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The Use of Guanfacine to Mediate Anxiety-related Reactivity and Reduce Associated Agonistic Behavior in Two Pigtail Macaques (*Macaca nemestrina*)

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Guanfacine, an α_2 adrenoceptor agonist, has been used to successfully treat self-injurious behavior in nonhuman primates, including macaques (*Macaca mulatta*) and baboons (*Papio anubis*). It does so by facilitating a correction to the dopaminergic system that mediates a reduction in impulsivity and reactivity. Given this, we assessed the potential efficacy of guanfacine to treat socially directed agonistic behavior in primates with an apparent reactive behavioral phenotype. We present data from 2 pigtail macaques (*Macaca nemestrina*): an intact adult male housed in a breeding group, and an experimentally naive adult female living in a research setting with her social partner. Baseline behavioral assessments suggested that both macaques showed extreme responses to external stressors that triggered them to aggress social partners often leading to wounding that required veterinary intervention. Both animals were tracked during the course of 1 y. Once treated regularly with guanfacine, both animals showed significant reduction in their agonistic behavior and the rate at which they wounded other animals. Indeed, in the year since the female has been treated with guanfacine she has never wounded her cagemate. By collecting regular and detailed behavioral observations on the male in the breeding colony, we were able to identify triggers for his aggression and to track the behavioral changes evidenced after guanfacine treatment. These data supported our hypothesis that his aggression reflected extreme reactivity to external stressors, rather than general anxiety. Importantly, we saw only a limited and short-lived reduction in the male's affiliative behavioral rates, and thus guanfacine had no sedative effect, but did successfully reduce his reactivity and resultant agonism and wounding.

Abbreviations and Acronyms: ADHD, attention-deficit/hyperactivity disorder; ANOVA, analysis of variance; CD, conduct disorder; GLMM, general linear mixed model; IM, intramuscular; ODD, oppositional defiant disorder; PO, per os (by mouth); SIB, self-injurious behavior; SID, once a day.

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Introduction

Guanfacine is an α_2 adrenoceptor agonist that moderates prefrontal cortex activation associated with the coordination of executive functioning. Guanfacine appears to drive a correction to the dopaminergic system and has been shown to mediate a reduction in impulsivity.[16] It is effective in treating symptoms of attention-deficit/hyperactivity disorder (ADHD) and in reducing hyperactivity, impulsivity, and distractibility in human patients with autism.[2,3,15] In nonhuman primates (*Macaca mulatta*), guanfacine has been shown to improve the efficiency of working memory and other prefrontal functions, highlighting the interplay between impulsive choice behaviors and prefrontal function.[4,8] Following the successful application of guanfacine in clinical contexts with human patients, and its ability to mediate impulsivity in nonhuman primates, guanfacine has been prescribed to treat self-injurious behavior (SIB) in nonhuman primates, including rhesus macaques (*M. mulatta*) and baboons (*Papio anubis*).[7,10]

While wounding related to SIB is a serious concern for those managing nonhuman primate populations,[9] wounds relating from social interactions are also a common consideration when forming and maintaining social pairs or groups. In nonhuman primates, some aggressive interactions represent species-typical behaviors, reflecting the mechanisms by which dominance hierarchies and rank relationships are established and maintained,[11] and most concerns about the risks of social wounding are overstated.[5] However, various extrinsic and intrinsic factors influence macaque wounding rates,[6,17] and some individuals show excessive agonism toward groupmates. If cases of extreme aggression are driven by anxiety-related reactivity, we theorized that guanfacine may offer a viable treatment.

For certain human patients, aggression, defined as "verbal or physical acts that are reactive or impulsive in nature," might derive from a comorbid psychiatric diagnosis or branch from a primary disorder such as ADHD.[12] Indeed, Patel and Barzman note that "aggression often comes from the impulsiveness associated with ADHD… [and] can be a symptom of disruptive behavior disorders including conduct disorder and oppositional defiant disorder (ODD)" (p. 408).[12] Accordingly, guanfacine

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has been applied as a treatment for aggression and agitation in individuals with Prader–Willi syndrome[15] and with ADHD.[13] Given the support for guanfacine effectively treating aggression in certain human clinical populations, and in treating SIB in macaques,[7] we aimed to test whether guanfacine also represented a suitable intervention for social reactivity in macaques that results in repeated and severe agonistic behavior and wounding.

Case Report

Case study 1. A SPF, experimentally naive pigtail macaque (*Macaca nemestrina*) male (age, 8-y-old; weight, 13.68 kg) was the focus of this case report. He was housed with a group of females as part of the University's breeding colony at the Johns Hopkins University Breeding Farm. Despite being a productive breeder, the male had a history of wounding the females he was housed with, often necessitating veterinary intervention. Various behavioral management strategies had been attempted to reduce the social wounding he exhibited. As it was hypothesized that the male's aggression was driven by anxiety, his group was moved to an enclosure at the facility that experienced less staff activity and other disturbances and he was maintained on fluoxetine (20 mg PO once a day [SID]). Eleven months prior to the start of this case study, the male was introduced to a new social group as part of our standard rotation process in which males are moved between breeding groups to increase population genetic diversity. A group of mature, and behaviorally calm, females was selected with the aim of further reducing triggers for the male's aggression. However, these interventions did not reduce rates of agonistic behavior observed: he wounded 6 of those 9 females he lived with on 15 different occasions, with 8 of those wounding events requiring a sedated examination and suturing. However, at all other times the male was socially appropriate and well bonded with the females with which he was housed.

