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ORIGINAL INVESTIGATION

Early life social complexity shapes adult neural processing in the communal spiny mouse *Acomys cahirinus*

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Abstract

Rationale Early life social rearing has profound consequences on offspring behavior and resilience. Yet, most studies examining early life development in rodents use species whose young are born immobile and do not produce complex social behavior until later in development. Furthermore, models of rearing under increased social complexity, rather than deprivation, are needed to provide alternative insight into the development of social neural circuitry.

Objectives To understand precocial offspring social development, we manipulated early life social complexity in the communal spiny mouse *Acomys cahirinus* and assessed long-term consequences on offspring social behavior, exploration, and neural responses to novel social stimuli.

Methods Spiny mouse pups were raised in the presence or absence of a non-kin breeding group. Upon adulthood, subjects underwent social interaction tests, an open field test, and a novel object test. Subjects were then exposed to a novel conspecific and novel group and neural responses were quantified via immunohistochemical staining in brain regions associated with social behavior.

Results Early life social experience did not influence behavior in the test battery, but it did influence social processing. In animals exposed to non-kin during development, adult lateral septal neural responses toward a novel conspecific were weaker and hypothalamic neural responses toward a mixed-sex group were stronger.

Conclusions Communal species may exhibit robust behavioral resilience to the early life social environment. But the early life environment can affect how novel social information is processed in the brain during adulthood, with long-term consequences that are likely to shape their behavioral trajectory.

Keywords Spiny mouse · Fos · pERK · Neural response · Social development · Communal breeding · Open field · Prosociality · Social behavior · Social reward

Introduction

The social environment is a landscape in which animals, including humans, make decisions with wide-ranging and potentially decisive effects on their future health and condition. For example, the decision to approach, avoid, or show aggression towards an unfamiliar individual has both immediate and long-term consequences. The real-time decision an individual makes is shaped by current factors such as their internal state (motivation, fear, anxiety) and by their external environment (characteristics of the other individual, familiar vs unfamiliar location). But the decision is also influenced tremendously by the individual's life history and prior experience.

There are many approaches to understanding how prior experience can shape social decision-making. One often-explored perspective has been to assess the role of the early life social environment on development and its long-lasting consequences far into adulthood. In some of the most robust studies in this area, investigations of maternal separation in rodents have shown profound effects on offspring stress responses (Macrì et al. 2008; Banqueri et al. 2017; Nishi 2020), socioemotional processing (Halladay and Herron 2022), dopamine signaling (Sasagawa et al. 2017), addictive

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behaviors (Moffett et al. 2007), memory (Tractenberg et al. 2016; Banqueri et al. 2017), aggression (Veenema et al. 2007), and depression-like behaviors (Tractenberg et al. 2016). More generally, prenatal environments (Kenkel et al. 2019) and early life social experience such as isolation or increased social complexity influence multiple dimensions of behavior in a variety of taxa including rats (Baarendse et al. 2013), marmosets (Dettling et al. 2002), and cichlids (Solomon-Lane and Hofmann 2019; Bannier et al. 2017).

Why does the early life social environment have such impactful and long-lasting consequences on offspring development? In mammals, early life is a particularly salient period of development due to levels of neurogenesis and plasticity in the brain that are not seen during adulthood (Wei et al. 2011; Bhardwaj et al. 2006; Gould 2007; Gage 2019; Bond et al. 2022). Yet, in most mammals (Derrickson 1992), including lab mice (see the NIH Office of Animal Care & Use JAX Mice Pup Appearance by Age Infographic), young are born altricial with closed eyes, closed ears, and little mobility. Precocial mammals, however, are much more mobile and independent early in life. For example, the spiny mouse *Acomys cahirinus* is born with open eyes and ears and sophisticated sensory and motor functions (Brunjes 1990). Thus, these pups experience and process a greater variety of stimuli at earlier developmental milestones. Despite this, extremely few studies have examined how the early life social environment affects social development of precocial mammals, likely due to a lack of precocial mammals that are amenable to keeping in labs.

While studies have examined the effects of a deprived social environment on adult behavior across a variety of contexts, much less is known about the role of socially complex environments that include non-family members. Communal breeding is an ecologically relevant social environment with a high degree of social complexity for young and can serve as a natural model for the question at hand. Previous literature has assessed the potential fitness benefits of communal rearing across a variety of species. For example, studies across rodents have assessed the presence of "helpers" on litter size and weight (degus: Ebensperger et al. 2007, prairie voles: Hayes and Solomon 2004, lab mice: Heiderstadt et al. 2014). And in house mice, communal nursing among familiar and related females improves lifetime reproductive success (König 1994). Yet, communal rearing does not ubiquitously provide benefits; Ebensperger et al. (2007) emphasize that net benefits or costs vary by species, likely dependent on the size of the breeding group, nest site availability, and costs of thermoregulation. Furthermore, communal rearing can increase competition between litters (Mennella et al. 1990) prompting competitive tendencies in adulthood (Fischer et al. 2018).

The communal and precocial spiny mouse (*Acomys cahirinus*) is an excellent model for exploring how social complexity shapes early life development and adult behavior. In this species, mothers exhibit alloparental care (Porter et al. 1980; Tučková et al. 2016) and fathers additionally exhibit some care (Tučková et al. 2016). Pups are therefore instantly exposed to a high degree of social complexity through communal rearing, and furthermore, these precocial young exhibit a high degree of neural development upon birth. Thus, these pups may indeed process, store, and act on social information in a way that their altricial counterparts do not. In the experiment conducted here, we reared spiny mice in either a simple social environment where subjects are exposed only to parents and littermates or a complex social environment where subjects additionally received visual, olfactory, tactile, and acoustic stimulation from a neighboring breeding group with its own litter. Upon adulthood, we characterized their behavioral phenotype across four behavioral tests: an open field task, novel object task, and social interaction tests with a same-sex conspecific as well as an opposite-sex conspecific. Previous research suggests that individuals exposed to greater social complexity during early life will exhibit "faster" behavioral phenotypes; thus, we expected offspring reared with a neighboring breeding group to exhibit higher exploration, activity, sampling, aggression, neophilia, and decision speed (Solomon-Lane and Hofmann 2019; Fischer et al. 2018; Curley et al. 2009). Given that spiny mice are highly prosocial, we expected all animals to actively interact with stimulus animals. But we hypothesized that exposure to non-kin during early development may make complex-reared animals more socially competent (Taborsky and Oliveira 2012), exhibiting more context-appropriate behavior and receiving less aggression from stimulus animals during novel social contexts.

To then determine if the neural mechanisms of social decision-making (Newman 1999; O'Connell and Hofmann 2012; Kelly 2022; Tremblay et al. 2017; Prounis and Ophir 2020) were influenced by rearing environment, we assessed behavioral and neural responses at two timepoints corresponding to two different social contexts. The first context was exposure to a single novel same-sex conspecific. This exposure allowed us to characterize neural responses to social novelty in a non-reproductive context. But in the wild, a spiny mouse may encounter multiple individuals at once when dispersing and assimilating into a new communal breeding group. Therefore, we exposed individuals to a second context with a novel, mixed-sex group of two males and two females to simulate contact with a new breeding group. Because socially complex-reared individuals had experience with non-family members in early life, we expected they may more readily prosocially interact with novel conspecifics as adults. Yet, we considered that in this highly social species, all individuals regardless of rearing environment may be highly prosocial towards a single conspecific. Therefore, it is

possible that rearing effects would manifest only under the more distinct and ecologically "high stakes" scenario of exposure to a novel breeding group.

