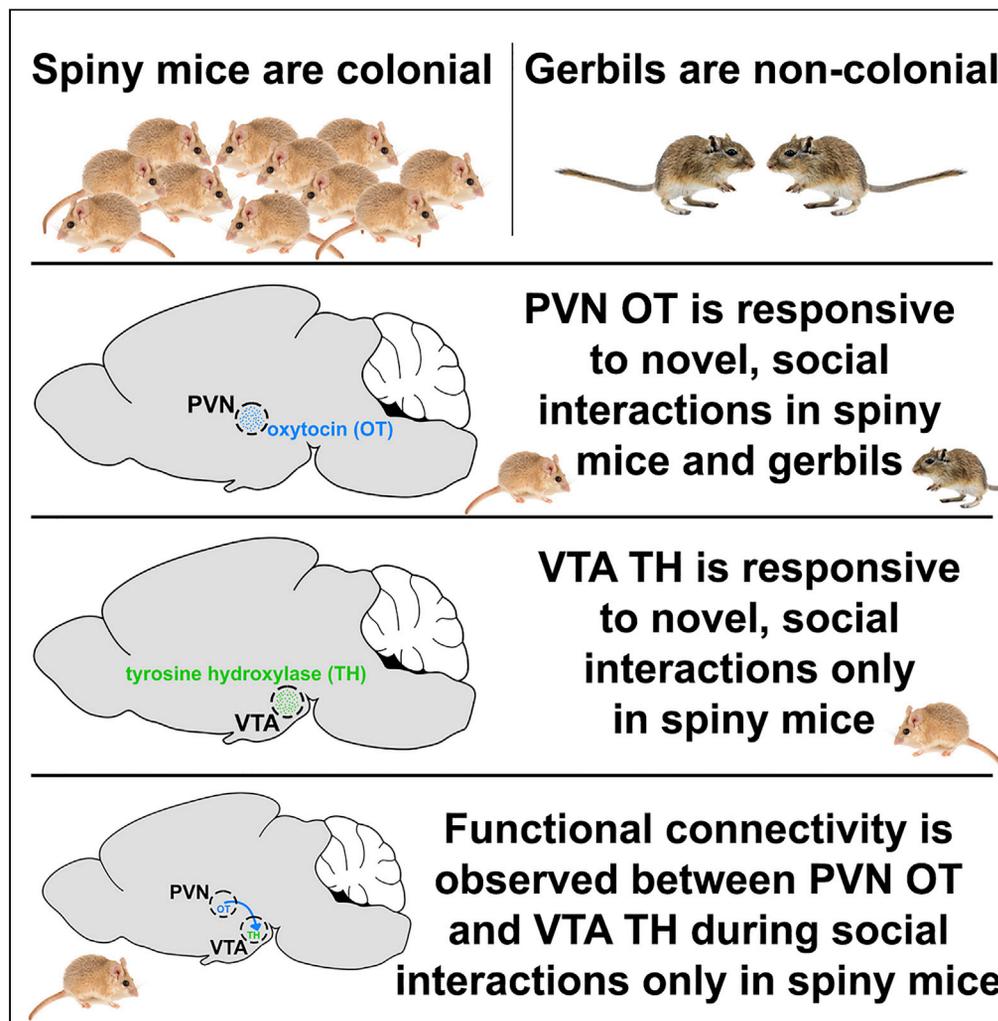


Article

Species-typical group size differentially influences social reward neural circuitry during nonreproductive social interactions



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Highlights

Spiny mice are more gregarious, more prosocial, and less aggressive than gerbils

PVN OT neurons are responsive to novel social interactions in spiny mice and gerbils

VTA TH neuronal responses are greater in response to social stimuli in spiny mice

PVN OT and VTA TH functional connectivity may gate social reward in spiny mice

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Article

Species-typical group size differentially influences social reward neural circuitry during nonreproductive social interactions

Jose A. Gonzalez Abreu,^{1,3} Ashley E. Rosenberg,^{1,3} Brandon A. Fricker,¹ Kelly J. Wallace,¹ Ashley W. Seifert,² and Aubrey M. Kelly^{1,4,*}

SUMMARY

We investigated whether nonreproductive social interactions may be rewarding for colonial but not non-colonial species. We found that the colonial spiny mouse (*Acomys cahirinus*) is significantly more gregarious, more prosocial, and less aggressive than its non-colonial relative, the Mongolian gerbil (*Meriones unguiculatus*). In an immediate-early gene study, we examined oxytocin (OT) and tyrosine hydroxylase (TH) neural responses to interactions with a novel, same-sex conspecific or a novel object. The paraventricular nucleus of the hypothalamus (PVN) OT cell group was more responsive to interactions with a conspecific compared to a novel object in both species. However, the ventral tegmental area (VTA) TH cell group showed differential responses only in spiny mice. Further, PVN OT and VTA TH neural responses positively correlated in spiny mice, suggesting functional connectivity. These results suggest that colonial species may have evolved neural mechanisms associated with reward in novel, nonreproductive social contexts to promote large group-living.

INTRODUCTION

In adapting to various environmental pressures, many species have evolved large group-living (i.e., colonial living). Large group-living is facilitated by gregarious phenotypes and high degrees of prosociality in contexts that are not necessarily driven by the motivation to mate (i.e., nonreproductive affiliation) (Goodson et al., 2012; Treisman, 1975). Exhibiting nonreproductive prosociality serves groups by aiding in greater foraging efficiency, predatory defense, more effective homeostatic regulation, and collective traveling (Krause and Ruxton, 2002). Despite the importance of prosociality in nonreproductive contexts for numerous species, little research examines brain mechanisms that promote such prosocial behaviors.

How an animal processes and responds to varying social contexts is strongly influenced by their species-typical group size (Goodson et al., 2005). Although affiliation in reproductive contexts (i.e., sexual behavior, pair-bonding, parenting) is commonly observed regardless of species-typical group size because of evolutionary drives directly relating to fitness, affiliation in nonreproductive contexts is more typically observed in large group-living species. Animals that live in large colonies are more likely to encounter nonreproductive interactions given the greater number of conspecifics they congregate with. Because it is important to maintain stability and group cohesion for successful large group-living (Papageorgiou and Farine, 2020), mechanisms have likely arisen to facilitate nonreproductive sociality in highly gregarious species.

A mechanism that plays a particularly important role in reinforcing specific behaviors is reward (Wise and McDevitt, 2018). Because of the evolutionary drive to maintain the presence of an animal's genes in the population, reproductive social contexts should be at least somewhat rewarding for all species regardless of group size. Indeed, ample literature demonstrates that sexual, pair-bonding, and parenting behaviors are rewarding for numerous species (Aragona and Wang, 2009; Becker et al., 2001; Champagne et al., 2004; Cornil et al., 2005; Heimovics and Ritters, 2005; Moncho-Bogani et al., 2005). However, nonreproductive social contexts may not be rewarding for every species. Although multiple mechanisms likely contribute to the evolution of cohesive large group-living, we hypothesize that one such

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mechanism is for large group-, but not small group-, living species to find nonreproductive social interactions rewarding.

The nonapeptides, oxytocin (OT) and vasopressin (VP), are evolutionarily conserved peptides that modulate a range of social behaviors including affiliation, parental care, aggression, gregariousness, pair-bonding, and anxiety-like behavior (Albers, 2012; Donaldson and Young, 2008; Kelly and Goodson, 2014b; Neumann, 2008). OT and VP are produced in distinct neuronal populations throughout the basal forebrain and midbrain, but the largest cell groups are those of the paraventricular nucleus of the hypothalamus (PVN) and the supraoptic nucleus of the hypothalamus (Landgraf and Neumann, 2004). The PVN and supraoptic nucleus of the hypothalamus (SON) cell groups contain the greatest amount of magnocellular neurons, which primarily send their axonal projections to the posterior pituitary where they release peptide into the periphery and affect the hypothalamic-pituitary-adrenal axis in response to stress as well as impact peripheral physiology, such as contributing to cardiovascular function, hydromineral balance, water retention, and smooth muscle function (Brown et al., 2013; Engelmann et al., 2004; Landgraf and Neumann, 2004; Sivukhina and Jirikowski, 2016). The PVN also contains parvocellular nonapeptide neurons, which send axonal projections throughout the brain (Landgraf and Neumann, 2004; Ludwig and Leng, 2006). Here we specifically focused on the PVN OT cell group because PVN OT directly facilitates gregariousness in birds (Kelly and Goodson, 2014a) and promotes affiliation in mice (Resendez et al., 2020) and voles (Ross et al., 2009). Furthermore, PVN OT sends axonal projections to the ventral tegmental area (VTA) of C57BL/6 mice where OT increases time spent with a constrained (i.e., under a cage) juvenile conspecific through direct effects on VTA dopaminergic neurons (Hung et al., 2017; Xiao et al., 2017). The study in C57BL/6 mice and an additional study in hamsters demonstrate that oxytocin actions in the VTA are necessary for the expression of the rewarding properties of social interactions (Hung et al., 2017; Song et al., 2016). VTA dopaminergic circuitry is crucial for processing reward (Ranaldi, 2014) as well as promoting social reward (Bariselli et al., 2018; Borland et al., 2018; Kawamichi et al., 2016). VTA tyrosine hydroxylase (TH) neurons reinforce courtship and sociosexual interactions in birds (Alger et al., 2011; Barr and Woolley, 2018), promote pair-bonding in prairie voles (Curtis and Wang, 2005), and facilitate sexual behavior in numerous species across taxa (Balfour et al., 2004; Charlier et al., 2005; Kramer et al., 2017). Fewer studies have examined the role of VTA TH in nonreproductive and grouping behaviors, however, estrildid finch species that flock in large groups have significantly more VTA TH-immunoreactive (Maldonado-Chaparro et al., 2018) neurons compared to related finches that are highly territorial (i.e., live in pairs or small family groups), suggesting that dopaminergic circuitry may play a role in promoting grouping behavior (Goodson et al., 2009). Therefore, to test our hypothesis that large group-, but not small group-, living species find nonreproductive social interactions rewarding, the PVN OT and VTA TH cell groups are excellent candidates for examination.

