Distinct developmental trajectories of autonomous behaviors and agency in rodents

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ABSTRACT

Behavioral interactions within the nuclear family may play a pivotal role in the emergence of autonomy and agency in mammals. While the emergence of a behavior may arise over weeks in line with nervous system maturation, individual events occur on sub-second time scales. This makes it uniquely challenging to track development in the lab where observations are made over minutes to hours or in ecological studies which lack individual specificity and sub-second precision. Here we study families of gerbils, a highly social rodent, raised in enlarged home-cage environments over weeks of development, using continuous video recordings to capture tens of millions of time points per family. Focusing on postnatal day 15 (when pups leave the nest) to day 30 (around the time when pups would disperse) we identify distinct developmental trajectories for both autonomous behaviors (exploration, food and water foraging), and social behaviors (huddling, approach, time spent together). Most of these behaviors emerge in concert with clear diurnal and crepuscular patterns and we find sex differences in both autonomous and social behaviors. Our work supports the emergence of distinct autonomous and social behavior phenotypes as the behavioral correlates of critical developmental periods of maturation of the rodent brain and can form the basis of future research on development from both neuroscience and behavioral biology perspectives.

INTRODUCTION

In humans, the development of autonomy can be understood as the development of three stages of agency: biophysical agency (capacity to regulate and sustain oneself), psycho-social agency (ability to generate intentional acts and select goals) and socio-cultural agency (sharing and creating actions based on cultural social norms) (Sokol et al 2015). While studies of the development of autonomy focus on human psychology (Lerner et al 2015) and sociological-cultural forces (Lewis 2014; Mayes & Lewis 2012), advancing our understanding of the neural basis of the development of autonomy and agency can also be carried out using non-human animal models. In most nonhuman mammals, the development of autonomy commences with increases of exploration outside the nest or home, and culminates with dispersal during which social bonds are severed (Howard 1949; Howard 1960; Gerlach 1990; Gerlach 1996; Groó et al 2013; Wang et al 2017). The neural bases for the development of autonomous behaviors have been studied in non-human animals such as rodents and correlate with maturation in sensory (eye/ear development: Altman & Sudarshan 1975; sniffing: Westerga & Gramsbergen 1990), motor (Blumberg & Adolph 2023) and spatio-cognitive functions (Wills et al 2013). There are also social or environmental factors that could drive the development of autonomy including an attraction to adults that are outside of the nest (Galef & Clark 1971; 1972; Alberts & Leimbach 1980), a decline in nest signals that promote affiliation (Grota & Ader 1969; 1970; Levin & Stern 1975; Goodwin & Yacko 2004), and instrumental learning about social variables (Chen & Amsel 1980). Studying the transition to autonomous and agentic behavior thus requires both an understanding of the development of sensory-motor-cognitive systems but also the role of the family and peers during such periods.

Studies of rodent family behavior have emphasized two rodent species, rats and mice, yet behavioral repertoires differ dramatically across rodent species, especially as a function of social structure. For example, social species, such as gerbils, form monogamous pairs and live in complex underground burrows as extended families of up to ~15 animals (Swanson and Lockley 1978; Ågren et al 1989; Scheibler et al 2004; 2006). This leads to unique family dynamics. For example, gerbil mothers do not display as much pup retrieval as other rodents (Kaplan & Hyland 1972), and pups spend many weeks exploring the local environment, yet returning to the burrow to alloparent their younger siblings (Payman & Swanson 1980; Ostermeyer & Elwood 1984). In addition, family dynamics must include a mechanism to promote dispersal during which the youngest members establish new colonies. Therefore, the neural mechanisms that ultimately yield independent behaviors and result in new families will require an approach that combines the advantages of field studies with the precise identity and

subsecond precision tracking available during transient lab observations (Lin & Wilbrecht 2022; Bordes et al 2023).

To gain new insights into the development of family behavior and the onset of juvenile independence and agency, we used uninterrupted home-cage monitoring in concert with a machine learning framework to continuously track individual Mongolian gerbil family members (2 adults and 4 pups) for two weeks, beginning when animals first leave the nest (~P15). We collected > 1000 hours of video data (~100 million video frames) and used contemporary pose estimation tools (Pereira et al 2022), behavior classifiers (Nilsson et al 2020) and custom algorithms for behavior classification. Our results reveal distinct timelines for the emergence of social and nonsocial behaviors, sex-based differences in the behaviors of both adult and developing pups, and the emergence of diurnal and crepuscular behaviors. We propose three stages of rapid development of behaviors in the first two weeks of autonomous behavior during which both social and nonsocial measures of agency develop and partially stabilize. Our work contributes a time-frame that may guide research on the maturation of neural systems underpinning elementary behavior in rodents.

RESULTS

Tracking behaviors of individual gerbil family members in home-cage environments

Capturing individual gerbil behavior across weeks with subsecond precision. We obtained video and audio recordings from three gerbil family cohorts from postnatal days (P) 15-30 (Fig 1; see also Methods). Prior to data collection, all gerbils were shaved with a distinct pattern to facilitate identification of individuals by SLEAP (Fig 1b). We began data collection on P15, the age at which gerbil pups begin to leave their nest (Ågren 1989), and ended recordings on P30, the age at which juveniles are weaned in our colony (Fig 1c). We captured more than 30 million frames of video data per cohort across three cohorts with varying numbers of female and male pups (Fig 1d).

Tracking individual gerbils behaviors. We used SLEAP (Pereira et al 2022) to track individual gerbils and gerbil-huddles across day and night cycles (Fig 1e; see also Methods). For tracking huddles, we trained SLEAP on manually annotated frames with groups of 2 or more nestling gerbils, and for open field behavior we annotated up to 6 body nodes on 1000 frames (Fig 1f). Because of idiosyncratic differences in shaving patterns and light conditions across the cohorts, we trained separate models for each cohort on day and night conditions, resulting in 6 body-feature models and 6 huddle models (Fig 1g).

We implemented several methods for improving animal identity tracking: (i) an identification-switch detection pipeline that automatically locates SLEAP identity errors on sequential video frames (Fig 1h; see Methods), (ii) a track fixing pipeline that interpolates short missing periods of huddling or individual tracks (Fig 1i, top) and (iii) an identity swap pipeline that detects and corrects clear track identity swaps (Fig 1i, bottom). We used 10-fold cross validation to evaluate SLEAP identity accuracy and found a true positive rate of 0.97 (0.04 std) for adult animals and 0.91 for pups (0.05) (Fig 1j). Feature errors (< 0.1 %) were not critical to our study because we used centroids that are not as sensitive to these errors (see Methods). Using our pipelines, we were able to track the huddling (Fig 1k, top) and open field exploratory behavior of multiple animals across weeks of continuous recording (Fig 1k, bottom).

Classifying animal behavior. We implemented two methods for classifying behavior: a heuristic-based method and a supervised classification method. For heuristic-based classifications we used

Regions-of-Interest (ROIs) to identify when animals were near the food hopper or water spout regions, and proximity metrics for detecting when gerbils were within 5 cm of one another (Fig 1l, top; see Methods). For approach behaviors we used a customized version of SimBA (Nilsson et al 2020) to train a classifier to capture approach behaviors from manually curated examples (Fig 1l, bottom) (see Methods). Using our pipelines, we were able to track individual gerbils over periods of two weeks with subsecond predictions (Fig 1m) and generate ethograms that capture postnatal day and hourly dynamics over several behaviors (Fig 1n for an example).

In sum, we found that unique shaving patterns alone enabled the tracking of multiple individuals using SLEAP's pose estimation and post processing pipelines. This enabled us to track the development of individual and group behaviors in gerbil pups during the period of development when animals adopt independent repertoires (See results below).

Distinct developmental trajectories for social and non-social behaviors

Using the processing pipelines and heuristics described above, we evaluated the development of (i) environment exploration and (ii) social behaviors in gerbil pups (Fig 2). We defined environmental explorations as behaviors which *occur independently* (i.e., without an apparent correlation to other animals' actions), including time spent in proximity to the food hopper or water spout or time spent exploring outside the nest. We defined social behaviors as those that *necessarily require another animal*, including (i) nest-related behaviors, (ii) social foraging, and (ii) social approaches.

The development of territory exploration in gerbil pups. We tracked the amount of time gerbils spent exploring their habitat anywhere outside the nest, in the food hopper region, and in the water spout region, as well as the total distance traveled per day (Fig 2a; see also Methods). We differentiated between periods during which behaviors displayed (i) *rapid development* (triangles) or (ii) *stabilization* (Fig 2b). For each behavior, we defined a postnatal day as a period of *rapid development* when it differed statistically from the previous 2 days and following day (2-sample K-S test; p-value < 0.05; see also Methods). As we were interested in the maturation of behavior, we evaluated pup behaviors against the average adult behavior on P29. We selected P29 as the adult reference date as we found all behaviors studied stabilized by this period.