In the two social contexts (single novel conspecific and novel mixed-sex group), we assessed neural responses across key regions associated with social behavior and aggression. This included regions that process social dynamics and influence prosocial responses in both reproductive and non-reproductive contexts: the preoptic area (POA), paraventricular nucleus of the hypothalamus (PVN), lateral septum (LS), and bed nucleus of the stria terminalis (BST) as well as brain regions implicated in the production of aggressive and avoidant behavior: the ventromedial hypothalamus (VMH), the anterior hypothalamus (AH), and the medial amygdala (MeA) (O'Connell and Hofmann 2011). We hypothesized that individuals that were reared with or without an unrelated neighbor breeding group would exhibit different neural responses to novel conspecifics.

Additionally, we previously found that PVN neural responses positively relate to prosocial behavior with novel conspecifics in spiny mice (Gonzalez Abreu et al. 2022), and thus, we expected that the PVN would be more responsive to a novel conspecific and to a novel group in animals reared in a socially complex environment. Further, we hypothesized that rearing in a socially simple environment would lead to a more avoidant phenotype during a novel social encounter (Walker et al. 2023), and thus, the VMH would be more responsive to novel conspecifics in simple-reared animals (Wallace et al. 2023).

The behavioral and neuromolecular approach employed in this experiment is designed to uncover previously undescribed mechanisms and developmental origins of social decision-making in an emerging model species for social neuroscience (Kelly and Seifert 2021; Fricker et al. 2022; Powell et al. 2023).

Materials and methods

Animals and housing

All subjects were spiny mice from our breeding colony, which were offspring from mice originally from the captive bred colony of Dr. Ashley W. Seifert (University of Kentucky). Subjects were housed in Tecniplast GR1800 double-decker polycarbonate rat cages (32 × 38 × 40 cm) lined with Sani-Chips better and were provided with rodent igloos and shepherd shacks. Upon weaning at postnatal day (PND) 21 (Young 1976), subject animals were housed with siblings (mixed-sex) in standard rat polycarbonate cages (41 cm × 20 cm × 20 cm). Animals were able to obtain food (Prolab RMH 1000) and water ad libitum. Animals were kept on a 14-h:10-h light-dark cycle. An ambient temperature was maintained at 24 ± 2 °C. All subjects were housed according to Emory University Institutional Animal Care and Use Committee (IACUC) regulations.

Experimental design and social rearing

To assess the role of early life social complexity on spiny mouse development, subjects were born and raised in either a simple or complex social environment. See Table 1 for experimental timeline. All animals were housed in double-decker cages that were divided in half by a clear plexiglass barrier. In the simple environment, subjects were housed with their parents and littermates on one side, and the side opposite the barrier remained empty. In the complex environment, subjects were housed with parents and littermates on one side of the barrier, and a "neighbor" breeding group consisting of one adult male, one adult female, and a litter less than 21 days old was housed on the opposite side of the barrier to prevent alloparental care toward subjects. This design allowed for the control of variation in parental care and instead specifically exposed subjects to, or not to, social

Table 1 Experimental timeline

Date	Event
Day 1	Focal parents introduced to home cage on opposite side of barrier
Day 4	Focal parent female moved to male side
Day 15	Neighbor pair introduced to empty side (*complex treatment only*)
--	*Daily observations for pup births*
PND 4, 6, 8	Home cage recorded
PND 21	Subjects weaned and housed with littermates
PND 55–65	Adult behavioral profiling on four tasks (all tasks conducted on the same day): open field, novel object, same-sex interaction, opposite-sex interaction
PND 55–65 + 3	Two-timepoint social exposure: single same-sex conspecific and 2M/2F mixed-sex group. Immediately followed by perfusion and brain tissue collection

complexity. The plexiglass barrier had 0.5-cm-diameter holes spaced roughly 1 cm apart to allow for some tactile interaction (nose poke or paw touching). At the beginning of the experiment prior to the birth of subject pups, the male parent and female parent were placed in the double-decker cage on the opposite sides of the barrier. Three days later, the female was transferred into the male's side to form a parent pair. This procedure was performed to prevent aggression (typically female aggression towards the male).

Twelve days after parental pairing, a neighbor pair (one adult male and one adult female, ear punched to distinguish from the parent pair) was added to the opposite side of the cage for the complex treatment. This neighbor pair had been housed together prior to the experiment and had given birth to at least one litter by the time they were introduced into the experimental housing. If the neighbor pair did not have their own litter by the day the subject pups were born, two or three pups from the youngest litter available in the colony were added to the neighbor pair to foster. Note that because spiny mice are communally breeding species, breeding mothers will readily accept pups from other litters. Throughout the experiment, any neighbor pups that reached PND 21 were weaned, and foster pups were added or removed as needed to ensure that subject pups in the complex treatment were reared with preweaning pups in the neighbor group.

On PND 4, 6, and 8, an hour-long front-facing video of the subjects in their home cage was taken to assess pup behavioral development and parental care. Because pup sex could not be determined until weaning, we did not examine sex differences in behavioral data collected from the home cage videos during PND 4–8. Subjects were weaned at PND 21. Subject weanlings were sexed via anogenital distance and presence of testes or a vagina and were then housed with mixed-sex littermates until adult behavioral profiling (PND 55–65). For each litter, the sex ratio of the litter (determined at weaning) was calculated as the number of males minus the number of females. Thus, a balanced sex ratio was recorded as 0, with positive values being more male-biased and negative values being more female-biased. Subjects were not housed with only same-sex littermates to avoid single-housing.

A total of 34 subject pups (simple-reared males 12, simple-reared females 7, complex-reared males 9, complex-reared females 6) were born to 14 parent pairs. Analysis of sample sizes per early life rearing condition is commensurate with prior literature in the field (Kelly et al. 2020; Prounis et al. 2018). Although we observe significant effects of sex, we acknowledge a lower than ideal sample size for complex-reared females (n = 6), and thus, future studies with higher sample sizes are required to confirm effects of sex on behavioral and neural responses to early life social complexity as presented below. Of the original 17 parent pairs, three were removed: one due to aggression between the parents upon pairing, one due to litter cannibalism, and one due to excessive delay between pairing and litter birth.

Adult behavioral profiling

PND 45 is considered the onset of sexual maturity in *Acomys* (Brunjes 1990; Haughton et al. 2016); thus, adult behavioral testing occurred between PND 55 and 65. This range in adult age was to allow multiple litters that had been born within ten days of each other to undergo behavioral profiling simultaneously. Upon adulthood, subjects were assessed in four contexts: an open field task, a novel object exposure task, a same-sex conspecific task, and an opposite-sex conspecific task. All tasks were conducted in the same day between PND 55 and 65, with a 2-h interval between tasks, excluding the novel object task, which immediately followed the open field task. Task order was balanced across individuals. Once adult testing began, subjects were individually housed for the remainder of the experiment to keep social exposure consistent across all subjects prior to the two-timepoint neural response tests.

The open field task occurred in a white plexiglass square arena (120 cm × 120 cm × 60 cm) with an overhead camera and room lighting. The arena was positioned such that there were no shaded corners. Subjects were collected from their home cage and gently introduced to the center of the arena via a 500-mL plastic beaker and allowed to roam freely for the 10-min duration of the task.