Here, using an explicitly nonreproductive context for two closely related rodents species that differ in group size – the colonial spiny mouse (*Acomys cahirinus*) and the small group-living Mongolian gerbil (*Meriones unguiculatus*) – we characterized and compare behavioral and neural responses between species. Although spiny mice are referred to as mice, phylogenetic and molecular studies place *Acomys* within Deomyinae, a distinct subfamily within Murinae (Deomyinae) that are more closely related to Gerbillinae (gerbils) than mice (Alhajeri et al., 2015; Fabre et al., 2012; Steppan and Schenk, 2017). Spiny mice are communally breeding rodents that live in interactive large groups of mixed-relation individuals (Haughton et al., 2016). They are highly prosocial and exhibit little to no aggression in both reproductive and nonreproductive social contexts (Fricker et al., 2021). In addition, spiny mouse males and females indiscriminately alloparent pups and lactating females nurse pups regardless of genetic relation (Tuckova et al., 2016). Further, spiny mice are gregarious (defined ecologically as a preference to affiliate in large groups (Goodson et al., 2012; Treisman, 1975)) and established breeding groups will welcome an unrelated newcomer with little aggression (Cizkova et al., 2011; Fricker et al., 2021). In contrast, Mongolian gerbils are territorial rodents that live in small family groups that consist of a breeding pair and a few litters of offspring (Agren, 1976; Deng et al., 2017). Although gerbils are affiliative in reproductive contexts with mates and offspring, they exhibit aggression during interactions with novel, same-sex individuals (Gromov, 2008; Liu et al., 2009; Pan et al., 2020; Roper and Polioudakis, 1977).

To determine whether nonreproductive social contexts may be rewarding to the colonial spiny mouse, but not the territorial gerbil, we first obtained behavioral profiles in nonreproductive contexts to examine species differences in gregariousness, prosocial, and aggressive behavior. We then conducted an immediate-early gene (IEG) study in which males and females of both species were allowed to freely interact with a

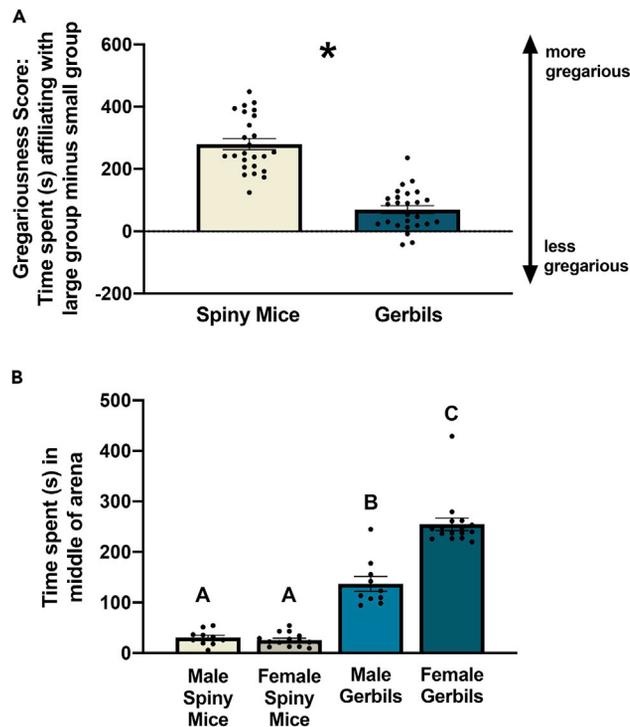


Figure 1. Group size preference test

(A) Mean (\pm SEM) time spent (s) affiliating with the large group minus the small group. Spiny mice (light beige) exhibited higher degrees of gregariousness than gerbils (blue).

(B) Mean (\pm SEM) time spent (s) in the middle of the arena not affiliating with any stimulus animals. Male (light beige) and female (dark beige) spiny mice spent less time not affiliating with stimuli than male (light blue) and female (dark blue) gerbils. Dots represent individual data points. * indicates statistical significance ($p < 0.05$). Letters (A,B,C) denote significant post hoc comparisons ($p \leq 0.05$), with shared letters indicating statistical similarity between groups; bars that do not share a letter were significantly different.

novel, same-sex conspecific or a novel object. Brain tissue was immunohistochemically processed for oxytocin (OT), tyrosine hydroxylase (TH; rate limiting enzyme for catecholamine biosynthesis), and Fos (a proxy marker of neural activity) in order to examine neural responses of social and reward circuitry.

RESULTS

Experiment 1

Behavioral profiling in spiny mice and gerbils

We first sought to determine species-specific behavior in a group size choice test to assess gregariousness. In an 8 min test, subjects freely explored a large arena that contained a group of 8 novel, same-sex conspecifics on one end and a group of 2 novel, same-sex conspecifics on the other end. All stimulus animals were constrained under wire mesh barriers. A gregariousness score was calculated as time spent affiliating with the large group minus time spent affiliating with the small group; higher scores reflect a higher degree of gregariousness. A general linear model (GLM) with Species and Sex as fixed factors revealed a main effect of Species ($F_{(1,50)} = 89.02$, $p < 0.01$; Figure 1A), with spiny mice exhibiting higher gregariousness scores than gerbils (Bonferroni-corrected posthoc $p < 0.01$). We also scored the amount of time spent in the middle of the arena where subjects did not actively engage with any stimulus animals. A GLM yielded a significant Sex by Species interaction ($F_{(1,50)} = 35.97$, $p < 0.01$; Figure 1B). Bonferroni-corrected posthoc analyses showed an effect of sex only in gerbils ($p < 0.01$), such that female gerbils were less affiliative and spent more time in the middle of the arena than male gerbils. Additionally, female and male gerbils spent significantly more time in the middle of the arena compared to female and male spiny mice.

Next, we examined prosocial and aggressive behavior in a freely interacting social interaction test. Subjects were placed in a novel cage and allowed to acclimate for 3 min before a novel, and same-sex conspecific

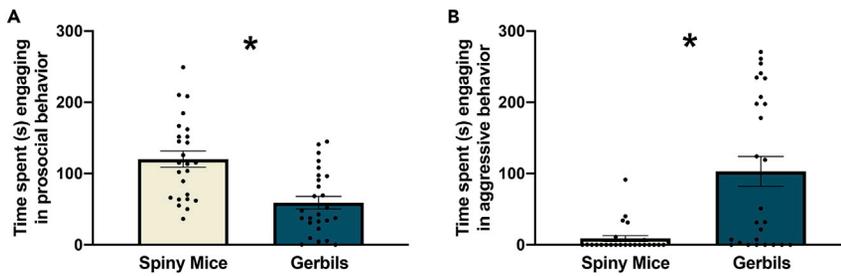


Figure 2. Prosocial interaction test

(A) Mean (\pm SEM) time spent (s) exhibiting prosocial behavior. Spiny mice (beige) exhibited more prosocial behavior than gerbils (blue).

(B) Mean (\pm SEM) time spent (s) exhibiting aggressive behavior. Spiny mice (beige) exhibited less aggression than gerbils (blue). Dots represent individual data points. * indicates statistical significance ($p < 0.05$).

was placed into the cage for an 8 min interaction test. For prosocial behavior, a GLM with Species and Sex as fixed factors revealed a main effect of Species ($F_{(1,50)} = 17.97$, $p < 0.01$; Figure 2A), such that spiny mice spent significantly more time than gerbils exhibiting prosocial behavior. For aggressive behavior, analyses also yielded a main effect of Species ($F_{(1,50)} = 18.35$, $p < 0.01$; Figure 2B), showing that gerbils exhibited significantly more aggression than spiny mice. We observed no effects of sex in either species in the social interaction test.

Together these behavioral findings reflect the behavioral ecology of spiny mice and gerbils, such that the large-group living spiny mouse is more gregarious, affiliative, and prosocial in nonreproductive social contexts compared to the small-group living gerbil.

Experiment 2

In a different cohort of animals than Experiment 1, we conducted an IEG study to examine neural responses to a nonreproductive social interaction compared to a novel object in female and male spiny mice and gerbils. Animals were placed into a novel cage, and then either a novel, same-sex conspecific or a novel object (fastened binder clips the size of a rodent) was immediately placed into the cage with the subject for 30 min. The conspecific or object was removed after 30 and 60 min later subjects were perfused to capture Fos expression in response to the stimulus exposure.

Spiny mice and gerbils exhibit similar PVN OT neural responses to nonreproductive social interactions

Brain tissue was immunohistochemically stained for Fos and OT to allow for quantification of OT-Fos colocalized cells in the PVN. The percentage of PVN OT cells colocalized with Fos were quantified for two brain sections and an average was obtained for analyses. We used a percentage of colocalized cells to account for species differences in overall OT cell number. This was conducted for both rostral and caudal portions of the PVN for which we have previously found functional differences in prairie voles (Kelly et al., 2018). Representative images of rostral and caudal sections are shown in Figure 3.

We first sought to examine species differences in the number of PVN OT-ir cells. An independent t-test showed that spiny mice have significantly more rostral PVN OT-ir cells than gerbils ($t(62) = -8.86$; $p < 0.01$; Figure S1A. PVN OT-ir and VTA TH-ir cell numbers), whereas the number of caudal PVN OT-ir cells did not differ between species ($t(62) = -1.87$; $p = 0.07$; Figure S1B. PVN OT-ir and VTA TH-ir cell numbers). Next, we examined PVN OT-Fos colocalization to examine responses to exposure to a novel conspecific or a novel object. A GLM with Species, Sex, and Condition as fixed factors revealed a main effect of Condition for the percentage of rostral ($F_{(1,63)} = 43.17$, $p < 0.01$; Figure 4A) and caudal ($F_{(1,63)} = 87.93$, $p < 0.01$; Figure 4B) PVN OT-Fos colocalized cells, such that both spiny mice and gerbils showed a higher percentage of rostral and caudal PVN OT-Fos colocalization when exposed to a novel, same-sex conspecific compared to a novel object. These findings suggest that PVN OT cells differentially respond to social and nonsocial novelty similarly in spiny mice and in gerbils.