It was hypothesized that the male's agonistic behavior was triggered by external stressors and represented an inappropriate or misdirected reactive anxiety response. To assess this, we collected detailed behavioral observations of the male in his social group (methods detailed in the Materials and Methods) for a month-long 'baseline' period, when he was maintained on fluoxetine, but without any additional/new interventions. During this baseline period, the male wounded 5 adult females across 7 events. In addition, it was observed that in 60% of the 10-min baseline behavioral observation sessions in which the male was recorded to engage in agonistic behavior (contact and noncontact aggression, with or without wounding), the aggression was preceded by recording of one of the following correlative events (that is, external stressors): staff activity in the indoor area of the building including cleaning neighboring animal enclosures, fights among animals in a neighboring group, or fights among other animals in the focal animal's group but not involving the focal animal. These data supported our hypothesis that this male's aggressive behavior was mediated by anxious arousal. Indeed, there were only 2 occasions in which one of these external stressors was recorded and the male did not subsequently exhibit aggressive behavior within that same 10-min observation session.

These observations prompted our evaluation of the efficacy of guanfacine to reduce anxious reactivity that results in aggression and wounding, as has been previously demonstrated in human patients. To do so, we began the male on a treatment course of guanfacine (10 mg PO SID) and collected detailed behavioral data over the course of a year. We note that this dosage is considerably higher than that prescribed to human patients,[3] but it reflects dosage that has been previously validated for use with macaques (for example, for rhesus macaques treated with guanfacine for SIB, Freeman and colleagues (2015) reported a dosage of 10 mg PO SID[7] and Macy and colleagues (2000) reported a dosage of 0.5 mg/kg SID delivered IM).[10]

Case study 2. In addition to the comprehensive study of the male macaque, we also provide a description of a second case study: an 11-y-old female SPF pigtail macaque (weight, 12.25 kg). This experimentally naive female macaque was housed at the Johns Hopkins Research Facility since August 2021 and pair-housed with her current social partner, an 8-y-old female pigtail macaque, since March 2022. Similar to the male pigtail macaque, this female was aggressive toward her social partner apparently in response to external stressors. Of concern, the focal female severely wounded her partner on 2 occasions in 7 mo, each time requiring veterinary intervention and multiday separation of the pair. On both of these occasions there were major disruptive events in her home room. Specifically, on both days, a large proportion of the animals were sedated for research sampling needs and, due to personnel training, more technicians were working in the room than typical, including a number of new, unfamiliar staff. At other times, however, the pair was behaviorally compatible. Moreover, anecdotal reports from the Johns Hopkins Breeding Farm, where this female was born and mother-reared, indicated that she had a history of directing aggression to cagemates in response to external triggers. Therefore, in October 2022 the focal female was started on 10 mg of guanfacine PO SID.

Materials and Methods

Case study 1. At the start of our evaluation, the male case study subject was housed in a breeding group with 9 adult females (average age, 12.46 y; SD, 4.31), 2 juveniles (average age, 2.06 y; SD, 0.01), and 6 infants (average age, 0.30 y; SD, 0.07). Due to births, deaths, and sales that occurred over the course of the year-long evaluation, the final composition of the male's group was 6 adult females (average age, 12.74 y; SD, 4.82), 6 juveniles (average age, 2.09 y; SD, 1.01), and 2 infants (average age, 0.48 y; SD, 0.06). The social group was housed in an indoor/outdoor enclosure (264 sq ft) at the Johns Hopkins University Breeding Farm, which is a USDA-licensed, Office of Laboratory Animal Welfare–assured, and AAALACi-accredited facility. The macaques had ad libitum access to water, were fed daily with LabDiet 5038 monkey diet, and were provided with food enrichment 5 times a week. The enclosure was furnished with raised perches both indoors and out, and the macaques were given numerous enrichment toys. Outside of the clinical evaluation of guanfacine, as described below (and see Table 1), no other changes were made to the macaques' housing or husbandry routine, care, or social group composition solely for the purpose of this evaluation. Thus, this represents an opportunistic case study. This evaluation was approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine.

The focal male's behavior was recorded via direct observation using a focal instantaneous sampling approach,[1] with data recorded by 4 observers (JVA, VH, MCP, and LMH) who had reached >85% agreement across a minimum of 3 sessions prior to formal data collection. Each observation session was 10 min long, with data recorded at 15-s intervals for most sessions. A subset of observations recorded by V.H., representing 19% of the total observations, used 30-s intervals. To account for this, all analyses examined the rate of behaviors per 'in view'

Table 1. Study phases showing the different treatment regimens studied and when behavioral data were collected for the male pigtail macaque housed at the Johns Hopkins University Breeding Farm