At the conclusion of the open field task, subjects were corralled underneath a Madesmart opaque plastic rectangular container (24 cm × 16 cm × 5 cm) and returned to the center of the arena to prepare the novel object task. Subjects remained under the container for approximately 30 s as the experimenter introduced the novel object to the center of the adjacent zone (roughly 35 cm away from the center of the arena). The novel object was an OXO Good Grips Triple Timer placed face-down (5.4 cm × 9.5 cm × 5.4 cm). The container was then removed and the subject was allowed to roam freely for 10 min. Following the task, the subject was returned to their home cage. The arena, beaker, novel object, and container were cleaned with Virkon S disinfectant between subjects to prevent the transmission of odor cues.

The same-sex and opposite-sex behavioral tasks were identical in procedure, with the only difference being the sex of the stimulus animal relative to the subject. The same-sex/opposite-sex task procedure was as follows: The subject was gently introduced into an empty standard rat polycarbonate cage with Sani-Chip bedding. A clear plexiglass lid with 0.5-cm holes was secured to the top of the cage via binder clips, and the animal was allowed to acclimate alone for 10 min. Following the acclimation, an unrelated stimulus animal was introduced to the cage and the social interactions between

the subject and stimulus animal were recorded for 12 min via an overhead camera. Prior to the task, the stimulus animal was labeled on their back with animal marker to distinguish between the subject and stimulus. After 12 min, the subject and stimulus were returned to their home cages. Note that we did not need to clean out cages for reuse as a new cage and bedding were used for each subject in each task.

Design for assessing neural responses at two timepoints/social exposures

Three days after the four-task behavioral profiling (thus PND 58-68), subjects underwent a two-timepoint social exposure to quantify neural and behavioral responses to two distinct social contexts. In the first timepoint, subjects were exposed to one novel, unrelated, same-sex conspecific. To do so, subjects were tested in a large white plexiglass arena (61 cm × 46 m × 38 cm) with four mesh cylindrical containers (pencil case size) placed in a 2 × 2 grid in the center of the arena. Prior to the beginning of the exposure, one novel, unrelated, same-sex conspecific was placed under one of the mesh containers. Then, the subject was introduced at one end of the arena using a 500-mL plastic beaker and was allowed to move freely in the arena for 15 min. At the end of the 15 min, the subject was returned to their home cage for 90 min. Perfusion of subjects in relation to the first timepoint was 105 min following onset of the exposure to the same-sex conspecific. This allowed for examination of the immediate early gene protein Fos, which serves as a proxy marker for neural activity. Fos functions by rapidly altering gene expression, either positively or negatively, in response to cell surface signals (Hoffman et al. 1993). Fos is induced from 30 to 90 min, reaching a maximum around 90 min and returning to baseline after 3–24 h, depending on brain region (Lara Aparicio et al. 2022). The arena was cleaned with wet paper towels between subjects and between timepoints.

Following the 90-min resting period, the subject then underwent the second timepoint exposure to a novel, unrelated mixed-sex group of two males and two females. The mixed-sex group often, but not always (dependent on availability of stimulus animals in the colony), contained sibling pairs. The mixed-sex group was housed in the double-decker cages prior to use in the experiment, with a divider separating the two males from the two females to prevent breeding. For the second timepoint, the two stimulus animals of a given sex were first placed in mesh containers diagonal to each other (not in adjacent containers) to prevent side biases. The subject was then placed in the arena and allowed to move freely for 15 min. Following this 15-min period, the subject was immediately euthanized for tissue collection. Timepoint two allowed for examination of phosphorylated endoplasmic reticulum kinase (pERK). pERK can be used as a common end point measurement for the activation of many classes of G protein coupled receptors (Garbison et al. 2015). pERK peak induction occurs within 2–10 min (Gao and Ji 2009), followed by a rapid decay after 45 min (Kukushkin et al. 2022). Because animals were euthanized 15 min after exposure to the mixed-sex group, the timing was likely too rapid for the induction of Fos protein by exposure to the mixed-sex group and subsequently detection of Fos via immunohistochemistry specifically in response to the second timepoint; thus, it is unlikely that the group exposure elicited a Fos response that would overlap with the timepoint one same-sex individual exposure. Similarly, because pERK rapidly decays after 45 min of induction, any pERK response to timepoint one would have been extinguished prior to timepoint two with little to no carryover.

Video scoring and behavioral quantification

For all adult behavioral assays, video images were taken from overhead cameras (Sony Handycam, HDR CX-405). Subject location and/or behavior data was recorded using the event-logging software CowLog (version 3.0.2). To assess location data in the open field task, novel object task, and two-timepoint social exposure, the applications "PictureIn-Picture" (Mac) or "OnTopReplica" (Windows) were used to overlay a transparent grid onto the video. In the open field and novel object tasks, the transparent grid consisted of a "wall" zone which was the outermost 11 cm of the arena. The inner section was then divided into nine square zones of equivalent size (33 cm × 33 cm) in a 3 × 3 grid. The animal was released into the middle of the center zone and the object was placed in the center of an adjacent zone which was 33 cm from the animal.

For the same-sex and opposite-sex behavior tasks, location data was not recorded. Instead, the following behaviors were recorded in CowLog: prosocial behavior (positive side-by-side contact, huddling, allogrooming) and aggressive behavior (chasing, being chased, biting, pinning, aggressive side-by-side contact), other social interaction (namely, investigation behavior not specifically characterized in the previous list), and non-interactive behavior (any behavior spent alone, such as jumping, grooming, or time away from conspecifics). This ethogram of behavior was modeled after previous experiments conducted in spiny mice (Gonzalez Abreu et al. 2022; Fricker et al. 2022).

Similar to the open field task, the two-timepoint social exposure was scored via CowLog using location data. The transparent grid was arranged as follows: The non-social area was the 25% of each end of the arena (outermost 23 cm each). The middle 46 cm was divided into four equal rectangular zones of 23 cm × 19 cm each, corresponding to each of the four mesh cylindrical containers. The containers were placed in the direct center of the arena flush against each other (not in the center of each rectangular zone); thus,

there was no central area where the subject could be between all four containers. If the individual climbed up on top of the containers, we labeled this as its own zone and this time was not analyzed. Note that due to the cylindrical shape of the stimulus containers, "time near" was determined by the scorer using cues in addition to the zone of the subject: time near was considered as time either within one body length (i.e., in the described zone), or within two body lengths if the head was oriented towards the stimulus cage. This allowed for individuals who were near two stimuli to be quantified based on their orientation, not simply slight differences in body positioning. To specifically assess if behavior differed between the single-animal exposure and the mixed-sex group exposure, we quantified time spent in the non-social ends of the exposure arena. This was the most directly comparable measure, as any quantification of social behavior would have needed to be adjusted for the difference in number of stimulus animals between the two contexts.

Tissue collection and immunohistochemistry

Following the second timepoint (exposure to a mixed-sex group), subjects were immediately euthanized by isoflurane overdose and were transcardially perfused with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde dissolved in 0.1 M borate buffer (pH 9.4). Brains were extracted, postfixed overnight in 4% paraformaldehyde dissolved in 0.1 M borate buffer (pH 9.5), and cryoprotected in 30% sucrose dissolved in PBS for 48 h. Brains were then frozen in Tissue-Tek O.C.T. (optimal cutting temperature) Compound in brain molds and stored at − 80 °C until sectioning. Brains were thawed and sectioned coronally at 40 µm into three series using a Leica CM-1860 cryostat. Sectioned tissue was stored in plastic 12-well culture plates with a lid at − 20 °C until immunohistochemical labeling.