We found that pup (i) distance traveled per day and (ii) open exploration time followed similar trajectories: they started at 8.72 ± 1.71 % (or 8.01 ± 1.53 meters/day) and 16.34 ± 5.48 % (or 0.88 ± 0.32 hrs/day), and increased continuously until P26 where they stabilized near adult levels at 85.90 ± 13.08 % (or 78.98 ± 9.91 meters/day) and 96.16 ± 12.82 % (or 5.17 ± 0.84 hrs/day), respectively (Fig 2b). We note that both distance traveled and environment exploration time underwent rapid development for most of the days between P17 and P25. These open environment exploration findings suggest a steady and continuous maturation rate with pup behavior becoming similar to adults by P26, 10 days after gerbil pups leave the nest.

In contrast, food hopper exploration increased slowly from P16 at 16.12 ± 14.59 % (or 0.14 ± 0.10 hrs/day) to P21 at 44.54 ± 24.92 % (or 0.44 ± 0.13 hrs/day); and underwent a period of rapid development between P22 until P26 stabilizing from P27 - P29 at 201.58 ± 68.18 % (or 1.92 ± 0.25 hrs/day) more than 100% higher than adult averages (Fig 2b).

Water spout region exploration also displayed three stages, but with a unique trajectory: (i) an initial stage with pups spending less than 10% of the adult averages from P16 at 2.75 ± 1.44 % (or 0.007 ± 0.004 hrs/day) to P23 at 13.75 ± 7.16 % (or 0.04 ± 0.02 hrs/day), (ii) a period of rapidly increasing water spout access from P24 at 31.28 ± 11.10 % (or 0.07 ± 0.02 hrs/day) to P26 at 83.68 ± 35.12 % (or 0.19 ± 0.07 hours), and (iii) a period of stability from P27 to P29 where gerbil pups spend 77.27 ± 30.26 % relative to the adults (or 0.18 ± 0.02 hrs/day). The rapid increase from P24 to P26 may reflect a reduction in the time spent nursing.

Distinct inter-behavior developmental trajectories for environmental exploration. We investigated whether individual behaviors were sufficiently distinct from one another by evaluating whether the intra-behavior variances were smaller than inter-behavior variances. We developed a simple method that uses PCA space and convex hull overlaps to quantify how much individual gerbil developmental trajectories were similar to each other within and across behaviors (Fig 2c-d; see Methods). We used PCA space to obtain a lower manifold on which we could quantify the overlap within each behavior against other behaviors (as opposed to kullback-leibler divergence or mahalanobis metrics which do not directly quantify overlap). For clarity, each point in PCA space represents the behavior trajectory of a single animal from P16 to P29. We used 3 PCs to compute the intersection as this provided approximately 80% variance explained for all the behaviors (see Methods). For environment exploration behaviors we found only minimal overlap between open exploration time (0.73%) and distance traveled per day behaviors (5.9%) confirming our qualitative observations that open exploration time and distance traveled per day curves were most similar to each other but that overall, all environmental exploration behaviors had distinct trajectories.

The development of social behaviors in gerbil pups. We additionally quantified social behaviors between gerbil family members, focusing on time spent together, approaches and sequences where individual animals followed each other out of the nest (Fig 2e).

With respect to *nest and huddling behaviors*, we computed the relative behaviors of pups to adults for (i) time spent per day in the nest, and (ii) *nest following* behavior as the ratio of the number of times pups were followed by adults after leaving the nest vs. adults following adults after leaving the nest (Fig 2f). We found that pups spent more time than adults in the nest on P16 at 121 ± 2 % (or 23.12 ± 0.02 hrs/day) and this value reached and stabilized at the adult average by P24 at 104 ± 6 % (or 19.80

± 1.05 hrs/day). Similarly, pups were followed out of the nest by parents significantly more than the adult average on P16 at 151 ± 0.17 % (or leading exits 76.09 ± 8.57 % of the time), and this nest following behavior stabilized also around P24 at 107 ± 12 % (or 53.53 ± 5.88 %). Overall, the behaviors had some overlap in PCA space (up to 20%) but were otherwise distinct (Fig 2g).

With respect to social *exploration,* we found that pups tended to spend significantly more time near pups than adults (Fig 2h). In particular, at P16 pup-pup pairwise exploration was low 37.07 ± 24.42 % (or 9.15 ± 4.15 minutes/day), increased rapidly to adult levels by P19 at 129.02 ± 63.34 % (or 28.03 ± 7.55 minutes/day) and stabilizing on P29 at 157.62 ± 44.69 % (or 33.67 ± 1.51 minutes/day) slightly above the average pairwise adult-adult social exploration of 27.48 ± 9.63 minutes/day. In contrast, adult-pup pairwise exploration time started lower on P16 at 29.52 ± 13.83 % (or 5.35 ± 1.88 minutes/day) and increased slower reaching a peak around P26 at 85.02 ± 32.78 % (or 16.02 ± 4.03 minutes/day). These findings suggest that pups interacted selectively with other pups throughout development as compared to adult interactions (e.g. pup-pup interactions were 148.38% greater than pup-adult). There was no overlap in the convex hull (Fig 2i).

For approach behaviors, we found a similar pattern between pup-pup and pup-adult approaches with some important differences. Pup-pup approaches were ~3-fold lower on P16 at 9.52 ± 7.96 % (or 3.52 ± 2.97 approaches/day), as compared to pup-adult approaches at 23.77 ± 15.28 % (or 10.08 ± 6.38 approaches/day). Both types of approaches increased gradually, with pup-pup approaches undergoing rapid development from P20 at 24.70 ± 13.35 % (or 11.14 ± 5.44 approaches/day) to P25 at 96.23 ± 23.47 % (or 39.76 ± 16.21 approaches/day) before leveling off to near adult levels P26 to P29 averages 92.24 ± 17.54 % (or 38.29 ± 13.29 approaches/day). Pup-adult approaches also underwent rapid development from P20 at 57.07 ± 22.18 % (or 28.21 ± 8.95 approaches/day) to P25 at 102.50 ± 21.26 % (or 35.67 ± 9.40 approaches/day) and leveling off to adult levels (P26-P29: 103.51 ± 32.10 %, or 44.18 ± 12.73 approaches/day for pup-adults, compared to 43.33 ± 9.50 for adult-adults). The convex hull overlap between pup-pup and adult-pup approaches was around 10% suggesting only limited similarity between the individual trajectories (Fig 2k). Taken together, pairwise approach analysis suggests that while pups approach other pups fewer times per day during early development (Fig 2j) they spend more time together following such approaches (Fig 2h).

Distinct rapid development periods. Over all behaviors studied, we found distinct sequences of rapid development and stabilization periods suggesting three types of development: <u>an early-only</u>

development period from P17-19, a broad continuous development period covering P17-P26 and a middle/late only period covering P22-26 (Fig 2l). We found that parental following behavior following pup nest exits and pup-adult social exploration fell into the early development class, suggesting these behaviors' early rapid change was a consequence of the capacity to leave the nest rather than developing social relationships and affinities for specific environmental locations. In the long continuous development class we find several behaviors: pup-pup social exploration (P17-P25 with 2 distinct stages), pup exploration time (P17-P25, with 2 distinct stages), pup nest behavior (P17-P25, with 2 distinct stages), and pup distance traveled (P17-P26). These longer spanning rapidly developing behaviors could be driven by increased capacities to explore the environment but also discovery of socially valuable spaces. In the last category of development, we find pup to adult and pup to pup approaches (both from P20-P25), food area proximity (P22-26) and water area proximity (P24-P26). This last period suggests an increase in the value of actively approaching other animals and of the rise of independent feeding and social interactions - especially between pups. Critically, there were no rapid development periods after P26 for any behaviors and all pup behaviors (excluding food hopper exploration and pup-adult social foraging time) stabilized after P26 to near adult-like levels. This suggests that pup behavior development across the axes we studied is largely complete.

In sum, we found that most environmental exploration and social behaviors had unique developmental trajectories, even between behaviors from the same class, with intra-behavior differences being larger than inter-animal differences. This supports the unfolding of different classes of behavior on distinct time courses correlating with the maturation of the nervous system in rodents between P15 and P30 (see Discussion). We also found that there are distinct rapid development periods during which pup behavior changed rapidly over a period of a few days or longer. This suggests that in addition to distinct behavioral correlates to the maturing central nervous system, there are narrow periods of a few days during which specific behaviors mature significantly.

Sex-based behavioral difference in adult gerbils and developing pups

Female pups and female adults explore for longer periods and travel longer distances than males. We quantified the amount of time spent exploring outside of the nest by sex for both adults and pups (Fig 3a). Across our 3 cohorts we had 3 adult males, 3 adult females, 3 male pups and 9 female pups. We found adult females on average spent 6.03 ± 0.90 hrs/day outside of the nest compared to males who spent 4.69 ± 0.83 hrs/day. For the 14 day recording duration, this yielded a total exploration time of 78.67 ± 5.05 hrs/cohort and 61.29 ± 1.44 hrs/cohort, respectively. The average daily difference in exploration between adult females and males was 1.35 ± 0.90 (t-test p-value: 5.51e-12; 2-sample K-S test against a shuffled distribution p-value: 1.67e-07; see also Methods). Similarly, female pups on average spent 3.73 ± 1.85 hrs/day outside of the nest compared to male pups who spent 2.94 ± 1.39 hrs/day outside of the nest, yielding a total exploration time of 48.96 ± 7.27 hrs/cohort and 38.25 ± 1.92 hrs/cohort, respectively. Female-pup to male-pup differences were smaller: -0.02 ± 0.87 (t-test p-value: 8.42e-01; 2-sample K-S test p-value: 9.54e-01; note; for pup exploration time the differences in Fig 3a were driven largely by our third cohort which did not have any male pups and was excluded in the computation of statistical significance; see also Methods).