Phase	Treatment	Date range	Number of data collection days	Total observation hours
Baseline	20 mg/d fluoxetine	15 Dec 21 to 14 Jan 2022	15	7.0
Transition	10 mg/d guanfacine while fluoxetine dosage was halved every other week until weaned	18 Jan 2022 to 28 Jan 2022	55	21.5
Guanfacine 1	10 mg/d guanfacine	01 Mar 22 to 14 Jul 2022	27	36.0
No treatment	No treatment	15 Jul 2022 to 18 Nov 2022	34	25.5
Guanfacine 2	10 mg/d guanfacine	21 Nov 22 to 03 Feb 23	17	18.0

observations. All observers also recorded the focal animal's agonistic interactions, anxiety-related behaviors, and abnormal behaviors ad libitum. Correlative events (for example, staff cleaning or feeding the study group or neighboring groups, agonistic interactions in neighboring groups) were also recorded ad libitum. At the start of each session, the observers recorded the following independent variables: treatment condition, date, time, temperature, the individuals present in the group, and what areas of their indoor/outdoor enclosure to which the animals had access. In addition to this detailed behavioral data, veterinarians and care staff also recorded all wounding events and any associated treatments or interventions.

To examine the potential effect of treating the focal male with guanfacine, we first collected behavioral data during a 2-wk baseline period, and during the subsequent month-long period when he was weaned off fluoxetine while being administered guanfacine (Table 1). We then monitored his behavior and wounding rates while the male was treated with guanfacine during a 4-mo period. Although the male was receiving guanfacine during the transition phase, we considered it separately to the first guanfacine treatment phase (guanfacine 1) due to the previously demonstrated lag time to effectiveness for guanfacine.[7] The results of the study by Freeman and colleagues (2015) also showed that SIB wounding rates remained at low levels for 4 wk after the cessation of guanfacine.[7] Given this reported legacy effect, we withdrew guanfacine treatment and continued to observe the male in a subsequent "no treatment" phase, as a washout to evaluate legacy effects. However, as the male macaque later showed increased rates of aggression when he was no longer being treated with guanfacine, we resumed his treatment and collected additional behavioral data in this final phase (guanfacine 2). A full timeline of the study phases is shown in Table 1. During the no treatment phase no placebo was administered. Therefore, not only was the provision of guanfacine withdrawn, but also the daily visits from the staff member administering the treatment. While it is possible that any resultant changes in the macaque's behavior may be a result in the withdrawal of treatment, the reduction in staff interactions, or a combination of both, we note that comparisons of the macaque's behavior during the baseline and guanfacine 1 phases allow us to compare the role of the drug in mediating his behavior, when staff interactions were kept constant as he was being treated daily with fluoxetine or guanfacine, respectively, during those phases.

We examined 3 behavioral categories of interest: agonistic behaviors (including both contact and noncontact aggression), anxiety-related behaviors (for example, yawning, self-scratching, and teeth grinding), and affiliative behaviors (for example, sitting in contact with groupmates, grooming groupmates, and playing with groupmates) (see Table 2 for a complete ethogram). We selected these behaviors to assess the efficacy of guanfacine on reducing rates of aggression, to understand the interplay between reactivity and agonism, and to assess potential sedation effects of guanfacine (that is, to determine whether affiliation was reduced, which would be detrimental in a breeding context).

To analyze the male's behavior, we first calculated the proportion of data points per study day that were either agonistic, anxiety related, or affiliative, creating a daily observed rate per each behavior of interest. Specifically, we calculated this daily rate as a proportion of data points in which the male was 'in view' and a behavior was identified and coded (see Table 2) (the male was coded as 'out of view' for 13.37% of all data points). To ascertain whether there were changes in the male macaque's behavior rates over time for each study phase, we correlated the daily rate that the male was observed engaging in each behavior of interest with study day. To examine relative changes in the propensity of the male to engage in agonistic, anxiety-related, or affiliative behaviors by treatment, we compared the daily rate of each behavior across the different study phases using an ANOVA with post hoc pairwise comparisons. To more broadly compare the male's likelihood of exhibiting agonistic behavior by treatment type (that is, fluoxetine, guanfacine, or no treatment) we coded each day as 1 if the male was recorded to perform agonistic behavior at any time or 0 if the behavior was never observed that day.[6] We compared daily occurrences using a binomial general linear mixed model by treatment. All analyses were run in R version 4.1.1.[14]

Case study 2. The female macaque case study subject was pair-housed with her social partner in standard primate caging (13.6 sq ft) at the Johns Hopkins University vivarium, which is a USDA-licensed, Office of Laboratory Animal Welfare–assured, and AAALACi-accredited facility. The pair had ad libitum access to water, were fed daily with LabDiet 5038 monkey diet, and were provided with food enrichment 5 times a week. As for the male (case study 1), outside of the clinical evaluation of guanfacine, no other changes were made to the macaques' housing or husbandry routine, or care for the purpose of this evaluation. Thus, this represents an opportunistic case study. We did not collect the same detailed behavioral data for this female as we did for the male at our breeding facility. Instead, we monitored the pair's behavior and wounding rates as part of daily health checks during the course of 6 mo following the commencement of guanfacine treatment. Accordingly, we report her results descriptively. This evaluation was approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine.