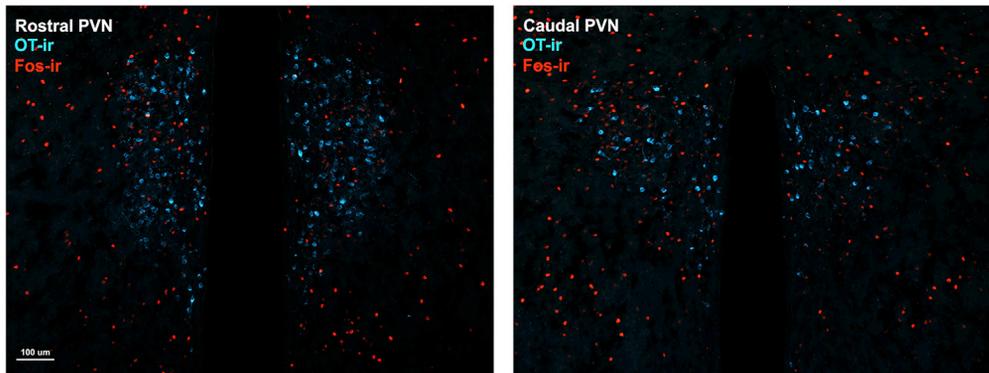


Figure 3. Representative images for PVN OT-Fos cell counts

Representative photomicrographs (10x magnification) of rostral and caudal brain sections containing the PVN. Regions of interest were not used for cell counts and instead all OT + cells in the PVN were quantified. Images were pseudocolored light blue for OT and red for Fos. Images shown are from a female gerbil. Scale bar represents 100μm.

Spiny mice and gerbils exhibit differential VTA TH neural responses to nonreproductive social interactions

Brain tissue was immunohistochemically stained for Fos and TH to allow for quantification of TH-Fos colocalized cells in the VTA. As has been shown for most species, VTA TH cells in spiny mice and gerbils have a dopaminergic phenotype (see [Figure S2](#). Colocalization of DOPA-decarboxylase and TH in the VTA), and thus this brain region reflects a key node in reward circuitry ([Ahmed et al., 2012](#); [Ranaldi, 2014](#)).

The percentage of VTA TH cells colocalized with Fos were quantified for two brain sections per brain and an average was obtained for analyses. We used a percentage of colocalized cells to account for any potential species differences in overall TH cell number. This was conducted for both rostral and caudal portions of the VTA. Regions of interest (ROI) are represented as white boxes in the images in [Figure 5](#). Unlike cell counts for the PVN OT cell group, we chose to use ROIs for the VTA TH cell group because the TH cell group of the substantia nigra is immediately adjacent to the VTA and we wanted to avoid accidental inclusion of those cells. Because we observed no differences in responses between rostral and caudal portions of the VTA, a single average of all four brain sections is presented below.

Again, we first sought to examine species differences in the number of VTA TH-ir cells. An independent t-test showed that spiny mice have significantly more VTA TH-ir cells than gerbils ($t(62) = -4.22$; $p < 0.01$; [Figure S1C](#). PVN OT-ir and VTA TH-ir cell numbers). Next, we examined VTA TH-Fos colocalization to examine responses to exposure to a novel conspecific or a novel object. A GLM with Species, Sex, and Condition (novel object or novel conspecific) as fixed factors revealed a significant Species by Condition interaction for the percentage of VTA TH-Fos colocalized cells ($F_{(1,63)} = 81.88$, $p < 0.01$; [Figure 6](#)). Within species, Bonferroni posthoc analyses showed that TH neural responses differed by Condition in spiny mice, but not gerbils, such that spiny mice that interacted with the novel, same-sex conspecific exhibited a higher percentage of TH-Fos colocalized cells in the VTA than those that interacted with the novel object ($p < 0.01$). Condition did not significantly influence gerbil VTA TH neural responses ($p = 0.67$). Between species, posthoc analyses revealed that in animals exposed to the novel, same-sex conspecific, spiny mice exhibited a higher percentage of VTA TH-Fos colocalized cells than gerbils ($p < 0.01$), whereas no difference between species was observed in animals exposed to the novel object ($p = 0.90$).

These findings suggest that spiny mice exhibit a greater reward response to social novelty in a nonreproductive context than gerbils.

Brain-behavior correlations suggest differential functions for social and reward circuitry in spiny mice and gerbils

In order to examine brain-behavior relationships in a nonreproductive social context, prosocial and aggressive behavior was quantified during the first 10 min of the IEG study for subjects that interacted with a novel, same-sex conspecific. Because of the absence of sex differences in neural responses just reported, we

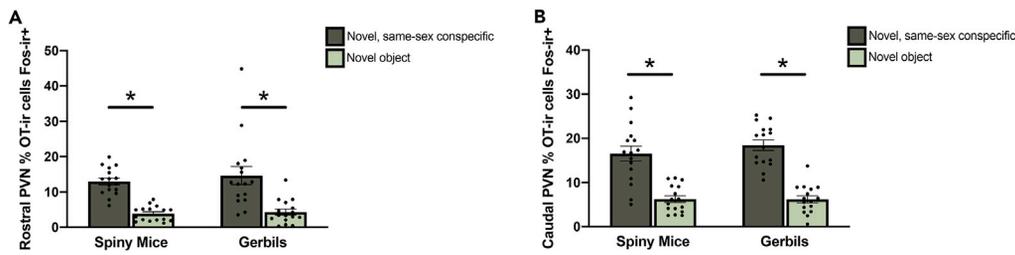


Figure 4. PVN OT neural responses

Spiny mice and gerbils exposed to a novel, same-sex conspecific (dark green) exhibited a higher percentage of rostral (A) and caudal (B) PVN OT-Fos colocalized cells than animals exposed to a novel object (light green). Data are represented as mean (\pm SEM). Dots represent individual data points. * indicates statistical significance ($p < 0.05$).

collapsed sex within each species, thereby increasing statistical power, and conducted linear regressions to determine the relationships between neural responses and behavior.

We analyzed brain-behavior relationships for PVN OT-Fos colocalization. Although magnocellular and parvocellular neurons have not been formally characterized in spiny mice or gerbils, the rostral sections quantified here primarily encompass the magnocellular component, whereas the caudal sections primarily encompass the parvocellular component, as described in rats by (Swanson and Kuypers, 1980; Swanson and Sawchenko, 1983). Notably, the magnocellular component of the PVN primarily sends axonal projections to the pituitary to exert effects on the periphery and modulate the stress response, whereas the parvocellular component primarily sends axonal projections throughout the brain to modulate behavior (Brown et al., 2013; Engelmann et al., 2004; Landgraf and Neumann, 2004; Sivukhina and Jirkowski, 2016).

Analyses did not yield significant correlations for the percentage of rostral PVN OT-Fos colocalization and prosocial or aggressive behavior for spiny mice (all $p > 0.15$). However, for gerbils, we did observe a positive correlation between rostral PVN OT-Fos colocalization and aggression (Pearson's correlation: $r = 0.65$; $p < 0.01$; Figure 7A) and a negative correlation between rostral PVN OT-Fos colocalization and prosocial behavior (Pearson's correlation: $r = -0.62$; $p = 0.01$; Figure 7B). We used a threshold of greater than or less than 3 standard deviations from the mean to identify outliers; no subjects exhibited values that were greater or less than 3 standard deviations from the mean. However, one gerbil subject was greater than 2 standard deviations outside the mean for the percentage of rostral PVN OT-Fos colocalization and thus may be driving our significant results. With this subject removed from analyses, we still observed a significant positive correlation between rostral PVN OT-Fos colocalization and aggression (Pearson's correlation: $r = 0.58$; $p = 0.02$). However, with this subject removed, the negative relationship between rostral PVN OT-Fos colocalization and prosocial behavior became non-significant (Pearson's correlation: $r = -0.42$; $p = 0.12$). These findings for the rostral portion of the PVN OT cell group may primarily reflect magnocellular OT neuronal modulation of stress response, suggesting that gerbils, but not spiny mice, may find nonreproductive social interactions stressful.

In spiny mice only, we did not observe a significant relationship with aggression (Pearson's correlation: $r = 0.38$; $p = 0.15$; Figure 7C), however, we found a positive correlation between caudal PVN OT-Fos colocalization and prosocial behavior (Pearson's correlation: $r = 0.79$; $p < 0.01$; Figure 7D). Caudal PVN OT-Fos colocalization did not significantly relate to behavior in the gerbils (all $p > 0.76$). These findings for the caudal portion of the PVN OT cell group may primarily reflect parvocellular OT modulation of social behavior in spiny mice.

Together, these findings suggest that although PVN OT neural responses are similar for both spiny mice and gerbils in relation to their responsiveness to social vs. nonsocial stimuli, the function of this neural activity may be quite different for the species and reflects their behavioral ecology.