Similarly, adult females traveled on average 109.64 ± 31.14 meters/day compared to 76.44 ± 19.25 meters/day for adult males, resulting in total distance traveled of 1435.60 ± 294.64 meters for females and 1001.35 ± 82.39 meters for males for the duration of each cohort. The daily average distance traveled by females more than males was 33.20 ± 33.67 (t-test p-value: 1.54e-07; 2-sample K-S test p-value: 8.28e-05). Female pups also traveled on average longer 50.08 ± 27.66 meters/day than male pups 40.08 ± 21.32 meters/day, resulting in total distances traveled of 657.23 ± 79.69 meters and 523.77 ± 43.19 meters, respectively. The daily average difference in distance traveled by the female pups more than male pups was 7.41 ± 9.52 (t-test: 1.22e-08; 2-sample K-S test: 1.63e-07).

Overall, adult females spent on average 29% longer outside of the nest and traveled 43% more than males during the 14 day period we recorded. Female pups also traveled 25% more on average than male pups. These findings suggest a difference in adult male and female roles during periods of pup rearing. Furthermore, since they were present to some extent in young female pups, this difference in longer activity periods for females may be partially inherited.

Female adults but not female pups spend more time in food hopper and water regions than males. Adult females spent almost 100% more time than males in the food hopper area: average 1.40 ± 0.74 hrs/day in the food hopper area vs. males 0.73 ± 0.27 hrs/day in the food hopper region (totalling 18.42 ± 4.11 hrs/cohort and 9.55 ± 1.16 hrs/cohort for the duration of our recordings; Fig 3c, left). On average, females spent 0.43 ± 0.40 hrs/day more than adult males in the food hopper region (t-test: 2.76e-08; 2 sample K-S test: 2.96e-04). Female pups spent on average 0.83 ± 0.65 hrs/day and male pups 0.76 ± 0.62 hrs/day in the food hopper region, resulting in totals of 10.72 ± 1.31 hrs/cohort and 9.82 ± 0.36 hrs/cohort respectively (Fig 3c, right). On average, female pups spent 0.08 ± 0.23 hrs/day more than male pups in the food hopper region (t-test: 7.64e-03; 2 sample K-S test: 2.46e-01).

We found similar trends with respect to water spout region with the adult female spending almost 80% more time in the water region than the adult male: average 0.27 ± 0.13 hrs/per day vs. males 0.15 ± 0.05 hrs/day (total times of 3.54 ± 1.36 hrs/cohort and 1.99 ± 0.36 hrs/cohort, respectively, over the entire recordings). On average, female adults spent 0.11 ± 0.13 hrs/day more than males in the water spout region (t-test: 6.36e-06; 2 sample K-S test: 1.50e-05). In contrast, female pups and male pups spent similar amount of time in the water spout region: female pups 0.06 ± 0.06 hrs/day, vs. male pups 0.07 ± 0.06 hrs/day (totals of 0.76 ± 0.21 hrs/cohort and 0.88 ± 0.25 hrs/cohort, respectively). There were no significant differences between pup water spout behaviors.

We note that the female adult - but not the male adult - time spent in the food hopper region decreased 26% from P24 (1.31 ± 0.19 hrs/day) to P29 (1.00 ± 0.58 hrs/day) and the time spent in the water region decreased 41% from P24 (0.34 ± 0.12 hrs/day) to P29 (0.20 ± 0.10 hrs/day). This is consistent with a decreased role of the adult female in nursing the pups which underwent rapid development periods for food hopper proximity (beginning at P22) and water spout region (beginning at P24) (see Fig 2).

Parental nest following behaviors. At P16, we found that adult gerbils (both female and males) tended to follow male pups out of the next 88.5 ± 2% more often than following female pups 76.7 ± 13% (Fig 3e; these differences were not statistically significant).

Pup-pup pairwise foraging time shows intra-sex bias. For social exploration behavior in pups, we found that female pups spent on average 30.48 ± 12.17 mins/day with another female pup and only 18.73 ± 10.91 minutes with male pups, i.e. 63% difference. On average, female pups spent 5.53 ± 5.30 mins/day

with other female pups more than with male pups (t-test: 2.28e-10; 2 sample K-S test: 4.89e-09). (We note that these values take into consideration the fewer numbers of male pups for this comparison; see Methods). While only a single cohort had two male pups, in that cohort the male pups also appeared to prefer within-sex time spent together (Fig 3f). On average, the male pups spent 18.66 ± 7.16 mins/day with each other more than with the female pups (t-test: 3.71e-07; 2 sample K-S test: 1.64e-03). Overall, this suggests sex-based differences in social behaviors are entrenched from very early development.

Adult to female pup social proximity time is significantly higher than proximity to male pups. We found that adult females spent an average of 15.23 ± 6.09 mins/day with female pups outside of the nest and 11.59 ± 4.29 mins/day with male pups, a difference of 31%. On average, the female adult spent 3.16 ± 4.91 mins/day more with female pups than male pups (t-test: 1.06e-06; 2 sample K-S test: 4.37e-04). The adult male had even more pronounced bias, spending 14.39 ± 6.00 mins/day with female pups and only 7.89 ± 2.97 with male pups, an 82% difference. On average, the adult male spent 5.57 ± 5.52 mins/day more with female pups than male pups (t-test: 3.57e-12; 2 sample K-S test: 1.07e-08). These results (Fig 3g) show the presence of a significant bias in socialization time with female pups over male pups, though it remains unclear what is driving such differences (e.g. male pups approach parents as frequently as female pups, but spend less time in the interactions; see also Fig 3i and Discussion).

Sex-specific approach behaviors between pups and between adults and pups. For adults, we found that males and females tended to approach each other a similar number of times per day, males approaching females: 42.17 ± 14.62 approaches/day and females approaching males: 38.10 ± 23.60 approaches/day (no statistically significant differences; t-test: 1.45e-01; 2 sample K-S test: 1.12e-01) (Fig 3h - left panel). In contrast, we found that male pup to male pup approaches occurred an average of 30.07 ± 20.64 times/day (cohort 2 data only), and female pups approaching female pups occurred 20.01 ± 16.66 times/day (Fig 3h, right). Our interpretation is that male pups tended to have shorter episodes of social interaction than female pups, suggesting a partially different type of social dynamic was at play (see Discussion).

We also found that adult females were approached significantly more by pups than the adult female approached the pups: average number of female pup to adult female approaches 29.07 ± 16.25 times/day and female adult approaches to female pups 14.46 ± 11.66 times/day, a difference of more than 100% (t-test: 3.55e-08; 2 sample K-S test: 2.46e-05). Male pups also tended to approach the adult

female more: 34.05 ± 17.41 times/day while adult females approached male pups 19.57 ± 11.54 times/day, a difference of 74% (t-test: 5.67e-07; 2 sample K-S test: 2.59e-04). In contrast, the adult male approached female pups 21.96 ± 14.16 times/day, while female pups approached the adult male 28.86 ± 16.33 times per day, a difference of only ~30 % (not statistically significant, t-test: 1.01e-01; 2 sample K-S test: 1.83e-02) (Fig 3i - right panel). And similarly, the adult male approached male pups 26.52 ± 14.24 times/day, while male pups approached the male adult 27.76 ± 14.15 times/day, a difference of only ~5% (not statistically significant, t-test: 5.50e-01; 2 sample K-S test: 9.87e-01). These findings support that pups seek (i.e. approach) the adult female significantly more than the adult male but that this seeking behavior between pups and the adults is not symmetric in both cases.

In sum, we identify sex-dependent behaviors within both adults and pups and between the age classes. We find adult females spend more time in the open than males, travel longer distances, and spend more time in the food hopper and water spout regions. These findings may suggest a division of labor during critical development periods for pups - though it is unclear if such behaviors are present once pups have left the family. Within pups, we find that pups prefer socializing with their own sex substantially more than with the other sex - suggesting very early sex-based socialization preferences. Across the age classes, we find that both adult females and adult males tend to prefer socializing with female pups.

The development of circadian rhythm based behaviors

Diurnal and nocturnal behaviors. Across most behaviors tracked we found that both adult and developing pups had similar light:day cycle exploration times or had slight preferences for light-cycle over dark-cycles (Fig 4). For example, the averages for adult foraging near the food hopper were 56.59% ± 0.53% during light cycle vs. 43.41 ± 0.46% for dark cycle while pup foraging was 59.6 ± 2.62% for day cycle and 40.4 ± 2.53% for night cycle (Fig 4a). In contrast, waterspout region averages were: adults 49.58 ± 0.7 % for day cycle and 50.42 ± 0.62 % for night cycle and pups: 48.8 ± 2.29% for light cycle and 51.2 ± 3.57 % for night cycle.