The immunohistochemical labeling protocol followed the protocol described in Kelly et al. (2022). After thawing, tissue was transferred into porcelain 12-well plates in 0.1 M Tris-buffered saline (TBS). A total of 16 sections per subject were then selected for further processing and each subsequent step described involved the transfer of these 16 sections into a new porcelain plate with wells filled with the relevant reagent. Tissue was rinsed in 0.1 M TBS five times for 5 min each in on a rocker (always set to low speed). Tissue was transferred into a block solution (0.3% Triton X-100 and 10% Normal Donkey Serum (NDS) in 0.1 M TBS) and incubated in a humid chamber (a closed Tupperware container with a wet paper towel) at room temperature for 1 h. Tissue was then transferred into a primary antibody solution diluted in TBS containing 5% NDS and 0.3% Triton X-100 and incubated in a humid chamber at 4 °C for 24 h. Primary antibodies used were guinea pig anti-Fos (Synaptic Systems; 1:500) and rabbit anti-pERK (Cell Signaling Technology; 1:500).

Following the primary antibody incubation, tissue was rinsed for 30 min in 0.1 M TBS twice on a rocker. Tissue was then incubated for 1 h in a biotinylated donkey anti-guinea pig secondary (Jackson ImmunoResearch; 8:1000) followed by two 15-min rinses in 0.1 M TBS on a rocker. Tissue was transferred into a secondary antibody solution containing streptavidin conjugated to Alexa Fluor 488 (3:1000) and a donkey anti-rabbit secondary conjugated to Alexa Fluor 594 (3:1000) and incubated in a humid chamber in the dark for 2 h. Tissue was then either (a) rinsed in 0.1 M TBS for 20 min on a rocker if the tissue was mounted immediately or (b) stored at 4 °C in 0.1 M TBS in a humid chamber for up to one week and rinsed once for 20 min in 0.1 M TBS prior to mounting.

Tissue was mounted onto microscope slides (TruBond 380 White 20-mm slides) and coverslipped (VWR Micro Cover Glass) with ProLong Gold antifade containing DAPI nuclear stain (ThermoFisher Scientific). Coverslipped slides were set out to dry at room temperature in the dark, and once dried (typically overnight), slides were sealed with clear nail polish and stored in the dark at room temperature until imaging.

Cellular imaging and quantification

Slides were imaged using a Zeiss Axio Image Microscope with ApoTome.2. Magnification varied by region and is listed below. Two sequential sections were imaged (left and right side separately if needed) and cell counts were averaged across either these two or four sections (four if the left and right hemispheres were imaged separately which was the case in the BST, AH, and MeA). Sections were selected based on morphological landmarks according to the Paxinos & Franklin Brain Atlas for Mice (Franklin and Paxinos 2019).

Cellular quantification methods varied by region due to two reasons: the ability/inability to draw a consistent ROI to capture the region across tissue and the level of background brightness. For all regions except the BST and VMH, the red channel and green channel images were first merged in FIJI (Schindelin et al. 2012) and the ROI was placed around the region. The "Clear Outside" function was used to subtract out any part of the image not within the ROI. Images were then passed through the FIJI "Adjust Minimum" and "Adjust Maximum" filter to subtract out background.

Based on level of background, cells were automatically counted in the POA, PVN, and LS using the software CellProfiler (Carpenter et al. 2006). For each channel of interest, we used the following pipeline: ColorToGray > IdentifyPrimaryObjects > ConvertObjectsToImage > SaveImages. We then added one CalculateMath module and

one ExportToSpreadsheet module to report the count data. For regions with high background (VMH, AH, BST, MeA), one scorer (KW) blind to treatment counted cells manually using the multipoint tool in FIJI.

Below, we list specific methodological considerations per region. For the BST, due to variation in anterior commissure morphology and high background, the scorer (KW) determined an approximated (unlabeled) ROI per image based on the Paxinos & Franklin Brain Atlas. For the VMH, an ROI was not used as the entire frame of view captured the region. While the methods varied by region, we chose these distinctions to best capture the full expression in each region. The slight variation in cell count methodology across brain regions does not interfere with interpretation of results given that we do not statistically compare brain regions in this study. Please note that for one individual, in one section, only one hemisphere of the VMH was viable for imaging, so their cell count was doubled prior to averaging. Dropping the subject from analyses did not make a statistically significant difference and thus, the subject was included in the analyses presented.

Statistical analyses

Data was first compiled in R (version 1.1.453) (R Core Team 2021, http://r-project.org). The R package "cowlogdata" (version 0.1.2) (Wallace 2020) was used to compile individual behavioral video logs into a summary spreadsheet that included durations of time spent in each zone, time of initiation of each behavioral event, and number of events recorded for each behavior and/or number of entries into a given area. Behavior and location data were analyzed as proportion of time spent exhibiting that behavior or in that location.

Unless otherwise specified, all statistical tests were generalized linear models (GLM) or linear mixed models (LMM) conducted in SPSS (version 29.0.0.0) (http://ibm.com/products/spss-statistics) with post hoc Bonferroni corrections. We chose these models as they are robust to outliers and bimodal distributions. We acknowledge that sample sizes per sex are not evenly distributed across all treatment groups (namely, complex-reared females, $n = 6$). However, we wanted to represent both males and females (as defined by gonadal sex) in our dataset, and current convention recommends controlling for sex as a variable in analyses (Garcia-Sifuentes and Maney 2021). In addition, a two-sided Pearson chi-squared was used to assess treatment differences in litter size, and a one-sample *t*-test to assess sex bias in litters was conducted in SPSS. Principal component analyses were conducted in R using the package "pvclust" (Suzuki and Shimodaira 2006) and all figures were generated in R. Effect sizes were either calculated using a direct formula or by using the R package "rstatix" (Kassambara 2020). Data analysis R code and primary data can be found on GitHub.

Results

Litter birth and early development

On average, the number of days between parent pairing and litter birth was 62.5 days ($n = 14$, SD = 34.8 days). The average litter size was 2.43 pups ($n = 14$, SD = 0.51). Because all parents gave birth to either two or three pups, we conducted a chi-squared test to compare litter sizes between treatments. In doing so, we found that parents in the simple treatment (i.e., without a neighbor family) gave birth to significantly larger litters than parents in the complex treatment ($p = 0.031$, $X^2 = 4.667$, $\varphi = 0.577$, Fig. 1A). Litters were significantly male-biased (one-sample *t*-test: mean = 0.50, SD = 0.76, $p = 0.029$, Cohen's $D = 0.66$), but did not differ between simple-reared and complex-reared litters ($p = 0.740$). When quantifying subject pup and parental behavior during PND 4–8 (home cage videos), we did not find any significant treatment effects in parental nursing behavior, pup self-grooming behavior, parent and pup nose pokes at barrier, or parent and pup transverses along the barrier (Supplemental Fig. 1).

Behavioral profiling in adulthood

Upon adulthood, subjects were assessed in four contexts: an open field task followed by presentation of a novel object, a same-sex conspecific task, and an opposite-sex conspecific task. Average proportions of time spent either in certain areas of the task arenas or exhibiting specific behaviors are visualized for each task in Fig. 1B.