Table 2. Ethogram used to record the male pigtail macaque's behavior

Category	Behavior	Definition
Agonistic	Bared teeth	Focal animal's lips are pulled back in an exaggerated manner exposing teeth. The animals' brows are pushed together in a way that makes the animal appear frightened; may or may not be accompanied by a scream.
	Chase	Focal animal runs threateningly after a recipient.
	Displace	The focal animal supplants a recipient and occupies the space vacated by the recipient.
	Display	Focal animal shakes or rocks the cage mesh, a structure within the cage, and/or bouncing on a structure or the ground.
	Noncontact aggression	Focal animal performs behaviors that are threatening, but do not involve physical contact with another animal. May include the following behaviors: head bob, open or round mouth threats, ear flap, stare, lunge, brow flash, slapping ground or cage, or lunging directed toward another animal.
	Contact aggression	Focal animal attacks another animal. Behaviors may include hitting, biting, grabbing, pinning, hair-pulling, and wrestling.
	Nonsexual mount	Focal animal grabs the hindlegs of another monkey with his own hind feet and places his hands on the lower back of the recipient, thus hoisting himself off of the ground; may include thrusting, but movements are less consistent and are of shorter duration compared with a sexual mount. Typically same-sex pairs, often male/male.
	Resource takeover	Focal animal takes food or other object(s) from a recipient.
	Receive bared teeth[a]	A group member directs a bared teeth display toward the focal animal.
	Retreat[a]	Focal animal moves away past arm's reach in response to approach or chase from another individual (compare with 'turn away').
	Turn away[a]	Focal animal performs a small movement or pivot away (within arm's reach) from another animal (compare with 'retreat').
	Receive noncontact aggression[a]	Focal animal is the recipient of noncontact aggression from another individual.
	Receive contact aggression[a]	Focal animal is the recipient of contact aggression from another individual.
	Receive nonsexual mount[a]	Focal animal is the recipient of a nonsexual mount.
Affiliative	Groom	Focal animal is picking through the hair or removing debris from the skin of another individual and using hands and/or mouth.
	Mutual groom	The focal grooms a group mate while simultaneously being groomed by that same individual.
	Lipsmack	Focal animal is performing rapid, repetitive opening and closing of the lips; teeth are covered by lips, may be audible.
	Present	Focal animal exposes their rump, neck, ventrum, back or other surface of the body toward another animal in an exaggerated way.
	Sexual mount	Focal animal grabs the hindlegs of another monkey with his/her own feet and places his hands on the lower back of the recipient, thus hoisting himself off the ground; must include consistent thrusting; the recipient often looks back, lipsmacks, or grabs the mounter. May be accompanied by screams.
	Social play	Focal animal performs nonaggressive chasing, bouncing, grabbing, wrestling, soliciting, and/or mock biting of another monkey. These behaviors are also often seen with a pucker (lips forward, ears back, neck extended, known as 'LEN') or 'play face' (that is, relaxed expression, typically not exposing teeth).
	Prosocial	Focal animal engages with another group mate in an affiliative way not previously defined, including directing a pucker (LEN) expression toward the recipient.
	Receive groom[a]	Another individual is picking through the hair or removing debris from the skin of the focal animal.
	Receive prosocial[a]	Focal animal receives any form of prosocial behavior not previously defined.
Anxiety related	Yawn	Focal animal yawns. This is often an extended or exaggerated yawn, with the mouth fully open and canines visible. Eyes may be closed.
	Self-scratch	Focal animal rubs fingers across his/her body part in a forceful and repetitive manner that is distinct from grooming (that is, the animal does not pick through his/her hair).
	Teeth grinding	Focal animal grinds his/her teeth together. This is typically identified via the sound of the teeth grinding, accompanied by a slight movement of the mouth. The mouth may appear closed.
Locomotion	Locomotion	Focal animal changes location in horizontal or vertical space by walking, running, climbing, or crawling. The change in location must be greater than one body length. The focal may locomote in any fashion including bipedally or quadrupedally.

(*continued*)

Table 2. (Continued)

Category	Behavior	Definition
Inactive	Neutral contact[b]	The focal animal is sitting or standing while in physical contact with another monkey, but not performing other social behaviors (for example, groom) or self-grooming.
	Neutral proximity	The focal animal is sitting or standing while within an arm's length of another monkey, but not performing other social behaviors or self-grooming.
	Self-groom contact[b]	The focal animal is grooming himself/herself and is in direct contact with another group mate.
	Self-groom proximity	The focal animal is grooming himself/herself while within an arm's length of another monkey, but not performing other social behaviors.
	Self-groom alone	The focal animal is grooming himself/herself and is more than one arm length from any group mates.
	Inactive alone	Focal animal is not moving and not engaged in any other behavior listed and is more than one arm length from any group mates. May or may not be sleeping.
Feed	Drink	Focal animal ingests liquid.
	Feed/forage	Focal animal is actively ingesting food items or searching for and/or collecting items for ingestion. Behavior includes instances in which focal is collecting food (for example, biscuits or produce) in his/her hand, foot, or mouth, without chewing or swallowing. Not 'object.'
Object	Food based	Focal animal interacts with a food-based enrichment device in some manner, includes holding it, lifting it up, banging it, picking out food from it.
	Toy	Focal animal interacts with a toy by touching it, picking it up, throwing, and so forth.
Staff interaction	Staff interaction	The focal animal engages with a staff member in some way (for example, receives medication).
Other	Other	The focal performs some other behavior not listed above.
Unknown	Out of view	The focal animal is out of view of the observer.
	Unknown	Focal animal is partially occluded so that his/her behavior cannot be determined.