Furthermore, analyses revealed a significant correlation for the percentage of VTA TH-Fos colocalization and prosocial behavior for spiny mice only (Pearson's correlation: $r = 0.70$; $p < 0.01$; Figure 8A). No significant correlation between VTA TH-Fos colocalization and prosocial behavior was observed for gerbils (Pearson's correlation: $r = 0.15$; $p = 0.58$; Figure 8B). Furthermore, VTA TH-Fos colocalization did not

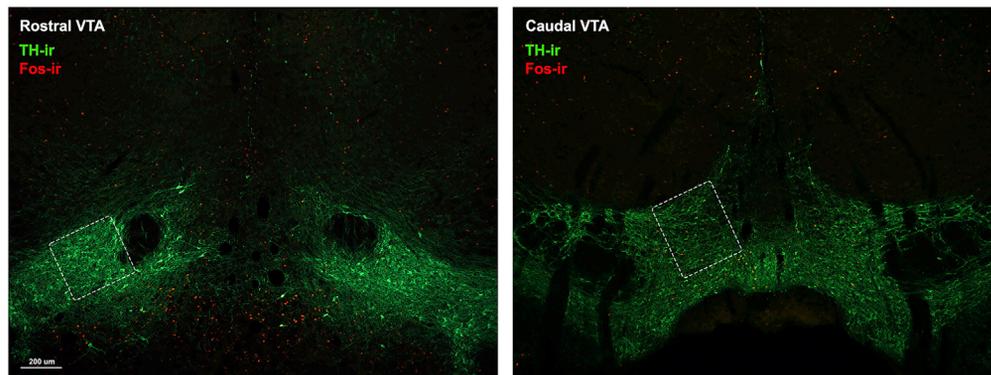


Figure 5. Regions of interest for VTA TH-Fos cell counts

Representative photomicrographs (5x magnification) of rostral and caudal brain sections containing the VTA. Regions of interest for cell counts are shown as a dashed white box. Images were pseudocolored green for TH and red for Fos. Images shown are from a male spiny mouse. Scale bar represents 200 μ m.

significantly relate to aggression in either species (all $p > 0.51$). These findings suggest that spiny mice, but not gerbils, may find social interactions with a novel, same-sex conspecific rewarding.

PVN-VTA correlations suggest functional connectivity in spiny mice

As has been shown for C57BL/6 mice, the PVN OT cell group contains neurons that send axonal projections to the VTA where OT is known to modulate dopamine release (Hung et al., 2017; Xiao et al., 2017). We injected Lumafuor retrobeads into the VTA of spiny mice and gerbils and found that, similar to rats and mice, both species also possess PVN OT cells that send projections to the VTA (Figure S3. PVN OT-retrobead colocalization; Figure S4. VTA retrobead injection). In both spiny mice and gerbils, only caudal PVN neurons showed OT-retrobead colocalization. Given that caudal PVN OT neurons send axonal projections to the VTA and both cell groups related to behavior in a nonreproductive social context in spiny mice, we investigated brain-brain relationships to reveal the potential for functional connectivity between the PVN OT and VTA TH cell groups. We found no significant relationships between the percentage of VTA TH-Fos colocalization and the percentage of rostral PVN OT-Fos colocalization in spiny mice or gerbils exposed to a novel, same-sex conspecific (all $p > 0.43$). However, we found that the percentage of VTA TH-Fos colocalization positively related to the percentage of caudal PVN OT-Fos colocalization in spiny mice (Pearson's correlation: $r = 0.92$; $p < 0.01$; Figure 9A), but not gerbils (Pearson's correlation: $r = 0.05$; $p = 0.87$; Figure 9B). Therefore, caudal PVN OT may mediate social reward in nonreproductive contexts via the VTA in spiny mice given that both cell groups positively relate to prosocial behavior and higher caudal PVN OT neural responses significantly relate to higher VTA TH neural responses.

DISCUSSION

In the present study, we show that spiny mice are more gregarious, more prosocial, and less aggressive than gerbils. Our findings from the group size choice test and the social interaction test illustrate the social phenotype of spiny mice and gerbils in nonreproductive social contexts, which mirrors the behavioral ecology of each species based on their species-typical group size in captivity. The examination of PVN OT neural responses yielded similar findings in spiny mice and gerbils, such that this cell group was more responsive to social than nonsocial stimuli, suggesting PVN OT may generally be involved in processing social salience. However, species differences in PVN OT relationships with behavior suggest functional delineation within the cell group, which likely correspond to parvocellular vs. magnocellular OT neurons that have differential projections and are largely involved in social behavior and stress response, respectively. Meanwhile, species differences in VTA TH responses suggest that spiny mice may find nonreproductive social interactions rewarding compared to nonsocial interactions, whereas reward circuitry does not differentially respond to social and nonsocial nonreproductive social interactions in gerbils. Finally, our finding that caudal PVN OT responses positively relate to VTA TH responses suggests functional connectivity between the cell groups and that PVN OT may gate social reward in nonreproductive contexts via influences on VTA reward circuitry in spiny mice.

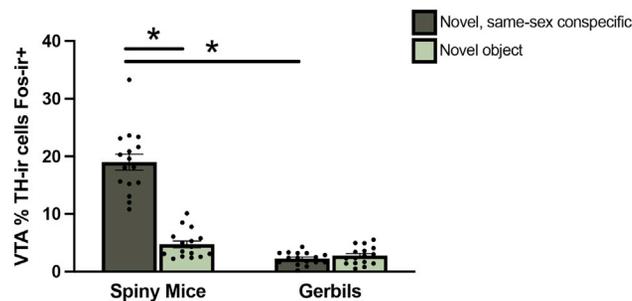


Figure 6. VTA TH neural responses

Spiny mice exposed to a novel, same-sex conspecific (dark green) exhibited a higher percentage of VTA TH-Fos colocalized cells than spiny mice exposed to a novel object (light green). Spiny mice exposed to a novel, same-sex conspecific also had greater VTA TH-Fos colocalization compared to gerbils. Data are represented as mean (\pm SEM). Dots represent individual data points. * indicates statistical significance ($p < 0.05$).

Species-typical group size and nonreproductive affiliation

Large group-living species evolved a gregarious phenotype for adaptive purposes and, as a consequence, animals that form large groups engage in nonreproductive social interactions more frequently than do those that live in small groups. Thus, social behavior in a nonreproductive context may have very different consequences dependent on species-typical group size. Nonreproductive affiliation aids in greater foraging efficiency, predatory defense, more effective homeostatic regulation, and collective traveling for large group-living species (Krause and Ruxton, 2002). Therefore, for a colonial spiny mouse, interacting in a prosocial manner with an unrelated and/or novel, same-sex conspecific has the potential to result in access to resources or safety. Additionally, because spiny mice are communal breeders and indiscriminately care for young pups regardless of genetic relation (Tuckova et al., 2016), a novel, same-sex conspecific may also serve as a potential alloparent to care for one's offspring. Conversely, for the territorial gerbil, an unrelated and/or novel, same-sex conspecific is more likely to be an intruder that poses as a threat for cuckoldry of one's mate, infanticide of one's offspring, or even death to the individual themselves (Agren, 1976; Elwood, 1977, 1980). Given the stark contrast in consequences of nonreproductive affiliation based on species-typical group size, it is no surprise that we observed that spiny mice were more prosocial and less aggressive than gerbils in a nonreproductive context in our study.

The social environment can serve as a stressor and/or as positive enrichment that relieves stress (Beery and Kaufer, 2015; Nakayasu and Kato, 2011). A stressful social environment can influence the hypothalamic-pituitary-adrenal (HPA) axis, leading to subsequent behaviors such as neophobia and social avoidance or flight (Carnevali et al., 2020), freezing (Roelofs et al., 2007), and/or aggression (Rodriguez-Romaguera and Stuber, 2018; Summers and Winberg, 2006). At a mechanistic level, the neural and physiological pathways mediating social behavior and stress are heavily intertwined (Beery and Kaufer, 2015; Dekkers et al., 2019). Here we observed a positive correlation in gerbils between rostral PVN OT-Fos colocalization and aggression, suggesting that stress may be mediating gerbils' responses to social novelty in a nonreproductive context. Conversely, we observed little to no aggression in the spiny mice, and rostral PVN OT responses did not correlate with spiny mouse behavior. If small and large group-living species experience differential costs and benefits to engaging with a novel conspecific, it is worth considering that the risk versus reward assessment of social novelty may also relate to a more domain-general distinction in risk/reward assessment and its underlying neuromolecular mechanisms (O'Connell and Hofmann, 2011). If the evolution of the social systems of these two rodents differentially co-opted relevant risk-reward neuromolecular mechanisms, the social behavior-reward integration would indeed diverge between spiny mice and gerbils given their differences in group size and life history as observed here. Notably, our results suggest that gerbils are not simply neophobic given that they spent a significant amount of test time engaging in either prosocial or aggressive behavior with the novel, same-sex conspecific. Similarly, in the IEG test, although we did not quantify behavior in animals exposed to the novel object (because we were specifically interested in social behavior), we found that every animal of both species approached and explored the novel object within just a few seconds of the novel object being placed into the subject's test cage. This suggests that behavioral differences, and differences in underlying neural mechanisms, may relate more to species-typical risk/reward assessment and is not solely driven by neophobia. Typically, the behavioral

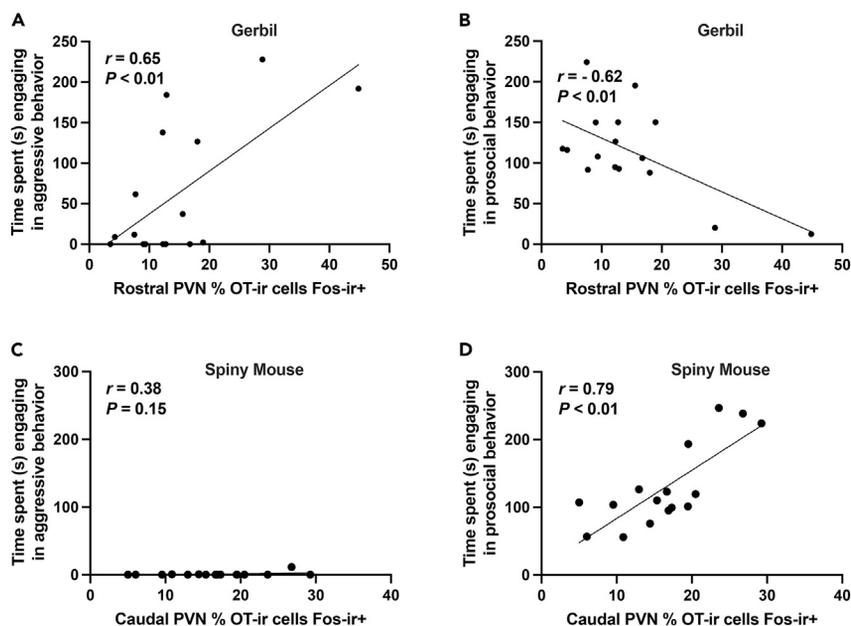


Figure 7. PVN OT – behavior correlations

In gerbils exposed to a novel, same-sex conspecific (A) aggressive behavior positively correlated with the percentage of rostral PVN OT-Fos colocalization and (B) prosocial behavior negatively correlated with the percentage of rostral PVN OT-Fos colocalization. (C) Spiny mice exposed to a novel, same-sex conspecific exhibited no relationship between aggression and caudal PVN OT-Fos colocalization, but (D) did exhibit a positive relationship between prosocial behavior and the percentage of caudal PVN OT-Fos colocalization.

ecology of risk/reward assessment has been explored through a reproductive lens (Wolf et al., 2007) or a foraging and predation lens (Lima and Dill, Verdolin, 2006). Our experiment provides a promising foundation for future investigations into differential risk/reward assessment strategies in large vs small group-living species in varying social contexts.