During the 10-min open field task, individuals spent an average of 78% of their time along the walls of the arena. In the subsequent 10-min novel object task, time spent near the wall was reduced to an average of 48% (Fig. 1B). When examining behavior in the open field task, a GLM with sex and treatment as fixed factors found that individuals significantly increased their time in the lower middle zone of the arena (where the object was placed) during the novel object task compared to the open field task ($p < 0.001$, $F = 75.224$, $\eta p^2 = 0.695$, Fig. 1C), confirming that the individuals approached and interacted with the object. No main effects of treatment or sex and no interaction were detected in GLMs of the time spent in the center of the open field or near the novel object (Supplemental Table 1).

In the same-sex conspecific test and opposite-sex conspecific test, subjects spent an average of 16% of their time behaving prosocially towards the same-sex conspecific and 14% of their time behaving prosocially towards the

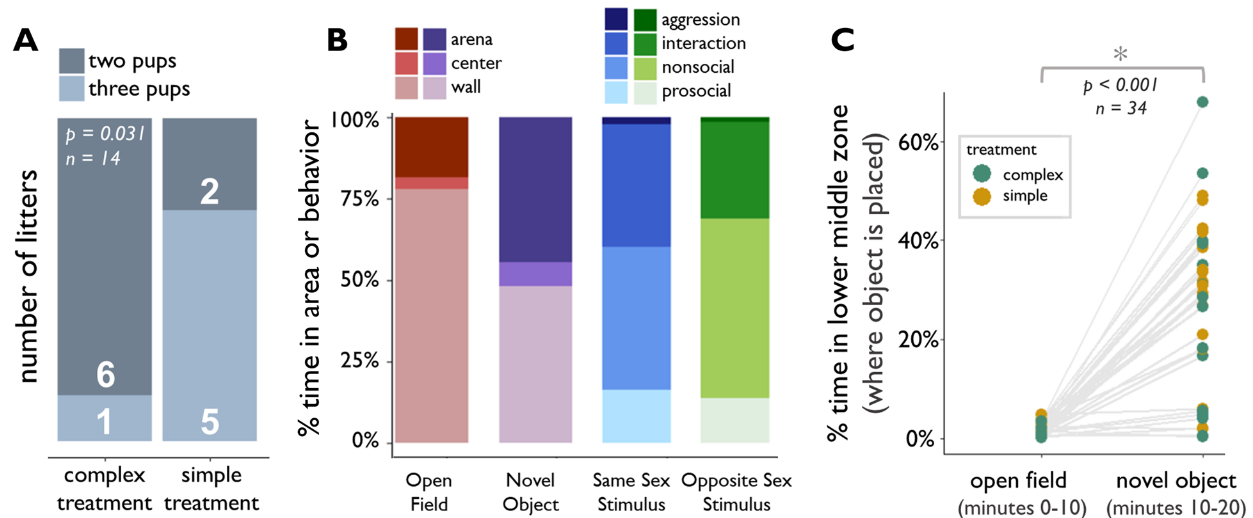

Fig. 1 **A** Parents without non-kin neighbors (simple treatment) gave birth to significantly larger litters than parents in the complex treatment. **B** Summary of behaviors during the four profiling tasks. Note that the open field and novel object tasks used the same zone quantification, and the same-sex stimulus and opposite-sex stimulus tasks used the same ethogram. **C** Upon adulthood, in the open field-novel object task, subjects noticeably responded to the introduction of the novel object as measured by increased time in the lower-middle zone of the arena

opposite-sex conspecific, and in both social tasks, subjects spent very little time aggressing the stimulus animal (an average of 2.1% and 1.6% respectively, Fig. 1B). GLMs of subject aggressive behavior and prosocial behavior did not identify a main effect of treatment, sex, or an interaction between sex and treatment in either social task (Supplemental Table 1).

To test for more comprehensive treatment differences in behavioral profiles, we conducted an exploratory principal component analysis combining the six terms mentioned previously: open field time in center, novel object time near object, same-sex prosociality and aggression, and opposite-sex prosociality and aggression (Supplemental Fig. 2, principal component loadings available in Supplemental Table 2). *T*-tests of the first two principal component scores found no significant treatment differences (PC1: $p = 0.606$, $T = 0.522$, Cohen's $D = 0.184$; PC2: $p = 0.314$, $T = -1.023$, Cohen's $D = -0.347$) or sex differences (PC1: $p = 0.269$, $T = 1.140$, Cohen's $D = 0.383$; PC2: $p = 0.121$, $T = -1.594$, Cohen's $D = -0.532$).

Behavior and neural response when exposed to a single same-sex conspecific

Following the behavioral tasks, subjects underwent a two-timepoint social exposure to quantify neural responses to distinct social contexts. The first timepoint consisted of exposure to a single same-sex conspecific and examined Fos responses. A GLM of time spent near the stimulus animal in this context identified a main effect of sex, with males spending more time near the stimulus animal than females ($p = 0.004$, $F = 9.710$, Fig. 2A). The model also identified a significant main effect of treatment, with simple-reared subjects spending more time near the stimulus than complex-reared subjects ($p = 0.036$, $F = 4.809$, Fig. 2B). The model did not yield a significant interaction effect between sex and treatment ($p = 0.849$).

Neural response to this single same-sex conspecific was quantified as the number of Fos+ cells in the following regions: POA, PVN, LS, AH, BST, MeA, and VMH. When each region's Fos expression was analyzed via a GLM that included sex and treatment as fixed factors, only the LS returned a significant main effect of treatment ($p = 0.019$, $F = 6.423$, Fig. 2C) and showed that simple-reared animals exhibited a greater Fos response in the LS in response to exposure to the same-sex conspecific. Fos expression did not differ in any region based on sex; further, we observed no interactions between sex and treatment (Supplemental Table 3, left). We conducted a post hoc pair of analyses to examine the visual bimodal distribution in LS Fos expression in the complex-reared animals (Fig. 2C). We found that a GLM of time spent near the same-sex stimulus animal comparing "high LS Fos" complex-reared individuals (LS Fos+ cells > 40, $n = 5$) and "low LS Fos" complex-reared individuals (LS Fos+ cells < 40, $n = 8$) did not yield a statistically significant main effect ($p = 0.112$, Supplemental Fig. 3A). Furthermore, when combining rearing groups in a linear regression, time spent near the same-sex stimulus animal did not significantly predict LS Fos expression across all individuals ($p = 0.092$, Supplemental Fig. 3B).