Note that affiliative behaviors were recorded as interval behaviors. Agonistic behaviors were recorded both as interval and ad lib, all occurrence behaviors. Anxiety-related behaviors were just recorded as ad lib, all occurrence behaviors.
[a]'Receive' behaviors were reverse coded for analysis, so that the focal animal was always defined as the actor in each interaction.
[b]These behaviors were included in the 'affiliative' category for analysis to generate a composite sociality index.

Results

Case study 1. When first treated with guanfacine (that is, during the guanfacine 1 phase), the male showed a significant reduction in his daily rates of agonistic behaviors over time (that is, by study day) ($r = -0.66$, $P < 0.0001$) (Figure 1). In addition, there was a significant difference in the male's daily rates of agonistic behavior across the 5 phases of the study ($F = 3.66$, $P = 0.007$). When the male was treated with guanfacine his daily rate of agonistic behavior was significantly lower than at baseline when he was treated with fluoxetine (guanfacine 1 compared with baseline: $t = 2.61$, $P = 0.018$; guanfacine 2 compared with baseline: $t = 2.25$, $P = 0.036$). Highlighting the time required for guanfacine to become efficacious, there was no significant difference in the male's daily rate of agonistic behavior during the transition phase and the baseline ($t = 1.24$, $P = 0.228$), and his daily rate of agonistic behavior was lower during guanfacine 1 as compared with during the transition phase ($t = -2.26$, $P = 0.028$) (Figure 1).

The male showed a significant increase in daily agonistic rates during the 4-mo no treatment phase when he was not treated with any drug (that is, study day and daily rates of agonism were positively correlated: $r = 0.57$, $P = 0.0004$). Given that Freeman and colleagues (2015),[7] who studied guanfacine as a treatment for SIB in macaques, reported a legacy effect of guanfacine, we wanted to examine whether the same pattern was reflected in our data. Specifically, Freeman and colleagues (2015) showed that when guanfacine treatment was stopped, the macaques' self-directed wounding rates remained low for 4 wk, but by 8 wk after the cessation of treatment, the macaques' SIB wounding rates had increased significantly.[7] We found the same pattern for the rates of socially directed agonistic behavior in the male macaque we studied: his daily rate of agonistic behavior was significantly lower in the first 4 wk after the male stopped receiving guanfacine as compared with weeks 5 to 8 of the no treatment phase ($t = 2.26$, $P = 0.032$; Figure 2).

Further supporting a potential legacy effect of guanfacine, the male's rates of agonistic behavior in the no treatment phase, following extended guanfacine treatment, were significantly lower than at baseline when he was treated with fluoxetine ($t = 2.39$, $P = 0.026$). However, this appears to be driven by the male's sustained low rates of agonistic behavior during the first 4 wk when he was not treated with guanfacine: restricting this comparison to the latter period of the no treatment phase (that is, week 5 onward) revealed no significant difference in his agonistic behavior rates with baseline rates (that is, during the baseline and transition phases combined) ($t = -1.01$, $P = 0.317$). This again reflects the results reported by Freeman and colleagues (2015) for macaque SIB as treated with guanfacine.[7]

Given the ultimate increase in agonistic behavior once no longer treated with guanfacine in the no treatment phase (Figure 1), the male was again placed on guanfacine (that is, study phase guanfacine 2) (Table 1). When guanfacine treatment was resumed, the male's rates of agonistic behavior again reduced over time (Figure 1). The male's daily rate of agonistic behavior in guanfacine 2 was significantly lower than at baseline when treated with fluoxetine ($t = 2.25$, $P = 0.036$), and there was no significant difference between the male's rates of agonistic behavior between guanfacine phase 1 and 2 ($t = -0.54$, $P = 0.593$).

