress in wild species is still poor (Lattin et al., 2019; Trainor, 2011). To further understand this question, research on the effect of dopaminergic drugs on birds that were socially or physiologically stressed compared to unstressed controls would be valuable. It would also be interesting to look at the effects of different dosages of these drugs, to see if the behavioral effects were specific to high dosages such as reported by Balthazart and colleagues (1997). Wild species are increasingly exposed to stress due to human disturbances (Birnie-Gauvin et al., 2016), and a greater understanding of their stress responses will be crucial to evaluating how these organisms manage these challenges.

Acknowledgements

We would like to thank Margaret Guy for her assistance in performing the experiment. We would also like to thank Drs. Jeffery Tomberlin, Sarah Hamer, and Gil Rosenthal for feedback on an earlier version of this manuscript.

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Table 1(on next page)

Pairwise comparisons between treatment/control types for the biting behavior model.

Statistical significance is indicated with an asterisk.

1

	Numerator df, denominator df	t-ratio (p-value)
Comparisons		
Control vs. Agonist	1, 45.1	-9.42 (<0.001)*
Control vs. Antagonist	1, 45.5	-0.45 (0.99)
Agonist vs. Antagonist	1, 45.3	9.67 (<0.001)*

2

3

Figure 1

Frequency of behavior before and after treatments.

Mean time (seconds) the focal birds (n = 20) spent on biting (A), movement (B), preening (C), and aggression (D) before and after injection with one of the drug treatments or control. Error bars depict the standard error of the mean and asterisks indicate statistically significant differences.

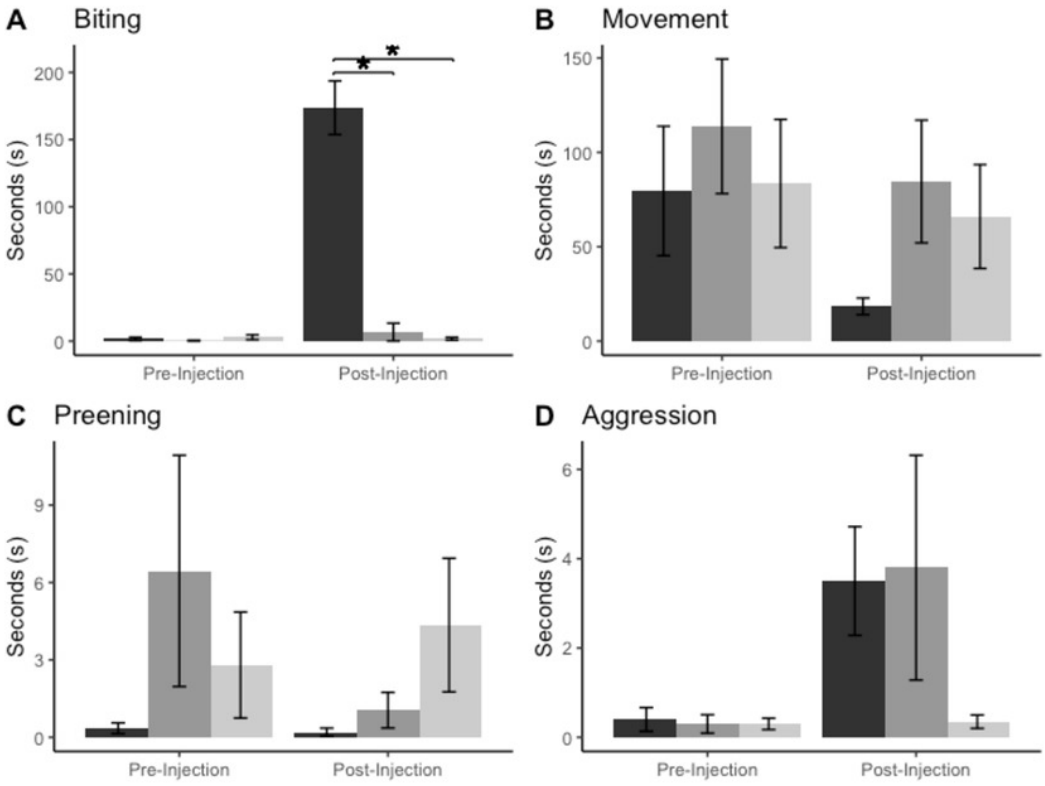
A
Biting
Seconds (s)
200
150
100
50
0
*
*
Pre-Injection
Post-Injection
B
Movement
Seconds (s)
150
100
50
0
Pre-Injection
Post-Injection
C
Preening
Seconds (s)
9
6
3
0
Pre-Injection
Post-Injection
D
Aggression
Seconds (s)
6
4
2
0
Pre-Injection
Post-Injection

Figure 2

Relationship between time (seconds) spent biting and general movement for each treatment/control.