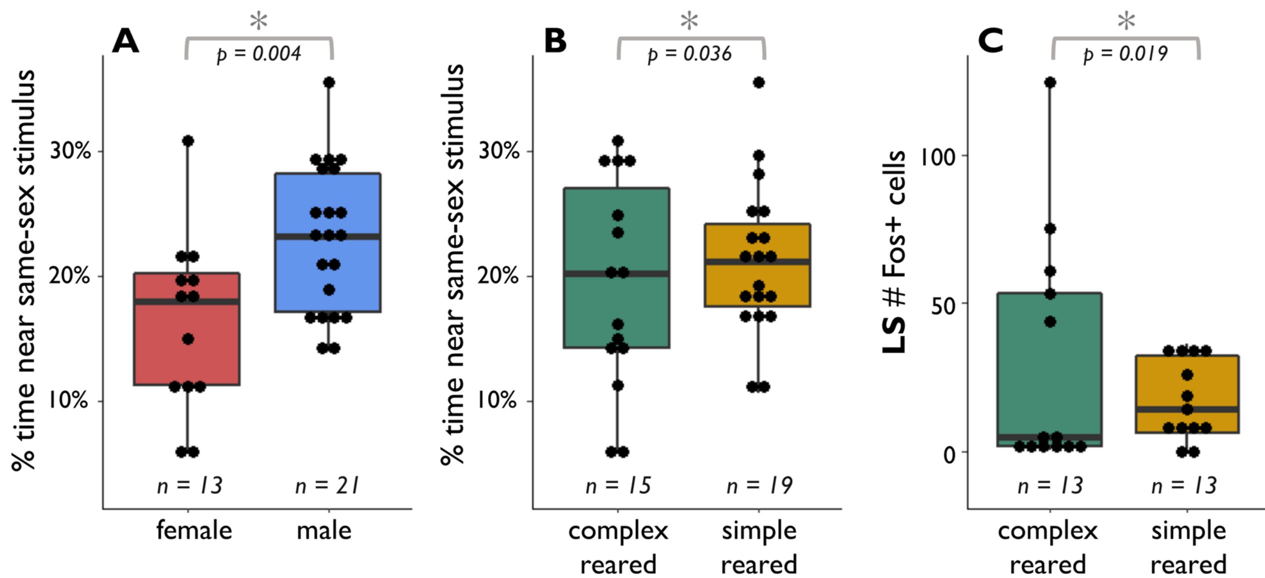

Fig. 2 When exposed to a single same-sex conspecific, **A** males and **B** simple-reared subjects spent more time near the stimulus animal. **C** Simple-reared subjects also exhibited a stronger neural response in the lateral septum (LS)

Behavior and neural response when exposed to a mixed-sex group

In the second timepoint of the exposure, subjects were exposed to a mixed-sex group of two males and two females. A GLM of time spent near the four stimulus animals did not show any significant effect of treatment (p = 0.181), sex (p = 0.642) or the interaction between treatment and sex (p = 0.811). In a GLM of the difference in time spent being non-social in the single-animal exposure (reported in the previous section) vs the mixed-sex group exposure, we did not identify any significant effect of treatment (p = 0.175), sex (p = 0.794), or the interaction between treatment and sex (p = 0.883), suggesting that an individual's behavior did not significantly differ between the two types of social exposures.

Neural response to the mixed-sex group was quantified as the number of pERK+ cells in the following regions: POA, PVN, LS, AH, BST, MeA, and VMH. When each region was analyzed via a GLM that included sex and treatment as fixed factors, a significant main effect of treatment was identified in both the POA ($p < 0.001$, F = 15.92, Fig. 3A) and the PVN (p = 0.039, F = 4.673, Fig. 3B). In addition to the main effect of treatment, the GLM examining PVN pERK expression yielded a main effect of sex (p = 0.037, F = 4.788, Fig. 3C), but no interaction (p = 0.158). No other regions significantly differed in pERK expression by sex and treatment, and no interactions between sex and treatment were observed (Supplemental Table 3, right).

Discussion

We aimed to assess the influence of early life social complexity on the development of the brain and behavior of the communally breeding spiny mouse *Acomys cahirinus*. To do so, we reared pups in either a simple social environment (parents and littermates) or a complex social environment (with a neighbor family opposite a barrier). Upon adulthood, pups raised in these treatments were assessed in a suite of behavioral tasks, then underwent a two-time social exposure to assess behavioral and neural responses to a single same-sex conspecific and a mixed-sex group.

Exposure to, or rather deprivation from, a communal breeding environment yielded significant results even prior to subject birth: parents with an adjacent non-kin breeding group had smaller litters than those without. We hypothesize that this reflects maternal investment, which is highly relevant for this communally breeding species (Frynta et al. 2011). In an experiment similar to the one conducted here, researchers assessed reproductive outcomes for pregnant degus in the presence or absence of a second breeding female with a litter. Subject litters that were born while another litter was already present were smaller in number than litters born when no other litters were present in the cage (Ebensperger et al. 2007). Ebensperger et al. (2007) conclude that this effect is likely a mechanism to promote seasonal synchrony in breeding and discourage large litters late in the season. Conversely, in cooperatively breeding African wild dogs, larger group size correlates positively to litter size (Gusset and Macdonald 2010). Importantly, we did

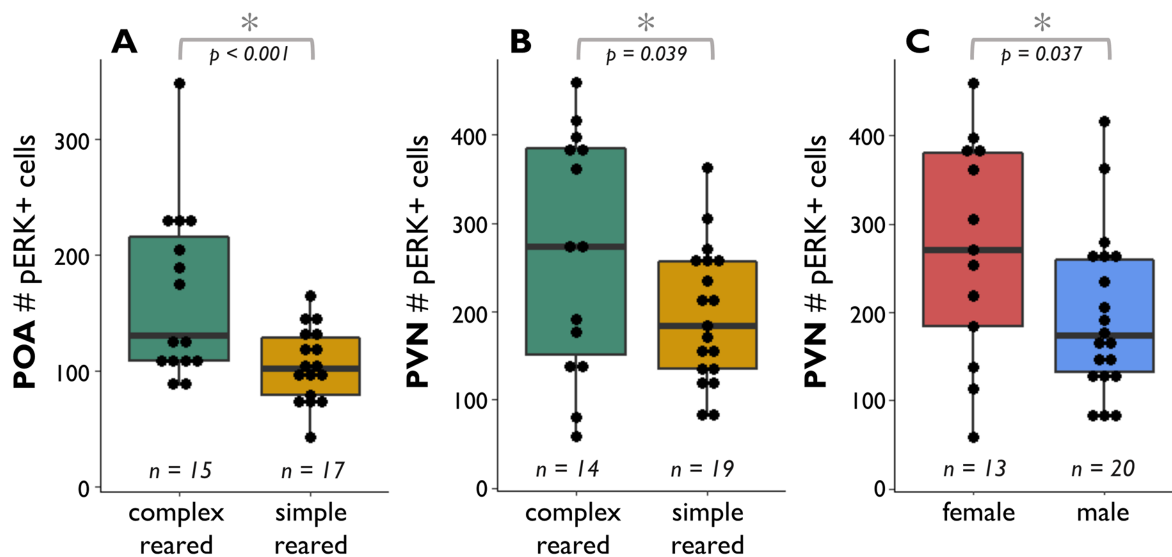

Fig. 3 When exposed to a mixed-sex group of two males and two females, complex-reared animals exhibit a stronger neural response in the **A** preoptic area (POA) and **B** paraventricular nucleus of the hypothalamus (PVN). **C** Females exhibit a stronger PVN neural response to the mixed-sex group than males

not identify differences in pairing-to-birth timelines, which suggests that there are no active suppression effects as seen in other rodent species. We also do not want to overlook the possibility that the spiny mouse parents cohoused with neighbor breeders may have given birth to smaller litters simply due to perceived space constraints—even though the square footage available for the subject breeding pair was the same in both treatments, the simple treatment did not have to "share" the cage with another breeder pair. This perception of less space and fewer resources alone could have been sufficient to influence litter size.