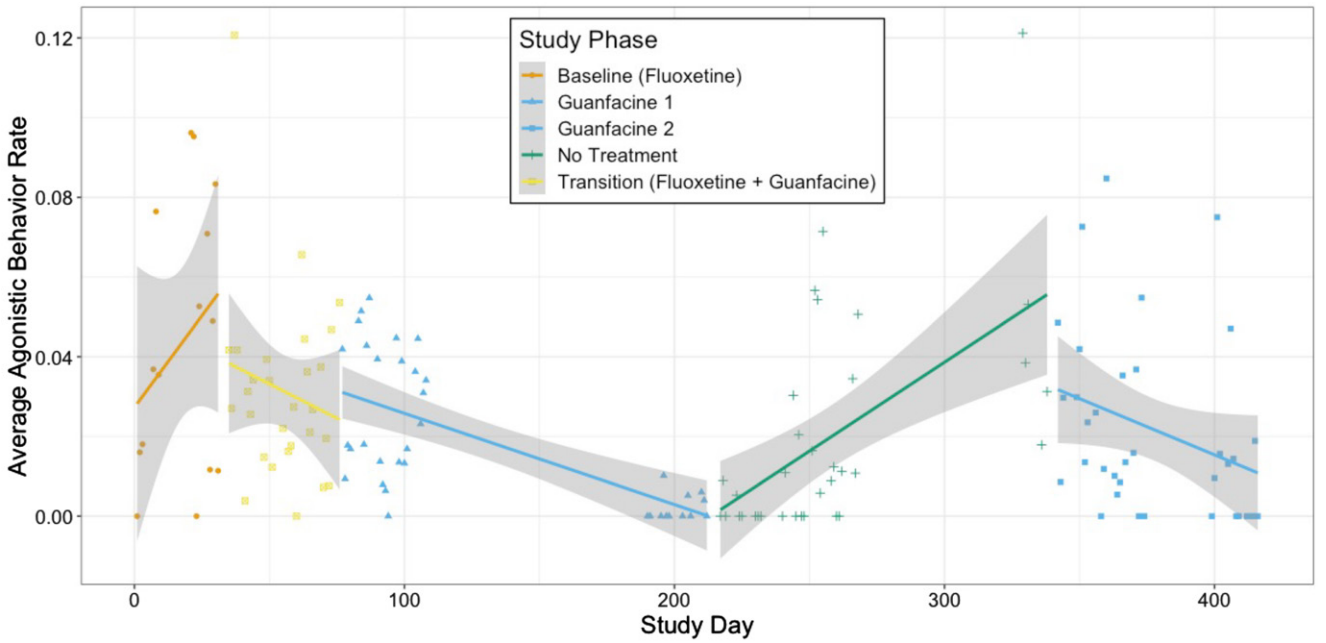

Figure 1. Average rates of agonistic behavior shown by the focal male pigtail macaque by study day across each of the study phases and treatment types. The line shows the linear relationship between study day and rate of agonistic behavior was observed per day, and the shaded areas show confidence intervals for the linear models.

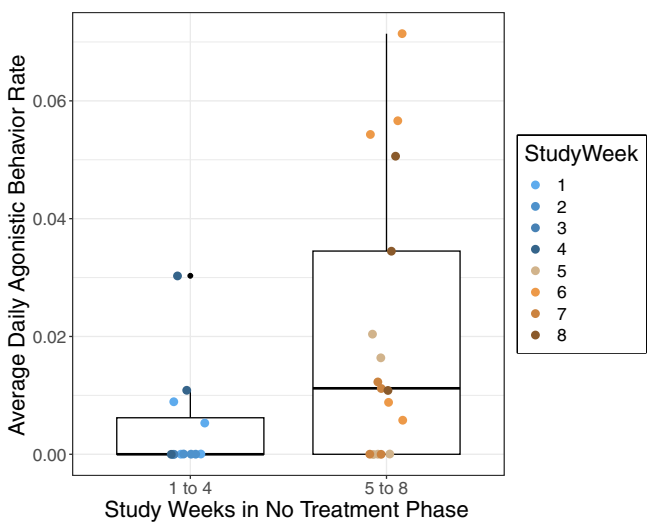

Figure 2. The male macaque's average daily rates of agonistic behavior in the first 8 wk of the no treatment phase of the study, comparing the first 4 wk following the cessation of guanfacine treatment (that is, weeks 1 to 4) to the second 4 wk (that is, weeks 5 to 8). Each point shows the daily average rate of agonistic behavior, color coded by study week.

However, during the guanfacine 2 phase the negative correlation between study day and daily rates of agonistic behavior was not significant ($r = -0.30$, $P = 0.075$). This nonsignificant reduction over time in agonistic behavior is likely due to the global reduction in his agonistic behavior following the first guanfacine administration (that is, guanfacine 1 phase).

Next, we examined the likelihood that the male would engage in any agonistic behavior each day by treatment type. As there was no difference in the proportion of days that the male engaged in agonistic behavior between the baseline and transition phases ($Z = 1.21$, SE = 1.27, $P = 0.262$), we considered them as a combined fluoxetine treatment phase for subsequent analysis. Similarly, as there was no difference in the proportion of days that the male engaged in agonistic behavior when treated with guanfacine in phase 1 or 2 ($Z = -0.07$, SE = 0.55, $P = 0.945$), we also combined these 2 phases for analysis as a single guanfacine treatment condition. Comparison of treatment types revealed that the likelihood of the male engaging in any agonistic behavior per day was significantly lower when he was treated with guanfacine (proportion of days when agonistic behavior was observed for guanfacine phases 1 and 2 combined, 0.75), as compared with when treated with fluoxetine (proportion of days for the baseline and transition phases combined, 0.93) ($Z = -2.30$, SE = 0.66, $P = 0.022$).