Genetically vs. environmental driven diurnal behavior structure. Our observations of rapid development periods suggested approximately three different developmental stages: an initial period up to P19, a longer period up to P26 and a final stage from P26 to P29 during which all pup behaviors stabilized. We accordingly used this observation to initialize our analysis of development of circadian rhythms (though we also sought to optimize these transition periods as well; see below and Methods).

In adults, we observed both nocturnal and diurnal behaviors, with a slight bias towards diurnal behavior and a systematic increase in the amount of behavior during daytime and peaking between 19:00 and 20:00 (prior to the night cycle start). This ramping was present for all developmental periods in adults and for most behaviors studied including exploration time (Fig 4a), distance traveled (Fig 4b, foodhopper proximity (Fig 4c), waterspout proximity (Fig 4d) and adult-adult approaches (Fig 4f). It was absent, or very weak in adult-adult proximity time (Fig 4e).

Similarly for pups, we also observed diurnal ramping in activity over the day cycle across most behaviors. However, this ramping up was present in all developmental periods only for exploration time (Fig 4a, right panel) and pup-pup time spent together (Fig 4e, right panel). For other behaviors, this substantial ramping effect developed gradually: foodhoper region exporation (Fig 4c), waterspout region proximity (Fig 4d), distance traveled (Fig 4b), and pup-pup approaches (Fig 4f). This suggests that the former behaviors (i.e. open exploration time and pup-pup social proximity) are likely driven by genetically encoded processes whereas the latter (food and water region exploration, distance traveled and pup - pup approaches) were driven by systems that develop later to detect valuable environmental and social interactions.

The development of crepuscular behaviors. We observed crepuscular behavior in both pups and adults for some, but not all, behaviors. In adults, crepuscular behavior was present in distance traveled (Fig 4b), weaker in water spout region proximity (Fig 4d), and mostly absent or very weak in foodhopper region proximity (Fig 4b), social foraging time (Fig 4e) and adult-adult approaches (Fig 4f). (We note that our observation of weak crepuscularity in waterspout region proximity is similar to Pietrewicz et al 1982). In pups, there were strong crepuscular behaviors in exploration time (Fig 4a), distance traveled (Fig 4b), foodhopper proximity (Fig 4c), pup-pup proximity time (Fig 4e). Waterspout region proximity had only weak crepuscularity (Fig 4d) and there were few and small pre-dawn peaks in pup-pup approaches (Fig 4f). Importantly, crepuscularity developed over time for food hopper, pup-pup time spent together, exploration time and distance traveled - being mostly absent from early development (i.e. P16-P20). Interestingly, we note that qualitatively, pups appear to develop much more pronounced pre dark-to-light cycle peaks generally around 4:00 and 5:00 than adults (dark-to-light cycle change was at 8:00).

Detecting the development of circadian rhythms. We sought to agnostically determine the transition from non-circadian to circadian rhythms by optimizing the times of three developmental periods. In particular, we sought to determine the times of transition between: an initial period D1, a middle period D2 and a final period D3 (initialized as P16-P20, P21-P25 and P26-P29 above). We optimized for the two time points, t1and t2, determining the transition from D1 to D2 and D2 to D3, respectively, by maximizing the difference between the individual animal circadian trajectories in each of these periods (see also Methods). For foodhoper region proximity, we found D1 as: P16-P19, D2 as: P20-P27, and D3 as P28-P29. (Fig 4g).

DISCUSSION

Studying the dynamics of family behavior in social mammals, such as gerbils, offers a unique opportunity for assessing the properties of the family unit and its effects on an individual's development. Furthermore, it provides the core findings with which to interpret central nervous system development. Rodent families have been studied both in natural environments to establish ground truth measurements (Ågren 1984; Ostermeyer & Elwood 1984; Hurtado-Parrado et al 2015), and in simplified laboratory enclosures to conduct mechanistic experiments (Lahvis 2017; Bordes et al 2023). Laboratory studies generally exploit brief, stereotyped behaviors in which one or two subjects are tested in a foreign environment. While this standardizes and simplifies the experimental design, it can disrupt diurnal cycles (e.g., feeding, sleep), perturb arousal or motivational states (e.g., human sensory stimuli; Sorge et al 2014), and introduce unanticipated variation (Crabbe et al 1999; Chesler et al 2002; Richter et al 2009). Some quantitative descriptions of rat family behavior in home cage environments have been attempted, but relied on human scoring of videos (Thiels et al 1990; study on weaning in rat pup behavior patterns). Using continuous recordings in an enlarged home-cage paradigm, we sought to quantify the development of autonomous and social behaviors in gerbil pups across a highly dynamic developmental window, i.e. P15-P30, during which gerbil pups go from being almost exclusively in the nest to exploring independently. We employed machine vision tools and developed methods for quantifying a set of unique developmental trajectories.

The gerbil family as a unique window into the development of behavior. Mongolian gerbils are a favorable species for the study of families as they exhibit unique behaviors (from mice and rats). These include: (1) monogamous pair-bonding (Tchabovsky et al 2019), (2) family member division into founder pairs, and integrated and expelled family members (Scheibler et al 2004), (3) dynamic association strategies to reflect life history (e.g., during food-hoarding season; Deng et al 2017), (4) coordination between male and female parents during pup rearing (Ostermeyer & Elwood 1984), (5) female mating preferences for relatedness (not kinship) (Valsecchi 2002), (6) increased prosociality towards pairbond partners - but not others - following testosterone boosts (Kelly et al 2022), and (7) expulsion of males with increased reproductive capacities from family group (Scheibler et al 2006). Gerbil pups largely remain in the nest until P16, and while they increasingly leave the nest from P18-P22 (Kaplan & Hylan 1972), they remain attracted to maternal nest odors until at least P42 (Yahr & Anderson-Mitchell 1983). During this time they do not increase vocalizations when the mother is absent

from the nest, and receive a similar amount of care from the father (Ostermeyer & Elwood 1984). By comparison, laboratory measurements in mice suggest that juveniles first leave their nest at postnatal day (P) 11-12, and begin to consume food and water between P15-18 (Bolles & Woods 1964; Gramsbergen et al 1970; Babický et al 1972; Thiels et al 1990; Renner & Pierre 1998).

Pup behaviors develop along narrow and distinct trajectories. For environmental exploration behaviors, such as time spent exploring and distance traveled per day, we observed a gradual pattern of development between P16 and P26, when they reached levels on par with adults. Food proximity behavior also developed gradually at first, but displayed a dramatic increase between P22-P26, then stabilizing at 100% more than the adult average. Water proximity behavior developed much slower, increasing substantially only on P24 and reaching < 80% the adult average by P26. Taken together, these results suggest the gradual development of exploration behaviors (e.g. distance traveled and exploration time), but a distinct value-driven emergence of food and water exploratory behavior. The timing of the rapid development periods for food exploration (P22) and water exploration (P24 onwards) coincide with the adult female decreasing time spent in the food hopper region (26% decrease from P24) and the time spent in the water region (41% decrease from P24). These findings provide independent confirmation that the pups' increase of food hopper and water spout proximity were driven, in part, by consumption behaviors and a decreased dependence on the adult female.

With respect to social behaviors, we found distinct trajectories for passive (e.g. nest), interactive (e.g. social proximity) and active (e.g. approach) behaviors. For nest behaviors, pups stayed in the nest approximately 20% more than adults at P16, and only reached adult levels at ~P24. Adults tended to follow pups out of the nest 50% more on P16, with this difference disappearing by ~P24. These behaviors confirm that pup exploration of the environment increased gradually, likely coinciding with maturation of critical sensory-motor systems over this period (Tan et al 2017). Interestingly, nest following behavior suggests that, early in development, the pups' decision to leave the nest is not based completely on a drive to follow parents. In contrast, parents had a higher tendency to leave the nest (within 10 sec) of a young pup leaving as opposed to the other way around. With respect to social proximity time, we found that pups tended to spend the same time together with each other and adults at P16 (<20% of the adult-adult average). However, these trajectories diverged significantly with pup-pup proximity time reaching 100% of the adult average relatively fast (~P19) and stabilizing slightly above this average. In contrast, pup-adult social proximity increased slowly and reached ~50% by P20 and

stabilized at ~60% of the adult average. Our interpretation is that pups value social interactions with other pups from very early on in development: by P17, pup-pup vs. pup-adult proximity time were substantially different (Note: it may also mean that pups tended to explore environment in similar ways to each other - whereas the adults had different types of environment exploration behavior). Lastly, pups tended to approach each other < 10% at P16 compared to approaching adults ~25%. This difference persisted until P24-25 when both approach behaviors stabilized to adult-like levels. To reconcile this with the larger time that pups spend together, we suggest that during earlier parts of development, pups tended to approach adults more often but stayed within proximity of adults for shorter periods of time.