Nevertheless, once the litters were born, the parents in our experiment provided equivalent amounts of care across the two social environments given that we observed no differences in parental behavior in the early life home cage observations. Similarly, pups did not exhibit detectible differences in behavior preweaning. This suggests that parental care is robust across both social environments and furthermore that both environments provide the resources and experiences needed for typical social development in spiny mice. Conversely, other forms of environmental manipulation influence parental care and subsequently offspring development. For example, in biparental prairie voles, parents that are forced with the tradeoff of choosing to feed themselves or brood their offspring yield less paternal care, which in turn produces male offspring that are slow to approach and investigate novel conspecifics once adults (Kelly et al. 2020). Additionally, complete paternal deprivation in prairie voles causes impairments in pair bonding, particularly in males (Kelly et al. 2020; Ahern and Young 2009). Similarly, parental deprivation in California mice alters offspring social vigilance behavior (Walker et al. 2023). Together, these findings demonstrate that manipulation of the parental care pups receive significantly influences development. Notably, in the present study, parental care was consistent across treatment groups, and instead, we induced variation in social complexity of the environment. Once our spiny mouse pups grew up to adulthood, we found no effect of rearing environment in our suite of behavioral tasks: an open field task, a novel object task, a same-sex conspecific interaction, and an opposite-sex conspecific interaction. The lack of rearing-environment effects on behavior in adulthood could reflect the lack of parental care differences. This would highlight the pivotal role of parental care in "setting the trajectory" of offspring behavioral profiles. Indeed, as we described previously, a wide variety of primary literature across taxa has described how changes to parental care, such as maternal separation or the presence of alloparental helpers, significantly influence the development of offspring (Kelly et al. 2020; Walker et al. 2023). Yet, our results suggest that the presence or absence of non-kin conspecifics does not cause any dramatic effects on behavior, at least as assessed in the tests conducted in the present study. Thus, spiny mouse pups may be resilient to a variety of conspecifics in their early life social environment, such that, for a communally breeding species, being exposed to kin or non-kin does not make much of a difference as long as some adult conspecifics are present to provide care. Alternatively, it is possible that the inability to fully interact with neighbors due to the barrier design muted any differences that would have been observed had the pups been allowed to fully interact with the other breeders and litter, as would happen in a natural communal environment. While allowing

subjects to directly interact (no barrier) during development would have certainly been a more ecologically relevant communal context, this would have introduced the variable of differential parental care as the neighboring parents would have provided alloparental care to the subject pups. We intentionally designed the experiment to separate the variable of social complexity exposure from the total amount of parental care received. We recommend additional studies to further disentangle the contributory roles of these two variables on offspring development.

Despite a lack of treatment effects on exploratory behavior and free interactions with conspecifics, we found that exposure to social complexity in early life significantly influenced neural processing of social information in adulthood. In the same-sex single conspecific exposure (the first timepoint of the two-timepoint neural response procedure), social engagement differed in relation to sex and treatment, with simple-reared individuals and males spending more time with the stimulus animal. We likely observed treatment and sex differences in this social exposure, but not the social interaction behavioral task because of differences in test design. In the same-sex social interaction test for behavioral profiling, the subject and stimulus were able to freely interact in a small arena, which is preferable for assessing prosociality and aggression. In the later same-sex conspecific social exposure that was part of the two-timepoint neural response procedure, the stimulus animal was restricted under a container within a much larger arena. This latter design not only removed the variable of stimulus animal behavior influencing subject behavior but also allowed the subject to more easily be non-social if desired. The finding that males engaged more with the same-sex stimulus under the container than females mirrors the prior observation in this species that both sexes prefer to affiliate with males (Fricker et al. 2022). Thus, the sex difference observed here likely reflects greater male interest in same-sex conspecifics and the preference for females to affiliate with opposite-sex conspecifics. Additionally, we also found that simple-reared animals, regardless of sex, spent more time near the stimulus container. Previous studies have indicated that spiny mice are neophilic (Gonzalez Abreu et al. 2022; Fricker et al. 2022). Given that the simple-reared subject's only prior experience with a non-kin conspecific was in the social interaction test, simple-reared subjects may have spent more time near the stimulus container due to the novelty of the encounter.

Upon examination of neural responses during the same-sex conspecific exposure, we found that the LS exhibited a higher Fos response in animals that were simple-reared. The LS is considered a hub for social processing (Menon et al. 2022). The role of the LS in social processing can be seen in investigations of discrimination: A study in rats demonstrated that neurons within the LS differentially process kin from non-kin (Clemens et al. 2020), which has also been observed in spiny mice exposed to novel kin vs novel non-kin (Fricker et al. 2023). Furthermore, LS activation reduces aggression and increases affiliation in prairie voles (Sailer et al. 2022). Previous studies have shown that the LS is influenced by the early life social environment. Kelly et al. (2020) demonstrated that in prairie voles, paternal deprivation resulted in epigenetic modifications to vasopressin receptors in the LS, which in turn related to delayed social approach behavior. In rats, maternal separation changes nonapeptide receptor (OTR and V1aR) binding (Lukas et al. 2010). Taken together, our findings here suggest that LS sensitivity to a same-sex novel conspecific is influenced by early life social experiences with non-kin.

To understand the role of early life social complexity in this communally breeding species, we wanted to explore adult responses to novel groups as a proxy for encountering a novel established group in which a dispersing spiny mouse could potentially join. Thus, in addition to assessing behavioral and neural responses during an exposure to a single novel animal, we additionally examined neural responses (via assessment of pERK) to a novel, mixed-sex group of two males and two females. Although simple-reared animals spent more time near just a single, novel same-sex conspecific, we found no effects of the early life rearing environment or sex on time spent near the group. It is possible that a novel group is equally novel to spiny mice raised in varying early life environments. Indeed, the social landscape is far more complex in a group, and there are likely very different consequences between encountering a single individual vs. a group. Therefore, it is possible that spiny mouse social competence may be robust across early life social experiences given that the consequences of behaving inappropriately could be dire. Whether lack of experience with non-kin in early life leads to less competent behavior when a spiny mouse freely interacts with a novel group remains to be determined.

Though overall interaction time during the mixed-sex group exposure was the same across animals, we observed noteworthy differences in neural responses to the group exposure. Two hypothalamic nuclei exhibited treatment effects in response to the mixed-sex group: the POA and the PVN. As with all nodes of the social behavior hetwork, the POA contains sex steroid hormone receptors and is reciprocally connected to the LS, VMH, AH, PAG (periaqueductal gray), MeA, and BST (Newman 1999). The POA has long been established as a center for the processing and coordination of sexual behavior (Paredes et al. 1993; Melis and Argiolas 1995). In our experiment, the mixed-sex group represented a reproductive context as it contained two individuals of the opposite sex to the subject. We identified a treatment difference in which complex-reared animals exhibited a greater POA neural response to this mixed-sex

group. This result, like those previously discussed, likely derives from the fact that the complex-reared animals were exposed to non-kin (i.e., future potential reproductive partners) during development. Thus, they may be “primed” to assess this mixed-sex group from the perspective of a reproductive opportunity. Conversely, the simple-reared animals may have exhibited a suppression of POA activity that could reflect the deprivation of exposure to non-kin conspecifics during development and lack of experience being able to readily identify potential mating opportunities.