Following the pattern of agonistic behavior, the male showed a significant reduction in anxiety-related behaviors when treated with guanfacine as compared with his baseline rates (Figure 3). When treated with fluoxetine, the male's average daily rate of anxiety-related behavior in the baseline and transition phases was 0.06, which dropped to 0.04 in guanfacine 1. During the guanfacine 1 phase the macaque's daily rates of anxiety-rated behaviors fell significantly over time (that is, daily behavioral rates were negatively correlated with study day: $r = -0.67$, $P < 0.0001$). Supporting our hypothesis that the male's agonistic behavior was related to reactivity, there was a significant correlation between daily rates of agonistic behavior and anxiety-related behavior in the guanfacine 1 phase ($r = 0.63$, $P < 0.0001$). Thus, when the male was treated with guanfacine (guanfacine 1), as his rates of agonistic behavior fell, so did his rates of anxiety-related behaviors.

Similar to agonistic behaviors, there was a significant difference in the male's daily rates of anxiety-related behaviors across the different study phases ($F = 3.79$, $P = 0.006$). As compared with the baseline rate, when treated with fluoxetine, the male showed reduced rates of anxiety-related behaviors when treated with guanfacine. This difference was significant for guanfacine 2 compared with baseline ($t = 2.77$, $P = 0.012$) but not for guanfacine 1 compared with baseline

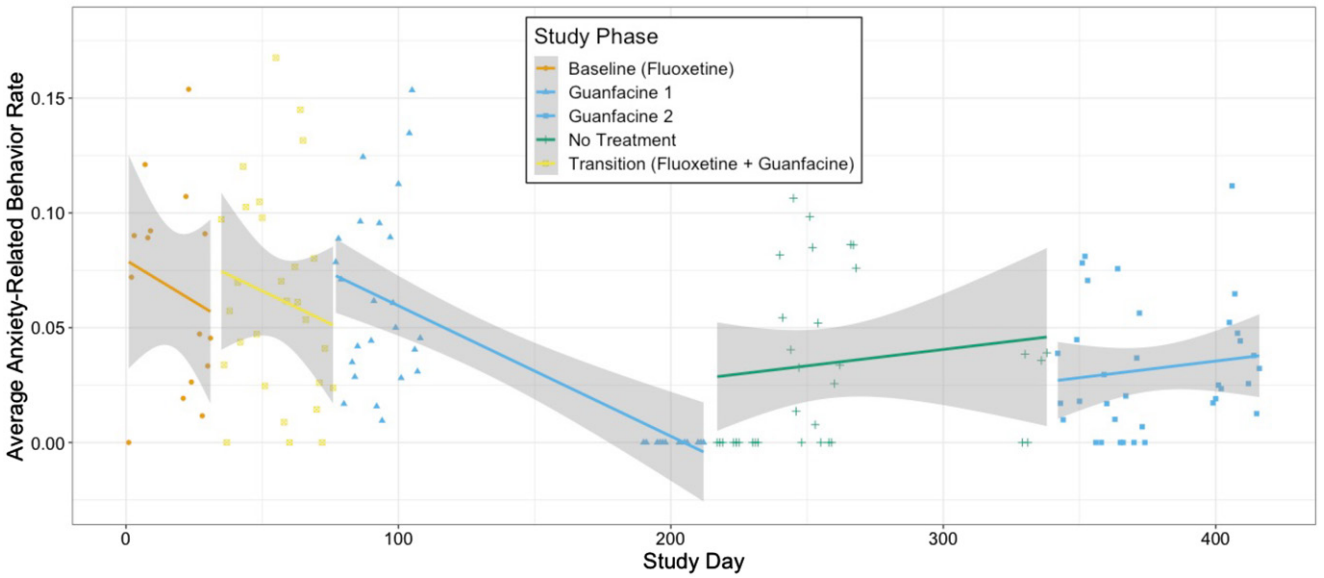

Figure 3. Average rates of anxiety-related behavior shown by the focal male pigtail macaque by study day across each of the study phases and treatment types. The line shows the linear relationship between study day and rate of agonistic behavior was observed per day, and the shaded areas show confidence intervals for the linear models.

($t = 1.71$, $P = 0.099$). Again reflecting the patterns observed for agonistic behavior, there was no significant difference in the rates of anxiety-related behaviors observed during the transition phase as compared with baseline ($t = 0.26$, $P = 0.794$), but the male showed reduced rates of anxiety-related behaviors during guanfacine 1 compared with the transition phase, which approached significance ($t = –1.73$, $P = 0.088$), and by guanfacine 2 this difference was significant ($t = –3.14$, $P = 0.003$), showing a reduction in rates of anxiety-related behaviors with long-term guanfacine treatment.

As the focal male was housed in a breeding group, we wanted to evaluate whether the efficacy of guanfacine to reduce the male's agonistic interactions was due to an overall reduction in all of his social behavior (that is, a sedation effect). Therefore, we also compared his rates of affiliative behavior across study phases and found that rates differed significantly by study phase ($F = 4.66$, $P = 0.001$). When first treated with guanfacine (that is, guanfacine 1), the male showed a significant reduction in the rate of affiliative behavior he engaged in as compared with baseline ($t = 2.36$, $P = 0.030$). However, his rates of affiliative behavior rebounded to baseline rates during the no treatment and guanfacine 2 phases; that is, there was no significant difference in the male's daily rates of affiliative behavior in the no treatment phase compared with baseline ($t = –0.49$, $P = 0.625$) or in the guanfacine 2 phase compared with baseline ($t = 0.22$, $P = 0.831$). There was also no difference between baseline rates and those during the transition phase ($t = –0.74$, $P = 0.466$).