Behavior development and stabilization during P15-30 is plausibly supported by the maturation of spatial-cognition and motor systems. A systematic finding in our study is that all behaviors commence at near zero levels at P16, undergo significant changes from P16 onwards, and stabilize by P26 (i.e. no significant changes occurring after P26). At P26, 7 of 10 behaviors stabilized within < 25% of adult average behavior. (The notable differences were: food hopper region: pups spent 100% more time than the adult average; pup-pup proximity: pups spent 30% more time together than adults did; and pup-adult proximity: pups spent 40% less time with adults than the adult average).

This rapid change and near complete plateauing of many behavioral development programs between P16 and P26 coincides with the maturation of neural pathways that subserve spatial memory especially in the hippocampal and medial-entorhinal-cortex axis (HPC-MEC). In mice, there are several systems that develop during this period: formation and maturation of head direction cells (P10-P20), place cells (P14-P40), boundary responsive cells (P16-17) and grid cells (P20-P21) (Tan et al 2017). In rats, hippocampus dependent learning also has been shown to commence around P18 for water maze learning (e.g. visual platform - P17-20 - and hidden platform - P19-21- learning), and T-maze learning (P19-P42) (Wills et al 2013). The behaviors that we have observed do not correlate with the onset of sensory transduction. The onset of transduction displays a similar sequence in most vertebrates, progressing from somatosensory to vestibular, olfactory, auditory and, finally, to the visual system (Gottlieb 1971). In mice, these systems emerge between birth and P12. However, a prolonged development of sensory processing has been demonstrated for many mammals (Sanes et al 2019). Therefore, one plausible interpretation is that the highly dynamic and specific periods of development that progress from a minimal state to near-adult levels between P16 to P29 are driven, in part, by maturation of HPC-MEC axis along with related areas.

A second plausible idea is that the changes and stabilization we observe arise as the motor cortex matures. In particular, the motor cortex is still relatively immature (e.g., as compared to sensory cortices), with an estimate that M1 starts producing a significant outflow only after postnatal day 20 (Blumberg and Adolph 2023). Cortical motor outflow appears to begin at ~P24 in rats (Young et al 2012; Ramanathan et al 2015; Singleton et al 2021), and there is a significant increase in stimulation-related motor activity after this age. For example, M1 activity recorded in vivo in P19-23 rats has yet to exhibit activity before wake or sleep movements (Dooley et al 2021). Therefore, an increase in exploration may also be tied to the maturation of the motor cortical pathway.

Possibility of discrete stages of behavior development. We sought to classify our findings by considering the stages of rapid development - which appeared to fall into discrete classes. In our view, it is likely that individual behaviors unfold on different time scales with rapid development occurring in narrow windows for some behaviors. We suggested three types of rapid development periods: a period of approximately three days ranging from P16 to P19, a prolonged period of development of several days ranging from P17 to P26, and a late development period of three-to-five days ranging across P20-26. This classification was based on our results and it is possible that slightly different categories could be obtained with different methods or with much larger behavior datasets. While it is intriguing to try and match these identified behaviors with specific developmental programs in neuronal maturation, our evidence only supports a very broad correlation with early developing behaviors likely driven by the early capacities of sensory systems, middle and prolonged periods of development driven by motor outflow and spatial systems (e.g., the HPC-MEC axis), and later behaviors being driven by increasingly sophisticated social-based factors that likely require additional CNS maturation. Importantly, we note that these types of sequential developmental behavior stages are also present in the sequential maturation of entorhinal-hippocampal networks which go from nearly 0% mature at P8-P14 to > 80% mature by P26 (Donato et al 2013; data from mice).

Sex-differences in behavior in environment explorations. In adults, we found that females explored for longer periods (29%) and traveled further (43%) than males, consistent with other studies (Roper 1976; Ru-yung & Shao-liang 1984). Similarly, females spent almost double the amount of time as males in the food hopper region and 80% more in the water region than males. Furthermore, there was a significant

decrease in these differences beginning ~P25, consistent with pups relying on mother care and nursing around this time. In pups, we did not find a significant effect of sex on exploration time, but there was a small, but significant difference for distance traveled. Taken together, these findings support other behavioral studies of a possible sex-based bias in animal foraging times possibly present very early in development prior to sexual maturation.

Sex-differences in social interactions between pups. We found several significant differences in pup-adult and pup-pup interactions based on pup sex. In social exploration, we found that female pups spent 30% more time with other female pups than with male pups. Similarly, we found male pups spent almost 100% more time with each other than with female pups (note: this came from a single cohort that had 2 male pups). Similar to above, we point out that these sex-based differences in pup-pup interactions appear to be present from very early development.

Sex-differences in social interactions between pups and adults. Adult females spent more time with female pups (31%) than with male pups, and the adult males spent 82% more time with female pups than with male pups. In contrast, we found that both female pups and male pups tended to approach the female adult significantly more than she approached them (> 100% and 74% respectively). This asymmetry was not present in adult male to pup approaches. These findings suggest a process by which female pups may be more interested in socializing with adults than male pups, with both pups being significantly more interested in proximity to the female adult than the male adult. While this finding is supported by the need for pups to nurse in early development, we did not observe a decrease in this asymmetry over development, suggesting a more persistent bias being present at least until the end of our analysis period (P29).

Circadian rhythms in adults. Our paradigm enabled us to track the circadian rhythm effects on adult behaviors and the development of circadian rhythm correlated behaviors in pups. Studies on behavior and circadian rhythms in gerbils indicate that light conditions can affect behavior (Pietrewicz et al 1982; Probst et al 1987; Refinetti & Kenagy 2018), with a crepuscular pattern for most behaviors except drinking (Pietrewicz et al 1982). In our study, we found adults were active during both light and dark cycles, with more behaviors during day time and a substantial ramping of activity prior to night cycle onset. We also observed crepuscular behavior patterns only in the amount of distance traveled with other

behaviors showing weak or no crepuscularity. We note that increased activity during the day is consistent with findings of greater maternal attention to pups during the day than at night (Grota & Ader 1969; Levin & Stern 1975).

Development of circadianity in pups. Beginning at ~P26, pups began to display diurnal patterns in all behaviors with peaks at night cycle onset. However, in contrast to adults, these older pups also displayed substantial crepuscular behaviors across most behaviors (note: pup crepuscularity was weak in the pup-pup approach behavior - similar to adult crepuscularity). This suggests that pups, unlike adults, had stronger drives to increase their activity pre light cycle onset than adults. Considering all periods of development, however, we found that some behaviors such as open exploration time and pup-pup time spent together changed much less between early and late development compared to food hopper region and waterspout proximity. We sought to use the heterogeneity in food hopper proximity in particular - as it showed the largest differences over time - to agnostically detect different developmental stages for circadian rhythms, and identified P16-19, P20-27 and P28-29 as the most likely partitioning of development into three stages.

Limitations of our work and directions for future studies. Our enriched and enlarged home cage environment enabled the tracking of several types of behaviors. However, our environment did not capture other behaviors observed in the wild such as gerbils' increased tendency for exploration towards novelty (Osborne 1977) and towards "open-arms" of mazes (compared to rats and mice; Rico et al 2016). Gerbils also show increased unpredictability in their escape behaviors than other rodents (Moore et al 2017), and it would be interesting to observe this escape behavior dynamics by simulating predators within our environment. Beyond home cage environments, we propose that contemporary machine learning methods are improving substantially and it may be possible to carry out similar studies in "limited size" environments such as scientifically-purposed barns (Lahvis 2017).

Understanding development from the micro to the macroscale. Our study supports the viability of studying rodent behavior across orders of magnitudes of temporal resolution during highly dynamic periods of behavior and development in family groups of rodents. Our findings reveal novel time courses for development in rodents, including dynamic periods of behavior acquisition, sex-based behavior differences, and the development of circadianity. We view that increasingly ethological (e.g.

group and family housed animals) and naturalistic (e.g. larger home enclosures with increased enrichment opportunities) behavior paradigms in the laboratory environment is an adequate and complementary method for the characterization of behavior and CNS development in health and disease.

METHODS

Experimental animals. Three gerbil families (Meriones unguiculatus, n=6 per family: 2 adults, 4 pups) were used in this study (Charles River). All procedures related to the maintenance and use of animals were approved by the University Animal Welfare Committee at New York University, and all experiments were performed in accordance with the relevant guidelines and regulations.

Audio recording. Four ultrasonic microphones (Avisoft CM16/CMPA48AAF-5V) were synchronously recorded using a National Instruments multifunction data acquisition device (PCI-6143) via BNC connection with a National Instruments terminal block (BNC-2110). The recording was controlled with custom python scripts using the NI-DAQmx library (https://github.com/ni/nidaqmx-python) which wrote samples to disk at a 125 kHz sampling rate. In total, 13.084 TB of raw audio data were acquired across the three families. For further analyses, the four-channel microphone signals were averaged to create a single-channel high-fidelity audio signal.

Home cage enclosure: We used an enlarged home enclosure (WxLxH: 35.6cm x 57.2cm x 38.1cm) with *ad lib* access to a food hopper and a water source.