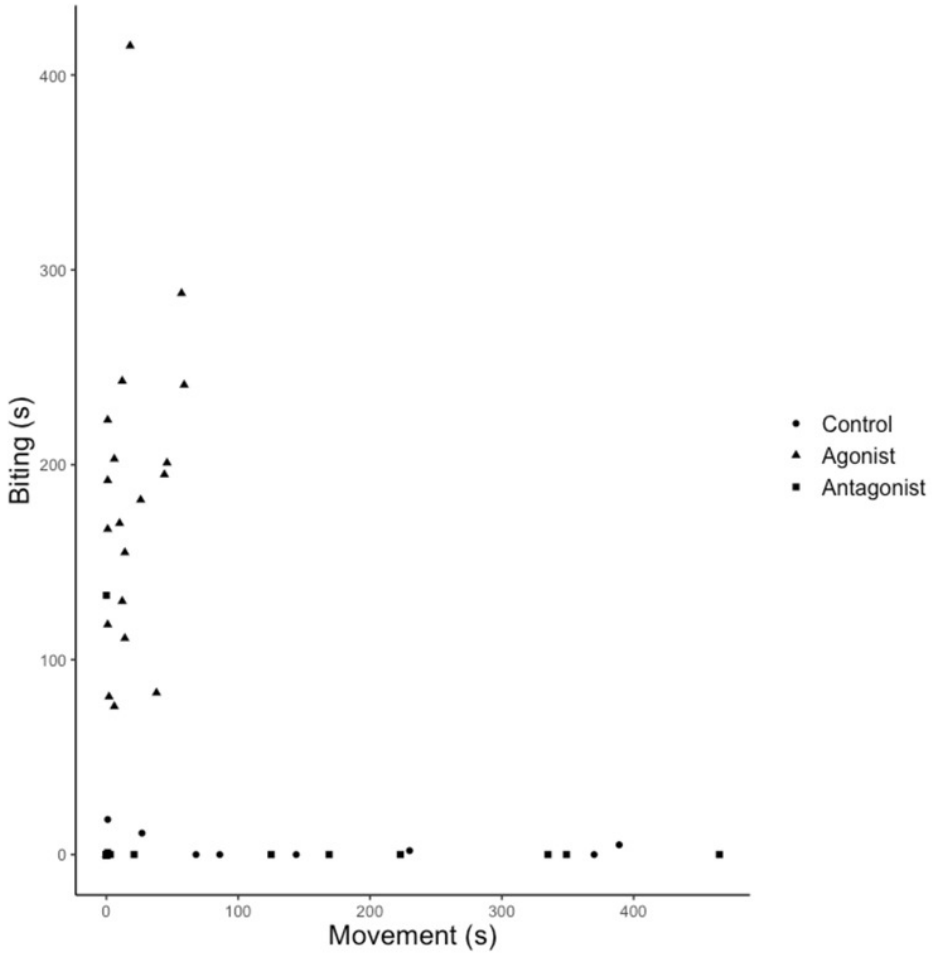
Biting (s)
400
300
200
100
0
0
100
200
300
400
Movement (s)
Control
Agonist
Antagonist

Table 2(on next page)

Results of the models for biting, general movement, preening and aggression

Statistical significance is indicated with an asterisk.

1

Response Variable	Independent variables	Numerator df, denominator df	F-value (p-value)
Biting after treatment/control			
	Treatment/control	2, 54.0	53.27 (2.7e-13)*
	Biting before treatment/control	1, 54.0	1.65 (0.20)
	Trial Order	2, 54.0	0.63 (0.53)
	General movement before treatment/control	1, 54.0	0.01 (0.96)
General movement after treatment/control			
	Treatment/control	2, 54.0	2.09 (0.13)
	General movement before treatment/control	1, 54.0	37.76 (9.9e-08)*
	Trial order	2, 54.0	0.60 (0.55)
Preening after treatment/control			
	Treatment/control	2, 54.0	1.88 (0.16)

	Preening before treatment/control	1, 54.0	0.27 (0.61)
	Trial order	2, 54.0	1.35 (0.27)
Aggression after treatment/control			
	Treatment/control	2, 54.0	1.31 (0.28)
	Aggression before treatment/control	1, 54.0	2.00 (0.16)
	Trial order	2, 54.0	0.08 (0.92)

2