In the present study, we observed no effects of sex on gregariousness or nonreproductive affiliation. This is similar to our previous findings that showed that spiny mice are highly affiliative in both reproductive and nonreproductive contexts, regardless of whether the conspecific stimuli are novel or familiar (Fricker et al., 2021). To our knowledge, this is the first study to conduct a group size preference test with gerbils. Although we did not find any effects of sex on gregariousness or behavior in the social interaction test, female gerbils spent significantly more time in the middle of the chamber, not affiliating with any same-sex conspecifics, in the group size preference test compared to male gerbils. Prior studies in gerbils have shown that male gerbils exhibit aggressive behavior with novel conspecifics in reproductive and nonreproductive contexts, however, less aggression is observed in male-male interactions (Swanson, 1974). Alternatively, although aggressive in both contexts, females exhibit no difference in aggressive behavior in female-male or female-female interactions. Interestingly, the author of this study noted that “attempts at escape by jumping was characteristic of individual females” (Swanson, 1974). Together with our findings, this suggests that female gerbils may be more socially aloof than males. Furthermore, infanticide is more pronounced in female than male Mongolian gerbils (Saltzman et al., 2009), so avoidance of novel, female conspecifics may be particularly advantageous, whereas the consequences of affiliation with males may be less dire.

Although the behavioral ecology of gerbils, as well as previous studies, demonstrate that gerbils are territorial and are generally aggressive with novel, same-sex conspecifics, in both Experiment 1 and Experiment 2 of our study we observed considerable levels of prosocial behavior in male and female gerbils. Notably, we considered positive investigation to be a prosocial behavior and the gerbils engaged in high amounts of positive investigation in addition to positive side-by-side contact. After revisiting the gerbil behavioral videos, we noted that social interactions always began as either socially avoidant or positive. We did not observe immediate aggression at the beginning of tests. Further, if aggressive behavior

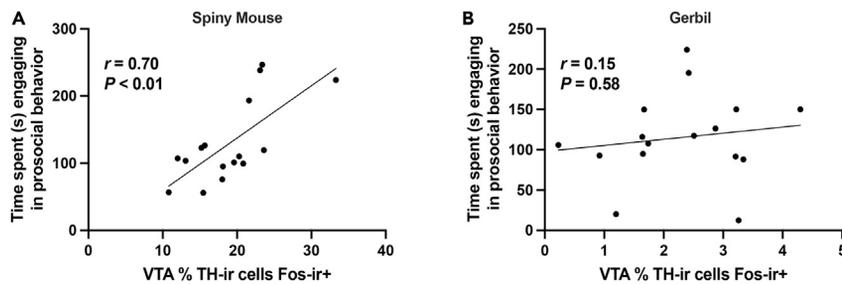


Figure 8. VTA TH – behavior correlations

(A) Spiny mice exposed to a novel, same-sex conspecific exhibited a positive correlation between the percentage of VTA TH-Fos colocalization and prosocial behavior, whereas (B) no significant correlation was observed for gerbils.

occurred, it followed a period of separation/avoidance, and not a period of positive investigation or side-by-side contact. Therefore, although certainly possible because we cannot interpret olfactory communication, we do not think that we mischaracterized prosocial behavior as aggressive behavior. It is also worth noting that data from our lab from a different study shows that once male and female gerbils are pair-bonded they engage in little to no prosocial behavior with novel, same-sex conspecifics (Kelly et al. *pers. obs.*). This would suggest a fundamental life history change in prosocial behavior that is associated with pair-bonding and the socially monogamous breeding systems of gerbils. Thus, our data here suggest that Mongolian gerbils may exhibit a more prosocial phenotype in nonreproductive contexts before pair-bonding.

PVN OT modulation of behavior

The OT cell group of the PVN is one of the largest nonpeptide neuronal populations in the brain. Containing magnocellular and parvocellular neurons allows this cell group to communicate with the periphery where it can regulate physiology as well as the CNS to modulate behavior (Engelmann et al., 2004; Landgraf and Neumann, 2004; Ludwig and Leng, 2006). Numerous studies have shown that PVN OT neurons are involved in modulating various social behaviors. For example, chemogenetic activation of PVN OT neurons enhances social investigation during a social choice test in transgenic mice (Resendez et al., 2020) and PVN OT neurons facilitate social learning and promote alloparental behavior in mice (Carcea et al., 2021). Similarly, PVN OT neurons are more responsive to exposure to a novel pup than a novel object in male prairie voles (Kenkel et al., 2012), suggesting this cell group may generally be involved in processing social over nonsocial stimuli. In zebra finches, PVN OT promotes both reproductive and nonreproductive social behaviors such that knockdown of OT synthesis in this region reduces gregariousness and impairs pair-bonding (Kelly and Goodson, 2014a). In addition to PVN OT-mediated social behavior, this cell group is also considered to have anxiolytic properties in mammals. Injection of OT into the PVN tempers anxiety and inhibits stress activation of the HPA axis in prairie voles during an elevated platform stressor (Smith et al., 2016) and in rats in an elevated plus maze and light-dark box (Blume et al., 2008). Furthermore, following a stressor, social support from a pair bond partner promotes OT release in the PVN of female prairie voles, suggesting that the PVN OT cell group mediates social buffering effects on the stress response (Smith and Wang, 2013). Although not quantified on a rostral-caudal axis, PVN OT neurons also exhibit greater Fos expression in California mice that experienced social defeat stress compared to those that were unstressed (Nasabuyan et al., 2018). Together, these findings demonstrate that the PVN OT cell group is multifaceted in how it modulates behavior.

Here we found that the PVN OT cell group was more responsive to novel social than novel nonsocial stimuli in both spiny mice and gerbils. Yet, how PVN OT neural responses relate to behavior varies by species depending on which component of the cell group is examined. We found that OT neural responsivity in the rostral PVN positively relates to aggression and negatively relates to prosocial behavior in the gerbils, whereas OT in the caudal PVN did not correlate with gerbil behavior. Because the rostral portion of this cell group primarily captures the magnocellular component of the PVN (as described in rats by Swanson and Kuypers, 1980; Swanson and Sawchenko, 1983), this finding suggests that the PVN OT activity we observed in the gerbils may reflect a stress response. Thus, gerbils may find novel, nonreproductive social interactions stressful, which aligns with their behavioral ecology given that gerbils are territorial (Gromov, 2008; Liu et al., 2009; Pan et al., 2020; Roper and Polioudakis, 1977). Future studies could specifically

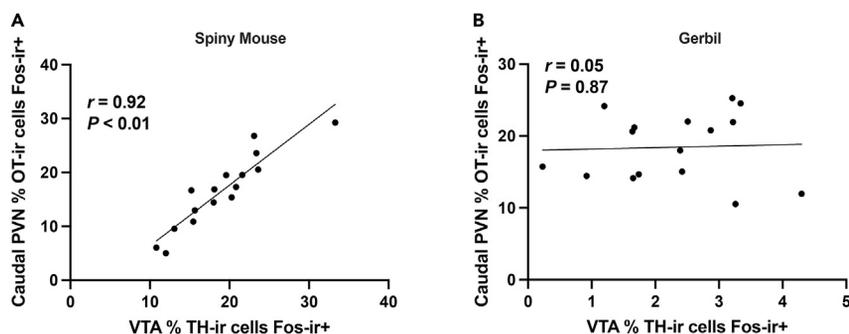


Figure 9. Caudal PVN OT – VTA TH correlations in spiny mice

(A) In spiny mice exposed to a novel, same-sex conspecific, the percentage of VTA TH-Fos colocalization positively correlated with the percentage of caudal PVN OT-Fos colocalization, whereas (B) no significant correlation was observed for gerbils.

examine HPA axis activity during novel social interactions in spiny mice and gerbils to determine whether novel social interactions are indeed stressful for gerbils, but not spiny mice. In addition to modulating the stress response, an increase in PVN OT has been linked with aggression in female rats, such that an infusion of OT into the PVN increases maternal aggression toward an intruder (Bosch et al., 2005) and PVN OT release is enhanced during maternal defense (Bosch et al., 2004). This is similar to our finding that rostral PVN OT neural responses positively correlated with aggression in the gerbils. Conversely, we observed no significant correlations between rostral PVN OT neural responses and behavior in the spiny mice. However, we did find that caudal PVN OT, which primarily captures the parvocellular component of the PVN, was positively related to prosocial behavior in the spiny mice. Although magnocellular PVN OT neurons send axonal projections mainly to the posterior pituitary as well as a few regions in the brain (i.e., caudate putamen, nucleus accumbens, and amygdala) (Zhang et al., 2021), parvocellular PVN OT neurons send axonal projections to the auditory cortex (Marlin et al., 2015) and throughout the mid- and hindbrain to modulate behavior (Liao et al., 2020; Swanson and Kuypers, 1980). Therefore, the relationship of caudal PVN OT neural responses to prosocial behavior in the spiny mice likely reflects a cascade of neuronal events downstream of the PVN OT cell group that led to the facilitation of prosocial behavior in the spiny mice.