Video recording. We recorded video using an overhead camera, FLIR USB blackfly S (FLIR USA), at 24 frames per second. We recorded individual periods of approximately 20 minutes (cohort 1) or 60 minutes (cohorts 2 and 3) following which video and metadata were saved for offline use. The saving period resulted in approximately 10% downtime between recordings. Continuous video recordings were obtained from an overhead camera (24 fps), and audio recordings were obtained from four ultrasonic microphones (125 kHz sampling rate).

Night:day cycle. Daytime began at 08:00 and night began at 20:00 during which an infrared light was activated.

Animal shaving patterns. We used a distinct shaving pattern across all our cohorts (as described in Fig 1b). We opted for use of shaving patterns instead of fur bleaching, implanted trackers or body piercing as we viewed shaving as a less intrusive method.

Tracking - Features. We manually labeled 1000 frames using the SLEAP (Pereira et al., 2022) labeling workflow. The skeleton consisted of 6 nodes: nose, spine1, spine2, spine3, spine4, and spine5; and 5 edges: nose to spine1, spine1 to spine2, spine2 to spine3, spine3 to spine4, and spine4 to spine5. Additionally, we labeled each instance with the animal's identity class as female, male, pup1, pup2, pup3, and pup4 to enable tracking unique identities. Due to idiosyncratic differences in shaving patterns, lens distortion and light conditions, each cohort and light condition (i.e. day and night) was labeled and trained individually, resulting in 6 feature models. The final results were trained using SLEAP bottom-up ID models that achieved 97% accuracy of adults and 91% for pups.

Track clean up - features. We developed an ID-switch error detection method to detect and correct some of the SLEAP ID-switch errors. We defined ID switch errors as those for which track segments were swapped between two animals. These errors were identified by evaluating sequential segments of tracks produced by SLEAP and determining whether (i) a large jump in track location occurred; and (ii) whether another segment fit better (usually within < 5 pixels of distance). We additionally implemented an interpolation method that connected SLEAP segments < 3 cm apart that belonged to the same animal. A custom python pipeline was developed to carry out this correction.

Tracking - Huddles. We manually labeled 1000 frames using the SLEAP (Pereira et al., 2022) labeling workflow. The skeleton consisted of 1 node: huddle; and no edges. We identified a huddle as a group of 2 or more animals grouped together in the nest area. Due to idiosyncratic differences in shaving patterns, lens distortion and light conditions, each cohort and light condition (i.e. day and night) was labeled and trained individually, resulting in 6 huddle models. We additionally implemented an interpolation method that connected SLEAP segments for huddles that were less than 30 seconds apart.

Bedding changes and exclusion of time points. We provided an interpolated measure of behavior on days of bedding changes. Briefly, on cohorts 1 and 2 we averaged the behaviors between P23 and P25

instead of using P24. On cohort 3 on P18 we averaged the behaviors between P17 and P19 instead of using the P18 day as we observed a substantial difference in the behavior just for that day.

Cross-validation of behavior tracking. We implemented 10-fold cross-validation to evaluate the precision of SLEAP identity tracking against a human annotator. Briefly, for all feature NNs we split our human labeled frames dataset (1000 labeled frames) into 950 - training and tested on the hold out of 50 frames. We repeated this 10 times by randomizing the training set frames and computed the average identity accuracy errors across adults and pups.

Convex hull overlap metric. We defined overlap volume between behaviors as the intersection between the convex hull generated by individuals in each type of behavior relative to the other behaviors. We computed the overlap as the intersectional volume of the first 3 principle components (using the python vedo package) or when this was not possible due to insufficient points to form a polyhedron - the first 2 principle components. We viewed the overlap volume as better at capturing and representing the inter-behavior similarity by providing a metric that quantifies when some of the individual behaviors were not distinguishable between behavior types. We note that comparing the individual behaviors at each time point was not practical due to only 3 samples in the behavior of the adult female and male and male pup.

Rapid development and stabilization periods. We defined a rapid development period as Pdays that had distributions that were statistically significant than the previous 2 Pdays. We accordingly used a sliding window to pool behaviors in groups of 2 days and compare them against the following 2 days. We used two days as a more conservative way to account for higher variability - than applying smoothing or other filtering techniques on the raw data. We used a 2 sample kolmogorov-smirnov test. For clarity, at each Pday, we pooled data from individuals (e.g. 12 pups or 6 adults) and computed the statistics at the level of multi-cohort.

Heuristics-based behavior classification. We implemented heuristics to compute the location of animals near the food hopper or waterspout and each other. For the food hopper we used an ROI of size 19.50 X 10.50 cm. For waterspout we used an ROI of size 8.50 X 8.00 cm. For inter-animal proximity

we used a distance of < 5 cm separation and a minimal proximity time > 200 ms. Animal locations were computed as the median centroid over (x,y) locations.

SimBA-based behavior classification. We defined an approach as an animal moving towards another - beginning when the initiator starts moving and ending when it stops moving (at most one gerbil length away from the other) or makes physical contact. We manually annotated 300 approaches (4578 frames) in 22 videos and implemented a modified version of SimBA (Nilsson et al 2020) for classifying pair-wise animal approach behaviors. We computed the threshold for approach behavior as the 99% percentile of the prediction distribution. We verified qualitatively that detected approaches matched with qualitative approaches.

Descriptive statistics of animal movements, angles, and distances in sliding time-windows were computed using runtime optimized methods available through the SimBA API and used in a downstream random forest classifier. We randomly sampled annotated frames from separate events in training and test set to reduce time-series data leakage. Within the training set, we further balanced the data by under-sampling the number of non-event observations to a 1:1 ratio of the event observations. Classifiers were validated and optimal discrimination threshold determined by evaluating performances on novel held-out videos excluded from the training and test sets.

Inter-sex differences. We used a t-test to evaluate the significance of the difference between our distribution (usually the difference between two groups) and a zero-mean distribution. Additionally, we used a 2 sample K-S test to compare the original distribution with a shuffled version where the identity of the animals was randomized. For example, for computing the distribution of distance traveled by adult females vs. adult males, we subtracted the distance traveled by each male from distance traveled by each female on each day of each cohort. For the shuffled version, we did the same but randomly switched between subtracting male from female and female from male distances. For pups we only used cohorts 1 and 2, which had male pups, for his comparison. In cohort 1, there were 2 pups and there we computed the average male behavior as the reference behavior and subtracted it from the individual female pup behaviors to get our distributions.