While the male's rates of agonistic behavior significantly reduced when treated with guanfacine, wounding events were still reported. In the 4.5 mo that the male was treated with guanfacine (guanfacine 1) he wounded 5 groupmates across 10 events, with one female wounded on 4 occasions. Thus, while guanfacine significantly reduced the male's reactivity and frequency of agonistic interactions, wounding was not eliminated. Total elimination of wounding is not likely in any macaque given the species' natural history and social repertoire; however, later in the study period when the male's guanfacine treatment was restarted (that is, the guanfacine 2 phase) only 2 wounding events were recorded during that 2.5-mo period. This emphasizes the efficacy of this treatment, especially when treatment is extended.

Case study 2. Considering our second case study subject, at the time of writing, the female pigtail macaque has been maintained consistently on guanfacine for >1 y without any pauses in treatment. In this time, she has exhibited no agonistic interactions with her social partner that have resulted in wounding. This is despite multiple external stressors being documented in the time period since her guanfacine treatment began, including a move to a new room, new animals moving into her room, multiple sedation events of her and her partner, and sedation events of other animals in the room in which she is housed. Thus, the pair have been cohoused successfully without any need for separation due to behavioral reasons or clinical treatment of wounding since guanfacine administration was started.

The female showed complete compliance with daily medication (and continues to do so). For both animals, while compliance has been high, given the large dose and aversive taste of guanfacine, the vehicle used to administer it has been of high value and, for the male in particular, we have had had to often change what treats we used to administer the drug in response to his preferences (including oatmeal, jelly, Nutri-Grain bars, and Starburst). Anecdotally, with other nonhuman primates that we have administered guanfacine to treat SIB, we have observed similar variability in compliance, with some animals showing complete compliance and other animals requiring very high value vehicles to mask the flavor, and with the need to vary the vehicles used over time.

Discussion

The male pigtail macaque treated with guanfacine showed a significant reduction in his agonistic behavior and associated wounding toward his group mates. The concurrent reduction in his rates of anxiety-related behaviors further supports our hypothesis that this male's agonism reflected an exaggerated response to external triggers due to a reactive behavioral phenotype. Importantly, we ultimately saw no overall reduction in the male's affiliative behavioral rates, thus guanfacine had no sedative effect. This is especially important when considering

the treatment of macaques in a breeding colony setting in which appropriate social behaviors are required to ensure stable group dynamics and successful breeding. In addition to the male, who was the core focus of our evaluation, we also tracked the behavioral response of a pair-housed female pigtail macaque living at our research facility, who has been treated with guanfacine for over a year as a mitigation intervention to her reactive aggression shown to her cage mate. As for the male, guanfacine was a successful treatment option—since guanfacine treatment was started, no wounding events have been reported, nor have any agonistic interactions between the 2 animals been observed during daily room checks.

The behavior of the male macaque after the cessation of guanfacine (that is, during the no treatment phase) revealed that there was an apparent legacy effect of the prior guanfacine treatment period. Specifically, for 4 wk after the cessation of guanfacine treatment, the male did not show an increase in agonistic behavior, and presumably his reactivity. Indeed, during the first 4 wk of the no treatment phase, the male never exhibited agonistic behavior following the occurrence of any environmental stressors recorded during observation sessions (that is, correlative events, such as care staff cleaning the room, animals in neighboring groups fighting). However, after this initial lag, the male's rate of agonistic behavior rates rose. This reflects the findings of Freeman and colleagues (2015), who used guanfacine to treat SIB in macaques, and who also reported an initial protective effect of guanfacine that lasted for about 4 wk after treatment was stopped.[7] The continued behavioral changes seen after guanfacine treatment is stopped and the lack of need to wean animals off guanfacine in a stepwise manner promote the use of guanfacine as a pulse therapy, which may offer greater flexibility for animals on specific research protocols that might not be compatible with continuous treatment. However, more work is needed to determine how long an animal must be maintained on guanfacine prior to withdrawal for such legacy effects to be evidenced.

In addition to highlighting the efficacy of guanfacine to treat social reactivity that leads to aggression in macaques, these 2 case studies also emphasize the importance of selecting the most appropriate pharmacological intervention when treating behavioral concerns. Initially the male had been treated with fluoxetine to treat anxiety. However, the male continued to show high rates of aggression, often leading to severe wounding, even when maintained on fluoxetine. Our behavioral assessments for this study, however, suggested that the male was reactive (to external triggers) rather than anxious; that is, he showed a behavioral overreaction to environmental and social stressors, rather than exhibiting chronic anxiety. The same apparent behavioral pattern was also observed in the female macaque. When not experiencing a stressor, both macaques were socially competent and affiliative with their social partners. Thus, selecting a drug (that is, guanfacine) that enhances executive function and self-control was more efficacious in reducing the macaques' agonistic behavior than was an antianxiolytic (that is, fluoxetine). These findings therefore demand the need for behavioral analysis to aid treatment selection and to help differentiate drivers of agonism (for example, typical dominance interactions, high anxiety, or increase reactivity). Future research should be run to determine the broader applicability of these results across settings, social dynamics, and species.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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