We observed a parallel finding regarding PVN pERK responses such that complex-reared animals exhibited a greater neural response to a mixed-sex group compared to simple-reared animals. Similar to our interpretation of POA neural responses above, it is feasible that early life social complexity primed complex-reared animals to more readily process novel mixed-sex group dynamics in adulthood, whereas such a response may have been blunted in simple-reared animals who were unaccustomed to processing such social information. Across a wide variety of animals, the PVN contains the largest population of oxytocin and vasopressin producing neurons (Kelly and Goodson 2014; Goodson and Thompson 2010; Kelly and Seifert 2021). PVN oxytocin has been shown to be particularly important for promoting a range of affiliative behaviors (Kelly and Goodson 2014; Goodson and Thompson 2010). In spiny mice, PVN oxytocin neural responses not only positively correlate with prosocial behavior toward novel conspecifics, but this cell group also sends direct axonal projections to the ventral tegmental area (VTA; a crucial node in reward circuitry), and PVN oxytocin neural responses positively relate to VTA dopaminergic neural responses in response to interactions with a novel, same-sex conspecific (Gonzalez Abreu et al. 2022). This suggests that PVN oxytocin may gate social reward to promote affiliative behavior in this species. While the study conducted here did not specifically assess oxytocin neural responses, future studies could determine if PVN oxytocin mediates the effect of social rearing on adult responses to novel mixed-sex groups. Given that the early life environment has been shown to shape oxytocin and vasopressin neuronal densities in other species (Perkeybile and Bales 2015), it is possible that, in the present study, spiny mice reared in simple and complex social environments may exhibit different nonapeptide neuroanatomical profiles.

Interestingly, we additionally uncovered a sex difference in the PVN neural response to the mixed-sex group in which females exhibited a stronger PVN response than males. This was the only neural sex difference observed in our experiment. Across many species, females are the more selective sex with regard to mating preferences (Gwynne 1991), and furthermore, communally breeding groups can have high reproductive skew such that dominant females give birth to the majority of offspring (Holekamp et al. 1996; Creel et al. 1997). Thus, when encountering a novel breeding group, females may invest more in processing the group social dynamics to ensure the correct decision to maximize fitness and reduce long-term costs. We have not yet quantified mating preferences and reproductive skew in spiny mice, but observations from our colony suggest that females are more dominant than males in mixed-sex groups. Additionally, the PVN sex difference observed here may reflect another ecological factor—dispersal (Schradin et al. 2010. While the specific dispersal rates of spiny mice are not yet reported, dispersal rates in this species are likely different between the sexes with males dispersing further and more frequently (Clutton-Brock and Lukas 2012). This sex difference in dispersal rate may influence the likelihood of encountering a novel breeding group (or multiple groups over the individual’s lifetime). If true, the females would have evolved to encounter new groups less than males, in which case it may be more novel and more important for females to carefully investigate the dynamics of a novel group.

The PVN not only is involved in processing social dynamics but also plays a role in stress responses (Lightman 2008; Jiang et al. 2019). It is possible that the higher PVN pERK expression in complex-reared animals and in females reflects a greater stress response. While we cannot rule this possibility out without direct measurement of correlates of stress (i.e., cortisol), we note that we found no difference in avoidance/non-social behavior during the mixed-sex group exposure. Furthermore, when considering the species more broadly, this highly prosocial (Fricker et al. 2022) communally breeding rodent likely has evolved not to perceive exposure to a novel group as stressful. In fact, it may even be rewarding. The hypothesis that exposure to a novel group may be rewarding is supported by the observation that spiny mice exposed to a novel conspecific exhibit higher dopaminergic neural responses in the VTA compared to spiny mice exposed to a novel, non-social object (Gonzalez Abreu et al. 2022). Future investigations of the mechanisms of social rewards, such as characterizing dopamine in social reward circuits in this species, are an important avenue of further exploration.

Taken together, the design of our social exposure procedure suggests that the neural responses observed in both the novel conspecific exposure and the mixed-sex group exposure reflect social information processing, rather than reflect the production of overt prosocial or aggressive behavior during a freely behaving interaction with conspecifics. Importantly, in our experiment, no significant effects of rearing were observed in the AH, BST, VMH, and MeA. The lack of influence of the early life social environment on neural responses toward same-sex conspecifics and toward mixed-sex groups in these brain regions that modulate avoidance and aggression may reflect the robustness of a highly prosocial phenotype in spiny mice. As communal breeders,

it is likely in the best interest of a spiny mouse to behave prosocially with novel conspecifics, regardless of variation in early life social experiences. Our data suggest that early life exposure to non-kin does not shape the perception of a novel same-sex conspecific or mixed-sex group as a threat. Indeed, exhibiting such resilience is more likely to result in the exhibition of prosocial behavior in such contexts, which could result in a potential mating opportunity or being accepted into a new group.

Conclusions

The spiny mouse is a communally breeding rodent that exhibits precocial development and very low aggression relative to other rodents. To understand how these traits shape the social development of offspring, we manipulated early life social complexity and assessed long-term consequences on offspring social behavior, exploration, neophilia, and neural responses to ecologically relevant social stimuli. We found that the lack of exposure to non-kin conspecifics during early life did not induce abnormal behavioral development, as all animals, regardless of rearing environment, exhibited species-typical behavior across two social and two non-social contexts. Despite a lack of gross behavioral differences, variation in the early life social environment did influence neural responses to novel social encounters, such that social information was differentially processed in the LS, POA, and PVN based on whether a spiny mouse was raised in a simple or complex environment. These differences in social processing support the powerful role of early life social complexity on offspring development. Furthermore, these differences likely cause downstream effects on brain or behavior in complex social scenarios yet unexamined. For example, an animal reared in a communal environment may assess reproductive dynamics differently in adulthood than one raised non-communally, leading to differences in mate choice and breeding decisions.

Beyond the specific perspective of this model species, the research described here supports the pivotal role that early life social development plays in shaping adult social decision-making and its underlying mechanisms. Early social manipulations in rodents have been increasingly used as models for depression (Réus et al. 2011) and stress-associated disorders (Nishi et al. 2013). But these methods have at times yielded inconsistent results (Nylander and Roman 2013; Tractenberg et al. 2016), pointing towards the need for additional experimentation and refinement such as the experiment conducted here. More specifically, this work emphasizes the importance of how differences in neural processing due to early life environments can influence how an animal responds to drug treatments as an adult. We previously demonstrated that PVN neurons project to the VTA and may gate social reward decisions in spiny mice (Gonzalzez Abreu et al. 2022), as has been shown in other rodents including lab mice and rats (Hung et al. 2017; Xiao et al. 2017; Northcutt and Nguyen 2014; Song et al. 2016). Given that we observed differences in PVN processing of social information, it is possible that downstream reward circuitry may be differentially regulated in animals that were reared in different social environments. Medications that target the dopamine system are used to treat conditions that influence social behavior (Mandic-Maravic et al. 2022; Kopec et al. 2019), and some of the most commonly abused drugs also influence dopaminergic signaling (Noble 1996). If the early social life shapes the development of connectivity to dopaminergic reward circuitry, then responses to drugs that target dopaminergic systems may have varying rates of efficacy depending on early life social experience. Therefore, it is of great importance to understand how variation in early life social experiences may influence neural development if we strive to develop effective treatments for disorders characterized by atypical socioreward signaling.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00213-023-06513-5.

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Author contribution Conceptualization: KJW and AMK.
Data collection: KJW, SD, and ML.
Statistical analysis: KJW.
Writing (original draft): KJW.
Writing (review and editing): KJW and AMK.
All authors have read and agreed to the published version of the manuscript.

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Data availability R code and raw data are accessible via http://github.com/kellyjwallace/Wallace-et-al-2023-Spiny-Mouse-Psychopharm.

Declarations

Institutional review board statement All procedures were approved by the Institutional Animal Care and Use Committee of Emory University. The IACUC oversees compliance with the Guide for the Care and Use of Laboratory Animals, Eighth Edition, published by the National Research Council of the National Academies (National Research Council 2011). Emory University is an AAALAC fully accredited institution (Association for Assessment and Accreditation of Laboratory Animal Care International).

Conflicts of interest The authors declare no competing interests.

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