The differences in brain-behavior relationships within and between the species we examined suggests functional delineation within the PVN OT cell group, which most likely corresponds to differential projections from magnocellular and parvocellular neurons. Although feasible, this does not explain why rostral PVN OT neurons were responsive to social stimuli, but did not correlate with behavior in the spiny mice, and why caudal PVN OT neurons were responsive to social stimuli, but did not correlate with behavior in the gerbils. However, perhaps rather than relating to the amount of behavior, PVN OT cells are directing attention, initiating signaling cascades, or increasing motivation to engage in a social interaction. Supporting this, the social salience hypothesis of OT was proposed as a theoretical framework to capture the role of oxytocin in regulating the salience of social cues; thus, OT directs attention to social context in order for the production of the most context-appropriate behavioral output (Shamay-Tsoory, 2010; Shamay-Tsoory and Abu-akel, 2015). Our finding that PVN OT-Fos colocalization was highest in both species in animals exposed to the social context fits within this framework. Therefore, PVN OT may, at first, simply direct an animal's attention to stimuli that are social in nature. Subsequently, downstream effects of that PVN OT neural activation may vary depending on social context and species-typical behavior.

Species-typical group size and social reward

In species that form large groups, prosocial behavior is more likely to lead to group cohesion and overall success of the group (Sumpter, 2006). Therefore, mechanisms may have evolved in large group-living species to reinforce prosocial behavior, not just in reproductive contexts, but also in nonreproductive contexts. In contrast, given that interactions with novel, same-sex conspecifics occur less frequently in small group-living species, and that territoriality and aggression in nonreproductive contexts are more common and potentially more advantageous, there is less of a need to reinforce prosocial behaviors during nonreproductive social interactions. Thus, we hypothesized that nonreproductive social interactions may be rewarding for large group-, but not small-, group living species. Indeed, our results show that the colonial spiny mouse exhibited greater VTA TH responses to a nonreproductive social interaction compared to a

novel object, whereas gerbil VTA TH responses did not differentiate between a novel, same-sex conspecific and a novel object. This suggests that spiny mice, but not gerbils, may find a nonreproductive social interaction rewarding.

There is a robust literature demonstrating the activation of VTA dopaminergic circuitry in response to [presumably positive] sociosexual behaviors. For example, sociosexual interactions increase TH-Fos colocalization in the VTA of birds (Bharati and Goodson, 2006; Charlier et al., 2005) and mammals (Balfour et al., 2004; Curtis and Wang, 2005; Kramer et al., 2017). However, fewer studies have examined VTA TH responses in nonreproductive contexts. A study in finches showed that species that flock in large groups have significantly more VTA TH neurons compared to species that are territorial (i.e., live in pairs or small family groups), suggesting that VTA dopaminergic circuitry may play a role in promoting grouping behavior (Goodson et al., 2009). This is consistent with our findings here that spiny mice have significantly more VTA TH-ir neurons than gerbils. It is worth considering whether spiny mice exhibited a greater reward response in our study simply because they have more VTA-ir neurons. However, we attempted to mitigate this possibility by quantifying a percentage of VTA TH-ir neurons expressing Fos in an ROI to avoid the confounding of a species difference in cell number. Regardless, having more cells available to modulate or reinforce behavior may nonetheless contribute to an animal's propensity to exhibit a particular behavioral phenotype. In male European starlings, densities of TH-ir in the VTA does not differ for animals within or outside of the breeding season, however, TH-ir density significantly relates to behavior only within the breeding season (Heimovics and Ritters, 2008). Such examples suggest that similarities may be observed in reward circuitry in reproductive and nonreproductive contexts; however, how those neural mechanisms titrate behavior may still vary based on intensity of a goal-direct behavior, which can be strongly influenced by social context. Furthermore, although familial in nature, social play between same-sex, juvenile female rat siblings elicit an increase in TH-Fos colocalization in the VTA (Northcutt and Nguyen, 2014). Because play behavior is reinforcing (Calcagnetti and Schechter, 1992; Douglas et al., 2004), this suggests that positive social behaviors outside the context of parenting or mating produce neural responses in reward circuitry similar to those of reproductive contexts. Together these studies suggest that VTA TH neurons respond to presumably positive stimuli, suggesting that spiny mice, but not gerbils, find nonreproductive social interactions rewarding.

Interestingly, some studies have shown that the VTA may also regulate agonistic behaviors. For example, a simulated territorial intrusion in male song sparrows increases the number of Fos-ir nuclei in the VTA, although notably this study did not specifically examine TH neural responses (Maney and Ball, 2003). Similarly, repeated exposure to social defeat results in greater TH neural responsivity in the ventral, but not dorsal, VTA of female California mice, suggesting that a component of the VTA is sensitive to aversive contexts (Greenberg et al., 2015). Although the nonreproductive social interaction in gerbils may have been at least somewhat aversive as indicated by the display of aggressive behavior and rostral PVN OT responses, we failed to observe significant responsivity in the VTA TH cell group. This may be because we did not quantify cell counts on a dorsal-ventral axis and/or a single exposure is not sufficient to induce a VTA dopaminergic response. Greenberg et al. reported that a single episode of social defeat in California mice was not sufficient to elicit increased TH-Fos colocalization in the VTA, and rather, repeated social defeat was required for such a response (Greenberg et al., 2015). Alternatively, it is also possible that gerbils may find a nonreproductive social interaction to possess a neutral valence or to be less aversive than California mice, which are highly aggressive and territorial outside interactions with pair bond partners and offspring (Ribble and Salvioni, 1990). Intuitively, it would be advantageous to reinforce any behavior, positively or negatively valenced, that is adaptive for a species. Future studies could incorporate repeated social exposures to examine the trajectory of agonistic behavior and VTA TH neural responses in gerbils to determine the degree of negative valence associated with nonreproductive affiliation and long-term neural consequences.

Oxytocin modulation of dopaminergic circuitry

OT has been widely studied in relation to social behavior (Caldwell, 2017; Donaldson and Young, 2008; Goodson and Thompson, 2010), and an increasing number of studies have been elucidating a role for OT actions on reward circuitry. OT receptors in the nucleus accumbens (NAc) are crucial for pair-bonding in the monogamous prairie vole (Young and Wang, 2004) and OT activity in the NAc facilitates the rewarding properties associated with performance in a social conditioned place preference (CPP) assay in mice (Dolen et al., 2013). Similarly, activation of OT receptors in the VTA is necessary for the rewarding properties of performance in a social CPP assay in male Syrian hamsters (Song et al., 2016). In addition, both

the application of OT in the VTA and optogenetic stimulation of OT terminals in the VTA increases DA neuron activity (assessed via electrophysiological recordings) in mice, demonstrating that OT has the potential to modulate VTA reward circuitry (Xiao et al., 2017). Tracing studies identified that a source (i.e., peptide producing neuronal population) of OT that is delivered to the VTA is that of the PVN OT cell group, which sends axonal projections to the VTA (Beier et al., 2015). A subsequent experiment then demonstrated that PVN OT modulates behavior via actions on the VTA, such that inhibiting PVN OT neuron projections to the VTA reduces the preference for a social over a nonsocial context and reduces the time a mouse spends interacting with a juvenile conspecific (note that juvenile stimuli are frequently used in C57BL/6 mice to reduce the potential for aggression (Grifols et al., 2020) (Hung et al., 2017)). This experiment also showed that OT release in the VTA specifically enhances the activity of a specific population of DA neurons that influence social behavior (Hung et al., 2017).

In mice, PVN OT neurons send axonal projections to the VTA (Hung et al., 2017; Xiao et al., 2017). Here we showed via retrograde tracing that PVN OT neurons also send axonal projections to the VTA in spiny mice and gerbils. Therefore, PVN OT is also poised to influence VTA dopaminergic activity in the species used for this study. We only observed retrobead-OT colocalization in the caudal PVN, which likely primarily captures the parvocellular component of the PVN. This may explain why we failed to observe a significant relationship between rostral PVN OT responses and VTA TH responses, yet did find a significant positive correlation between caudal PVN OT and VTA TH responses in spiny mice. This correlation between the cell groups suggests the potential for functional connectivity that may occur via anatomical connectivity. Therefore, in spiny mice, given that caudal PVN OT neural responses and VTA TH neural responses both positively correlated with prosocial behavior, and because caudal PVN OT responses positively related to VTA TH responses, our data suggest that caudal PVN OT neurons may gate social reward in a nonreproductive context via influences on VTA dopaminergic circuitry in the colonial spiny mouse.

There are several possible explanations for the lack of significant findings for functional connectivity in the gerbils. First, we may have failed to observe any PVN OT – VTA TH correlations in the gerbils because they mounted a very minimal VTA TH response to exposure to a novel, same-sex conspecific.

Alternatively, it is possible that the PVN OT neurons that were active in the gerbils were not the OT neurons that send axonal projections to the VTA. So, despite finding that the PVN OT neural responses were similar to social vs. nonsocial stimuli in spiny mice and gerbils, distinct neuronal ensembles within the PVN OT cell group may have been differentially activated across the species. It is also worth considering whether there is the potential for something that is inherently not rewarding to a species to result in downregulation of OTR receptors on the membrane or an increase in local inhibition within VTA, therefore inhibiting reward circuitry from being activated.