FIGURES

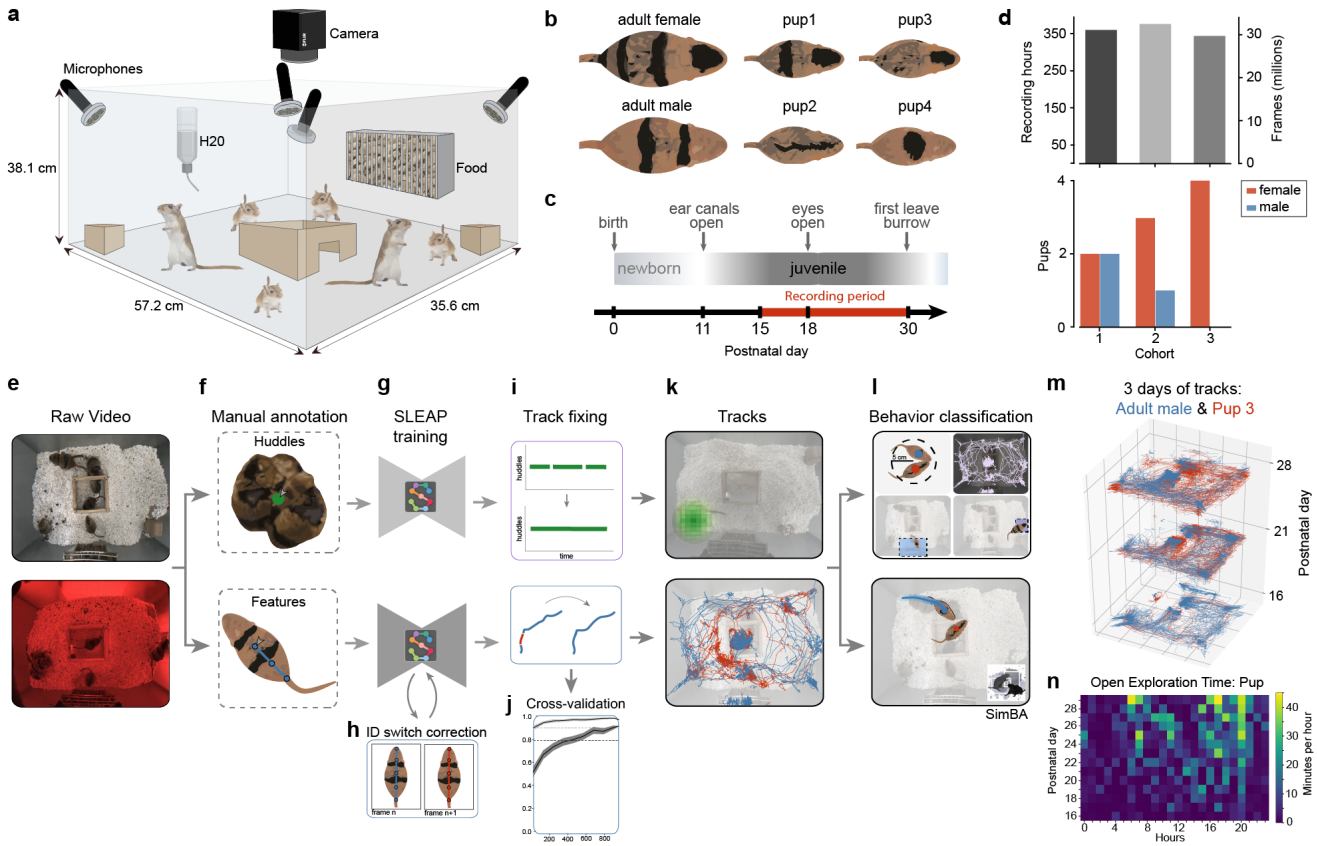

Fig 1. Capturing individual gerbil behavior across weeks of development with subsecond precision. a Homecage and recording setup. Continuous, synchronized video (single overhead camera at 24 fps) and audio (4 ultrasonic microphones at 125 kHz at corners of the enclosure) recordings. Food hopper and waterspout permitted ad libitum access to food and water, and enrichment was provided by one wooden hut and two blocks. **b** Individual gerbil identification based on distinct shaving patterns. **c** Experimental timeline. Three cohorts with the same family composition (2 adults, 4 pups) were recorded continuously for two weeks, from postnatal (P) day 15 to 30. **d** Video data and pup sex distribution. Recording hours and frames per cohort (top). Number of female and male pups per cohort (bottom). **e - l** Workflow of animal tracking and behavior analysis. **e** Acquisition of raw video in a 12 hr light / dark (infrared) cycle. **f** Manual annotation of two categories: huddles (2 or more gerbils huddling together in the nest area) and features (individual animals with a 6-node skeleton). **g** SLEAP training phase. Due to unique variations in the videos, each light condition (light and dark / infrared) and cohort required separate training. This resulted in six feature models and six huddle models. **h** Correction of identity

switches between consecutive frames and re-training to ensure accurate tracking. **i** Track fixing pipelines. Interpolation of small missing periods and fixing id-swapped tracks. **j** Cross-validation procedure to evaluate the model's performance. **k** Visual representation of nest location and animal tracks. **l** Behavior classification using heuristics (top; e.g. time spent in a region of interest and distance traveled) and a SimBA classifier (bottom; for pairwise approaches). **m** Two animal tracks (adult male and pup) across 3 days of development. **n** Example ethogram. Open exploration time per hour for one pup across 15 days of development.

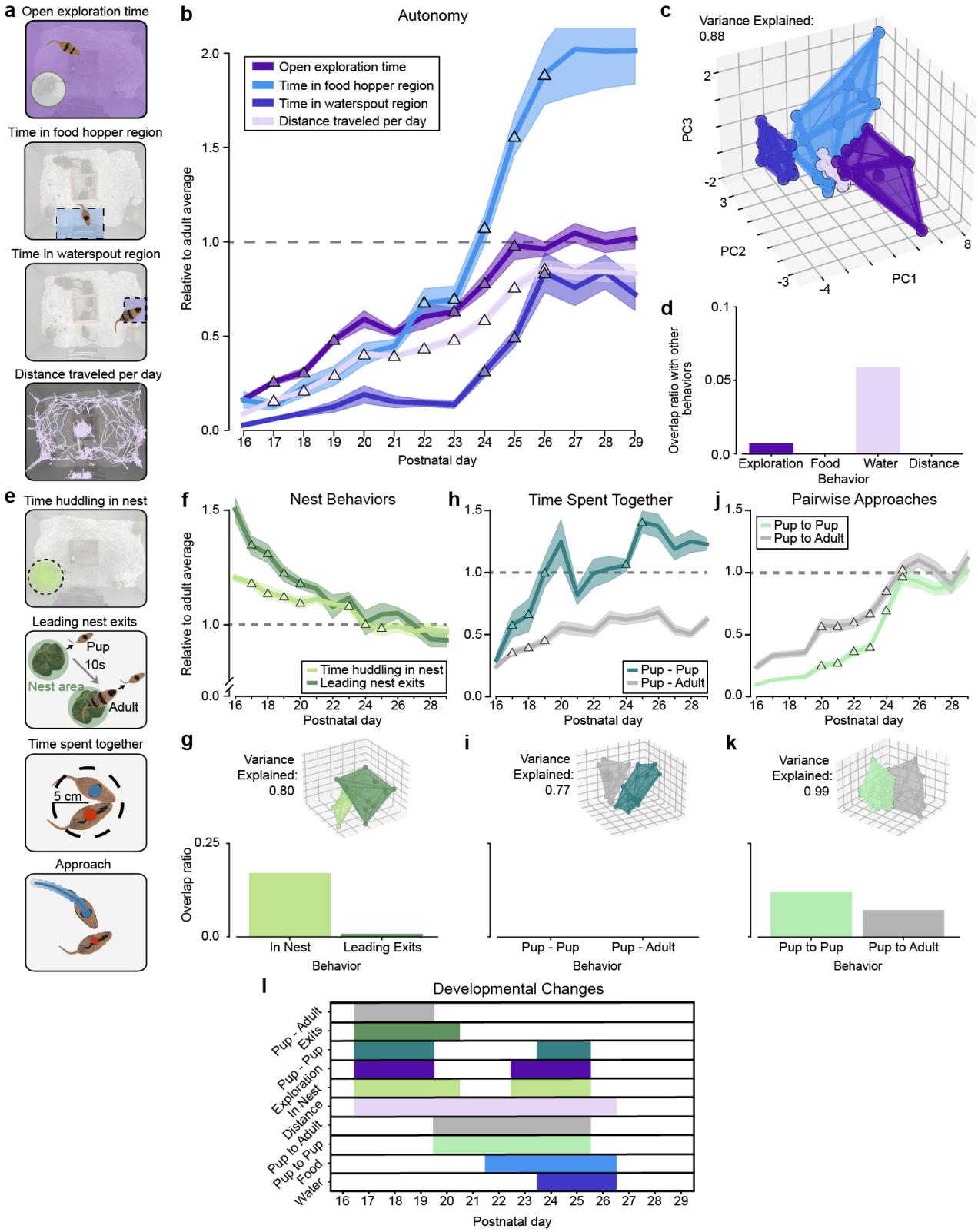
a
Open exploration time
Time in food hopper region
Time in waterspout region
Distance traveled per day
b
Autonomy
Open exploration time
Time in food hopper region
Time in waterspout region
Distance traveled per day
Relative to adult average
Postnatal day
c
Variance Explained:
0.88
PC3
PC2
PC1
d
Overlap ratio with other behaviors
Exploration
Food
Water
Distance
Behavior
e
Time huddling in nest
Leading nest exits
Pup
10s
Nest area
Adult
Time spent together
5 cm
Approach
f
Nest Behaviors
Time huddling in nest
Leading nest exits
Postnatal day
h
Time Spent Together
Pup - Pup
Pup - Adult
Postnatal day
j
Pairwise Approaches
Pup to Pup
Pup to Adult
Postnatal day
g
Variance Explained:
0.80
Overlap ratio
In Nest
Leading Exits
Behavior
i
Variance Explained:
0.77
Pup - Pup
Pup - Adult
Behavior
k
Variance Explained:
0.99
Pup to Pup
Pup to Adult
Behavior
l
Developmental Changes
Pup - Adult
Exits
Pup - Pup
Exploration
In Nest
Distance
Pup to Adult
Pup to Pup
Food
Water
Postnatal day

Fig 2. Developmental changes in gerbil independent and social behaviors. a Independent behaviors. Sketches illustrating open exploration time, food hopper and waterspout regions of interest, and total distance traveled. **b** Development of environment exploration in gerbil pups. The average value of each behavior relative to the adult average on P29 (lines) and standard error (SEM; shading). Pdays classified as rapid development days were denoted with triangles. **c** Inter-behavior developmental trajectories. Implementation of a convex hull overlap method within PCA. 3 principal components (PCs) were used to compute the intersection as this provided ~80% variance explained for all the behaviors. **d** Overlaps in independent behaviors between open exploration and distance traveled. **e** Social behaviors. Images illustrating nest behaviors (time huddling in nest) and sequences where individual animals followed each other out of the nest, time spent together in the open, and approaches. **f** Development of nest behaviors. The average value of time huddling in the nest (light green) and leading nest exits (dark green) relative to the adult average on P29 and SEM. **g** Overlap for nest behaviors. **h** Development of pairwise time spent together in the open. The average value of pup time spent in close proximity with each other (turquoise) and with the adults (gray) relative to the adult average on P29 and SEM. **i** Overlap for proximity time. **j** Development of pairwise approaches. The average value of pups approaching other pups (green) and adults (gray) relative to the adult average on P29 and SEM. **k** Overlap for approach behaviors. **l** Delineation of developmental phases showing rapid development periods (colors).