Although there are an increasing number of elegant studies demonstrating that OT gates social reward by acting on reward circuitry, our findings are unique such that we used species that naturally exhibit higher degrees of prosociality and our results reflect the natural behavioral ecology of spiny mice and gerbils. We were able to examine neural responses to freely behaving animals interacting with one another, whereas the experiments described above in mice (Dolen et al., 2013) and hamsters (Song et al., 2016) used social CPP assays where neural metrics were not obtained when two animals were freely interacting. This is likely because Syrian hamsters and C57BL/6 mice are quite aggressive with same-sex conspecifics (Elidio et al., 2021; Gaskill et al., 2017; Grifols et al., 2020; Weber et al., 2017). The preference to spend time in the socially conditioned chamber in a CPP assay may reflect social investigation or territoriality, and not necessarily prosocial (i.e., positive social) behavior. Given that data shows that agonistic stimuli can be rewarding to some species in certain contexts, it is interesting to consider whether the OT actions on VTA dopaminergic circuitry in those studies was the result of rewarding aspects related to aggression or territoriality, which would be more species-typical for Syrian hamsters and C57BL/6 mice. Our study contributes to this growing body of literature by adding an ethologically-relevant behavioral perspective, and suggests that the PVN OT cell group may reinforce prosocial behavior in highly gregarious, large-group living species via actions on VTA dopaminergic circuitry.

In our study, we did not observe effects of sex on any neural responses examined. It is possible that the lack of sex effects on PVN OT and VTA TH responses relate to the social context in which our studies were conducted. For example, perhaps evolutionary consequences of same-sex interactions are largely similar for

males and females, at least within the rodent species we examined. Reproductive contexts can be complex and may result in more variable outcomes (i.e., mate, co-parent, extrapair copulation opportunity, cuckoldry, infanticide, etc.) with different consequences for males and females, certainly physiologically, but also behaviorally, particularly for non-biparental species where females carry a greater burden for reproducing. Conversely, nonreproductive contexts may be more straightforward and novel individuals may be viewed as either friend or foe, and therefore there is less of a need for effects of sex to influence behavior in such contexts. However, our lack of observation of effects of sex on neural responses does not necessarily rule out differential regulation of nonreproductive social behavior (De Vries, 2004). Importantly, we only examined two neuronal cell groups in the brain, and thus it is quite possible that there are other nodes of social and reward circuits that are influenced by sex and simply were not identified in this study.

Conclusions

Using the gregarious, large group-living spiny mouse and small group-living Mongolian gerbil, here we show that social circuitry exhibits similar, but reward circuitry exhibits different, neural responses to a nonreproductive social context between the species. Brain-behavior relationships further reveal that the gerbils may find social interactions with same-sex conspecifics stressful, reflecting the territoriality they exhibit in the wild. Meanwhile, our results suggest that spiny mice, which are highly gregarious and live in large groups, find interactions with same-sex conspecifics rewarding. Further, caudal PVN OT responses positively relate to VTA TH responses in the spiny mice, suggesting that there is functional connectivity between the cell groups and that PVN OT may gate social reward in nonreproductive contexts via influences on VTA reward circuitry in spiny mice. Together these findings suggest that a mechanism that may have evolved to promote large group-living is for colonial species to find nonreproductive social contexts rewarding.

Limitations of the study

In this study, we compare the brain and behavior of spiny mice and Mongolian gerbils. Although these species are related, an ideal comparison would be to use two species within the genus *Acomys*. However, the most closely related, non-colonial species to *A. cahirinus* that is readily available for testing in a laboratory is the Mongolian gerbil. If studies in the field identify non-colonial *Acomys* species, and if it is feasible to obtain permits to trap and transport said species to begin an in-house breeding colony in the United States, then future studies could compare species within the same genus to determine how species-typical group size influences the brain and behavior. Another limitation is that the results presented here are from an IEG study, which is correlational in nature. Further manipulative research is needed to obtain causal data to determine if and how the PVN-VTA circuit gates social reward in spiny mice.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.104230>.

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AUTHOR CONTRIBUTIONS

J.A.G.A. conducted behavioral tests, scored behavioral videos, and cryosectioned brains. A.R. designed the study and scored behavioral videos. B.A.F. scored behavioral videos and conducted retrobead tracing surgeries. K.J.W. contributed to writing the Discussion of the manuscript. A.W.S. provided feedback on and edited the manuscript. A.M.K. designed the study, conducted antibody validations, conducted immunohistochemistry, conducted microscopy and cell counts, analyzed the data, wrote the manuscript, and as principal investigator, obtained the funding.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-oxytocin	Millipore	Catalog# MAB5296; RRID: AB_2157626
Mouse anti-tyrosine hydroxylase	Millipore	Catalog# MAB318; RRID: AB_2201528
Rabbit anti-Fos	Synaptic Systems	Catalog# 226 003; RRID: AB_2231974
Rabbit anti-AADC	Millipore	Catalog# AB1569; RRID: AB_90789
Bacterial and Virus Strains		
Red retrobeads	Lumafuor	https://lumafuor.com/Home_PAGE.php
Experimental Models: Organisms/Strains		
Mongolian gerbil (<i>Meriones unguiculatus</i>)	Charles River Laboratories	https://www.criver.com
Spiny mouse (<i>Acomys cahirinus</i>)	Colony bred at Emory University by Kelly Lab	N/A
Software and Algorithms		
Prism 8.0	GraphPad	RRID: SCR_002798
SPSS 27	IBM	RRID: SCR_002865

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Aubrey Kelly (aubrey.kelly@emory.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

For Experiment 1, ten adult male and fifteen adult female spiny mice (*A. cahirinus*) ranging from PND 80–170 and ten adult male and sixteen adult female Mongolian gerbils (*M. unguiculatus*) ranging from PND 80–140 were used for the purposes of the behavioral study. For Experiment 2, an additional sixteen adult male and sixteen adult female spiny mice and sixteen adult male and sixteen adult female gerbils ranging from PND 100–200 were used for the immediate-early gene study.

All spiny mice were obtained from our breeding colony, and breeders were obtained from the captive bred colony of Dr. Ashley W. Seifert (University of Kentucky). All Mongolian gerbils were obtained as young adults (PND 50–65) from Charles River Laboratories. Spiny mice were group housed (2–5) with same-sex littermates in standard rat polycarbonate cages (40.64 cm × 20.32 cm × 20.32cm) or two-level polycarbonate cages (32 cm × 38 cm × 40cm). Gerbils were group housed (2–4) with same-sex littermates in standard rat polycarbonate cages (40.64 cm × 20.32 cm × 20.32cm). All rodent cages were lined with Sani-Chips bedding and included nesting material, rodent igloos, and shepherd shacks. Animals were able to obtain food and water *ad libitum* and were kept on a 14-h light: 10-h dark cycle. Ambient temperatures were maintained at 24 ± 2°C. All procedures were approved by the Institutional Animal Care and Use Committee of Emory University (Protocol, 201,900,126).

METHOD DETAILS

Behavioral tests and quantification

For the group size preference test, subjects were given a choice to affiliate with either a large (8) or small (2) group of novel, same-sex conspecifics; we previously validated this test in our lab (Fricker et al., 2021). This test is used to assess the degree of gregariousness (a preference to affiliate with large groups) of an individual or species (Fricker et al., 2021; Kelly and Goodson, 2013). Subjects were tested in a large Plexiglas arena (60.96 cm × 45.72 cm X 38.1cm) with a large stimulus group on one end and a small stimulus group on the opposite end. All stimulus animals were restrained under wire mesh containers and were allowed to acclimate for 3 min prior to the start of testing. At the start of testing, subjects were placed under a plastic beaker in the center of the arena and were subsequently released and video recorded using Sony Handycam cameras for 8 min. Time spent affiliating within one body length of the large and small groups of conspecifics was quantified. Additionally, time spent in the middle zone of the chamber, equidistance away from the large and small stimulus groups, was quantified.

For the social interaction test, subjects were placed in a novel standard rat polycarbonate cage (40.64 cm × 20.32 cm X 20.32cm) and allowed to acclimate for 3 min. A novel, same-sex conspecific was then placed into the testing cage at the opposite end of the subject. The subject and stimulus animal were allowed to freely interact during an 8 min test, which was video recorded with Sony Handycam cameras. Behavioral videos were scored for prosocial (positive investigation, positive side-by-side contact, huddling, allogrooming) and aggressive (chasing, biting, lunging, pinning, aggressive side-by-side contact) behaviors.

Immediate-early gene experimental design

This study aimed to assess behavioral and neural responses during a social interaction in a nonreproductive context. Subjects underwent a modified social interaction test as described above. Subjects were placed into a novel standard rat cage and then either a novel, same-sex conspecific or a novel object (fastened binder clips the size of a rodent) was immediately placed into the cage with the subject for 30 min. Behavior was video recorded using Sony Handycam cameras. Prosocial and aggressive behavior were scored as described above for the first 10 min of the social interaction; this time period most closely corresponds to the immediate-early gene responses quantified. The conspecific or object was removed from the test cage after 30 and 60 min later subjects were perfused to capture Fos expression in response to the stimulus exposure. Sample sizes for treatment groups in the IEG experiment were: n = 8 male spiny mice exposed to a novel, same-sex conspecific; n = 8 female spiny mice exposed to a novel, same-sex conspecific; n = 8 male spiny mice exposed to a novel object; n = 8 female spiny mice exposed to a novel object; n = 8 male gerbils exposed to a novel, same-sex conspecific; n = 8 female gerbils exposed to a novel, same-sex conspecific; n = 8 male gerbils exposed to a novel object; n = 8 female gerbils exposed to a novel object.

Histology and immunohistochemistry

At the end of the IEG experiment, subjects were euthanized by isoflurane overdose and were transcardially perfused with 0.1M PBS (PBS) followed by 4% paraformaldehyde dissolved in 0.1M borate buffer (pH 9.5). Brains were extracted, post-fixed overnight in 4% paraformaldehyde dissolved in 0.1M borate buffer (pH 9.5) before cryoprotection in 30% sucrose dissolved in PBS for 48 h. Brains were then frozen in Tissue-Tek O.C.T. Compound (Sakura Finetek) in Peel-A-Way molds and stored at −80°C. Prior to immunohistochemical processing, brains were thawed and sectioned coronally at 40μm using a Leica cryostat, with every third section being saved for use in the present study.

Hypothalamic tissue sections were immunofluorescently stained for OT and Fos and tissue sections containing the VTA were stained for TH and Fos following previously established protocols (Hiura et al., 2018; Kelly et al., 2018; Kelly and Seifert, 2021). Tissue was rinsed 5x for 10 min in 0.1M PBS (pH 7.4), incubated for 1 h in a blocking solution (PBS +10% normal donkey serum +0.03% Triton X-100), and then incubated for approximately 48 h in primary antibodies diluted in PBS containing 5% normal donkey serum +0.03% Triton X-100. Primary antibodies used were mouse anti-OT (Millipore; 1:1000), rabbit anti-Fos (Synaptic Systems; 1:500), and mouse anti-TH (Millipore; 1:1000). The primary incubation was followed by two 30 min rinses in PBS. Tissue was then incubated for 2 h at room temperature in a donkey anti-mouse secondary conjugated to Alexa Fluor 488 (3:1000) and a donkey anti-rabbit secondary conjugated to Alexa Fluor 594 (4:1000). All secondary antibodies were diluted in PBS containing 5% normal donkey

serum +0.03% Triton-X-100. Alexa Fluor conjugates were obtained from ThermoFisher Scientific (Waltham, MA). Following two 30 min rinses in PBS, sections were mounted on microscope slides and cover-slipped with Prolong Gold antifade containing a DAPI nuclear stain (ThermoFisher Scientific).

Although tyrosine hydroxylase (TH) is widely used as a marker for dopaminergic neurons, many TH cells in the brain do not synthesize dopamine (DA) or catecholamines (Bjorklund and Dunnett, 2007). In order for cells to exhibit a dopaminergic phenotype they must colocalize TH and aromatic L-amino acid decarboxylase (AADC) – the enzyme necessary for the conversion of dihydroxyphenylalanine (DOPA) to DA (Ahmed et al., 2012; Bjorklund and Dunnett, 2007; Kitahama et al., 1990; Skagerberg et al., 1988). We examined the existence of cells that were immunoreactive for both TH and AADC in the ventral tegmental area (VTA) of one male and one female spiny mouse and one male and one female gerbil. Tissue was immunofluorescently stained for TH and AADC. Tissue was rinsed 5x for 10 min in 0.1M PBS (pH 7.4), incubated for 1 h in a blocking solution (PBS +10% normal donkey serum +0.03% Triton X-100) to prevent non-specific binding, and then incubated for approximately 48 h in primary antibodies diluted in PBS containing 5% normal donkey serum +0.03% Triton X-100. Primary antibodies used were mouse anti-TH (Millipore; 1:1000) and rabbit anti-AADC (Millipore; 1:1000). The primary incubation was followed by two 30 min rinses in PBS. Tissue was then incubated for 2 h at room temperature in a donkey anti-mouse secondary conjugated to Alexa Fluor 488 (3:1000) and a donkey anti-rabbit secondary conjugated to Alexa Fluor 594 (4:1000). All secondary antibodies were diluted in PBS containing 5% normal donkey serum +0.03% Triton-X-100. Alexa Fluor conjugates were obtained from ThermoFisher Scientific (Waltham, MA). Following two 30 min rinses in PBS, sections were mounted on microscope slides and cover-slipped with Prolong Gold antifade containing a DAPI nuclear stain (ThermoFisher Scientific).

Western blot characterization of antibodies

We previously validated the efficacy of the OT antibody used in the present study in spiny mouse brain tissue (Kelly and Seifert, 2021). To confirm that the OT (for gerbils), TH, Fos, and AADC antibodies bind specifically to spiny mouse and gerbil OT, TH, Fos, and AADC, respectively, we provided fresh frozen whole male and female spiny mouse and Mongolian gerbil brains and antibodies to RayBiotech (Atlanta, GA) for auto-western processing. Briefly, total cellular proteins were extracted with phosphate inhibitors and protease. To denature proteins, samples were prepared using a standard boiling procedure with SDS and beta-mercaptoethanol. Samples and reagents were loaded into an assay plate and placed into a capillary immunoassay western machine. A minimum of 40nL of sample was automatically loaded into the capillary and separated by size as they migrated through a stacking and separation matrix. The separated proteins were immobilized to the capillary wall via proprietary, photoactivated capture chemistry. Target peptides were identified using the primary antibodies and immunoprobed using HRP-conjugated secondary antibodies.

Western blots yielded similar results for male and female tissue (Figure S5. Western blot characterization of antibodies). Representative results from the western blots run on female tissue are shown below. For the mouse anti-OT, we observed a dark band at 2 kDa (kDa). The National Institute of Health's (NIH) National Library of Medicine reports the molecular weight of OT as 1.007 kDa (NCfB, 2020). Additionally, western blot analysis revealed a dark band at ~60 kDa for the mouse anti-TH; TH has been reported to have a molecular weight of 60kDa (Mogi et al., 1986). For the rabbit anti-Fos, we observed a faint band at 61kDa; Fos has been reported to have an observed molecular weight between 60 and 70kDa (Chiu et al., 1988). Furthermore, for the rabbit anti-AADC, we observed bands at ~56-58kDa; AADC has been reported to have a molecular weight of 58 kDa (Yuwen et al., 2013).

Antibody characterization

We conducted specificity controls on the primary antibodies, all of which yielded expected molecular weights in the western analyses (e.g., mouse anti-OT, mouse anti-TH, rabbit anti-Fos, and rabbit anti-AADC). Primary antibodies were preadsorbed with 50 μ M OT (Sigma Aldrich), 50 μ M TH (Novus Biologicals), 50 μ M Fos (Invitrogen), and 50 μ M AADC (generated by Genscript from Entrez Gene Number NM_000,790.3, which corresponds to the sequence used for antibody development) at room temperature for 4 h prior to overnight tissue incubation. Immunoreactive staining was eliminated in preadsorbed controls confirming specificity of primary antibodies in spiny mouse and gerbil tissue.

Neural quantification

Photomicrographs were obtained using a Zeiss AxioImager II microscope outfitted with an AxioCam MRm, z-drive, and an Apotome optical dissector (Carl Zeiss Inc., Gottingen, Germany). z stack images were

flattened and processed with Zen Pro software (Carl Zeiss Inc., Gottingen, Germany). Designation of neuro-anatomical structures was based on Paxinos and Franklin's mouse brain atlas (Paxinos et al., 2009). Cell counts were conducted in Photoshop CS6 (Adobe Systems, San Jose, CA) and ImageJ (National Institutes of Health, Bethesda, MD).

For PVN OT cell counts, we quantified the total number of OT-ir neurons and the number of OT-ir neurons that expressed Fos-ir in the PVN at rostral and caudal levels. For each the rostral and caudal levels, cell counts were quantified for two tissue sections that were 200 μ m apart and an average of the cell counts from the two sections was used for statistical analysis. The distance between the last rostral tissue section and the first caudal tissue section was 440 μ m. As had been found previously (Kelly et al., 2018), functional differences were observed for rostral and caudal components of the PVN OT cell group and were therefore analyzed separately in the present study.

For VTA TH cell counts, we quantified the number of TH-ir neurons and the number of TH-ir neurons that expressed Fos-ir in regions of interest of the VTA at rostral and caudal levels. For each the rostral and caudal levels, cell counts were quantified for two tissue sections that were 200 μ m apart and an average of the cell counts from the two sections was used for statistical analysis. The distance between the last rostral tissue section and the first caudal tissue section was 320 μ m. We observed no significant differences between rostral and caudal cell counts and thus an average of all four tissue sections quantified was used for analysis.

Surgical procedure

We used red fluorescently tagged beads (Retrobeads; Lumafluor) as retrograde tracers to map anatomical projections to the VTA. Briefly, one gerbil and one spiny mouse were administered analgesics and anesthetized under 2–4% isoflurane. Once deeply anesthetized, animals were maintained at 0.5–4% isoflurane in oxygen via a metal mask. Animals were secured in a digital stereotaxic frame (Kopf) and body temperature was maintained at 37 °C using a far infrared heating pad (Kent Scientific). A midline incision in the scalp of the animal was made with microscissors, allowing access to the skull. 1mm holes were drilled into the skull using a surgical drill (Kopf). Coordinates for Mongolian gerbils were obtained from the gerbil brain atlas (Radtke-Schuller et al., 2016) and rostral and caudal sites of the VTA were targeted (rostral coordinates: from bregma, –3.75 anterior-posterior, 0.7 lateral, –6.50 ventral; caudal coordinates: from bregma, –4.10 anterior-posterior, 0.40 lateral, –6.80 ventral). Coordinates for spiny mice were modified accordingly based on of the Paxinos and Franklin mouse brain atlas (Paxinos et al., 2009) and beads were injected at rostral and caudal sites of the VTA (rostral coordinates: from bregma, –2.98 anterior-posterior, 0.3 lateral, –5.25 ventral; caudal coordinates: from bregma, –3.18 anterior-posterior, 0.40 lateral, –4.10 ventral). A pulled glass pipet containing 1 μ L of red retrobeads was lowered through the holes in the skull using the stereotactic manipulator. A volume of 400nL of retrobeads diluted 1:1 in sterile isotonic saline was injected into each target region at a rate of 0.1 μ L/min via a Nanoject injector. After injection, the needle remained in place for 10 min while the injected substances diffused through the tissue. After completion of surgical injections, the skin was closed via sutures (for gerbils) or VetBond (for spiny mice) and disinfected.

After a survival period of 7 days to allow for axonal transport of the tracer, animals were overdosed on isoflurane and transcardially perfused. Brains were post-fixed overnight in 4% paraformaldehyde dissolved in 0.1M borate buffer (pH 9.5) before cryoprotection in 30% sucrose dissolved in PBS for 48 h. Brains were then cryosectioned at 40 μ m and processed via immunohistochemistry to stain for OT as described above.

QUANTIFICATION AND STATISTICAL ANALYSIS

Behavioral and neural data were analyzed using General Linear Models (GLM) and Pearson correlations. All posthoc pairwise comparisons were adjusted using the Bonferroni correction. All data were analyzed using SPSS 27 (IBM Analytics, USA) and graphs were made using Prism 8 (GraphPad, USA).