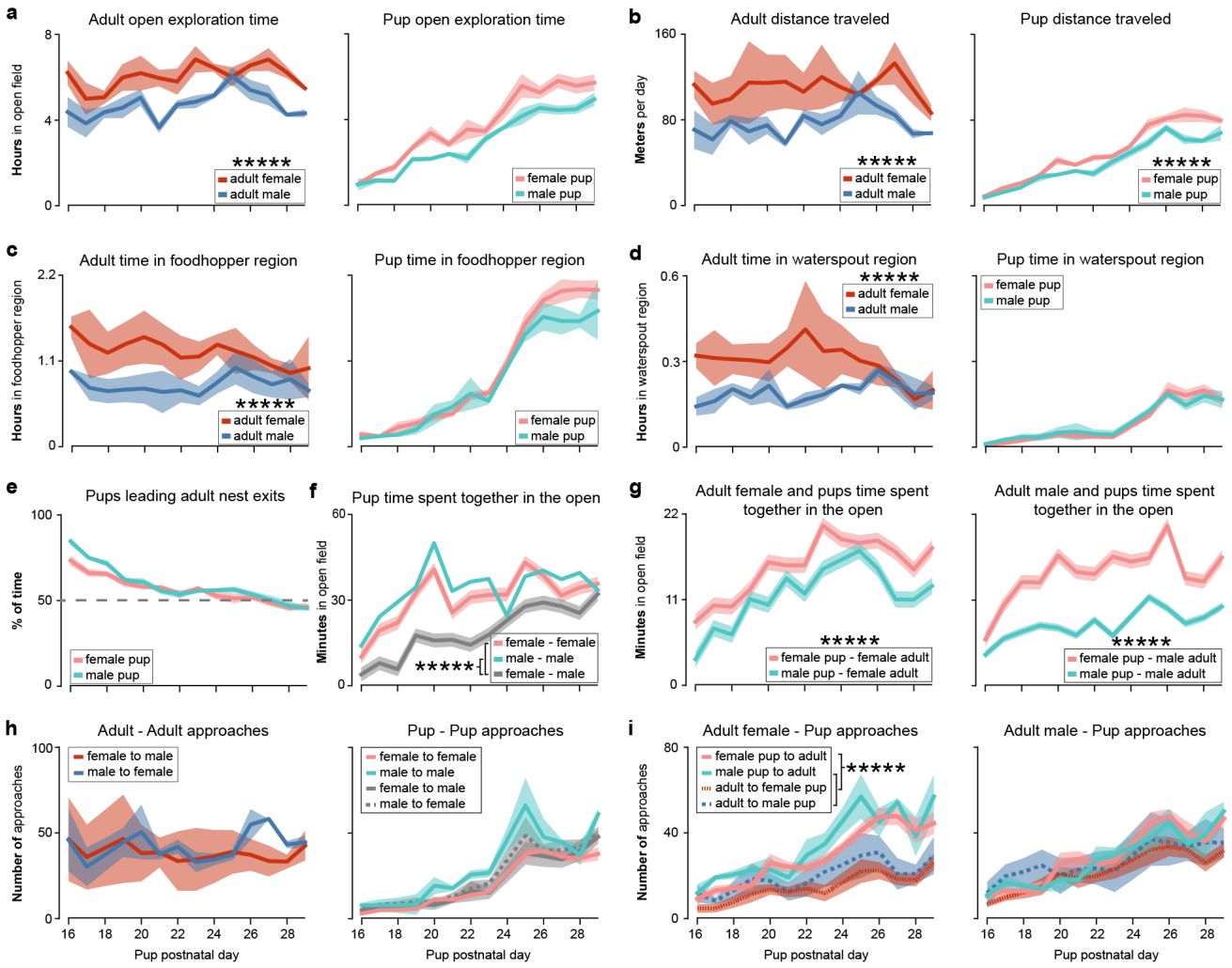

Fig 3. Sex differences in behavior. a The average (lines) and SEM (shading) of the time adult females (red) and adult males (blue) (left) and female pups (pink) and male pups (baby blue) explored outside of the nest. Asterisks indicate statistical significance on both t-test and 2 K-S test (e.g. p-value < 1e-05 = *****; see also Methods). **b** Distance traveled by adults and pups. **c** Time spent inside the food hopper region. **d** Time spent inside the waterspout region. **e** The ratio of the number of times pups were followed by adults after leaving the nest. **f** Time spent in close proximity to each other in the open (pink: female with female; baby blue: male with male; gray: female with male). **g** Time the adult female (left) and adult male (right) spend in close proximity to female (pink) and male (baby blue) pups in the open. **h** The number of adult to adult approaches and pup to pup approaches. **i** Approaches between the adult female (left) and adult male (right) and pups.

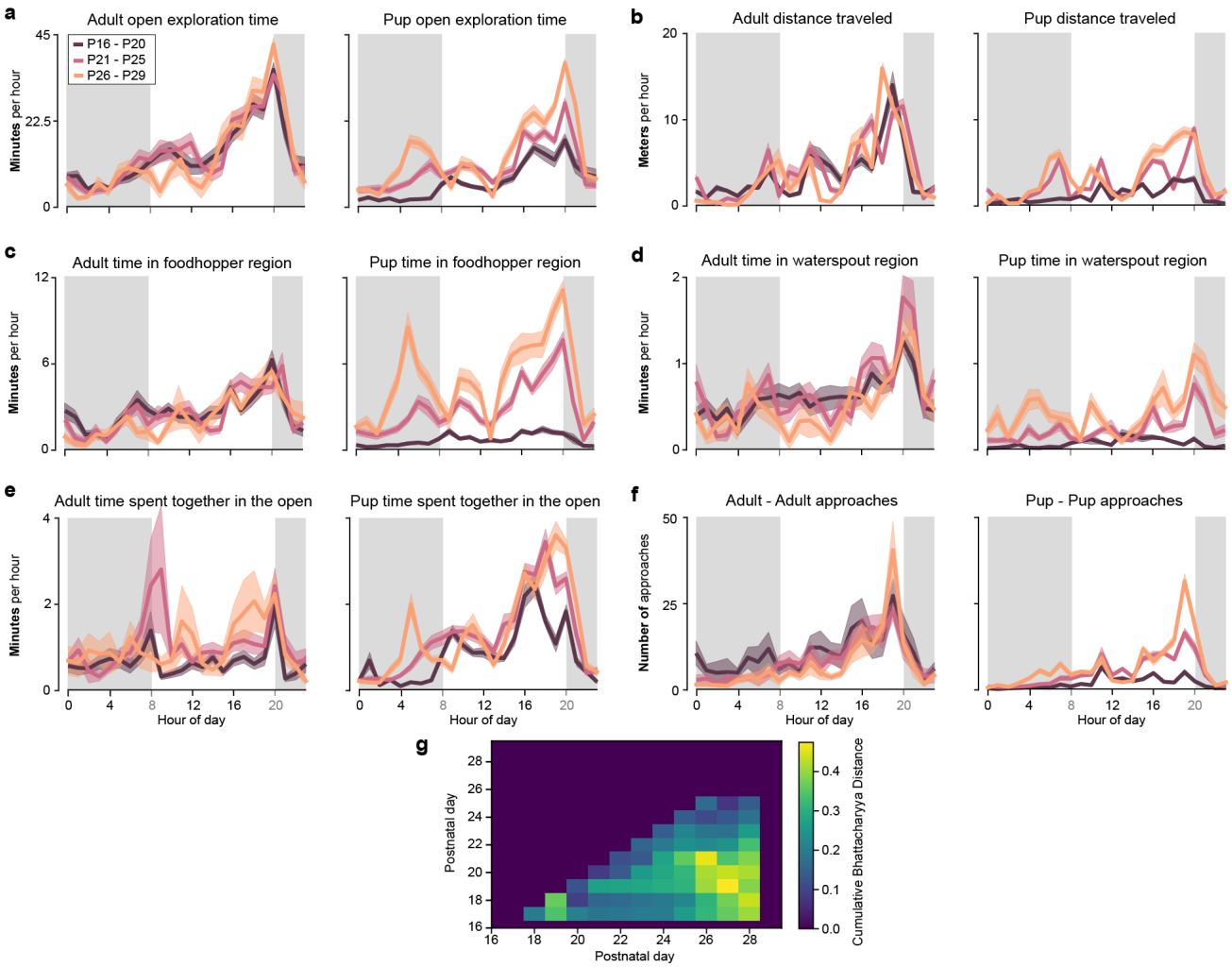

Fig 4. Circadian rhythms in adult and developing gerbils. a Average adult (left) and pup (right) open exploration time per hour (lines) and SEM (shading), across three developmental periods: P16 to P20 (dark purple), P21 to P25 (pink) and P26 to P29 (light orange). **b** Average adult (left) and pup (right) distance traveled per hour and SEM, across the three developmental periods. **c** Average adult (left) and pup (right) time spent in the food hopper region per hour and SEM, across the three developmental periods. **d** Average adult (left) and pup (right) time spent in the waterspout region per hour and SEM, across the three developmental periods. **e** Average time in proximity for adults with adults (left) and pups with pups (right) per hour and SEM, across the three developmental periods. **f** Average adult to adult (left) and pup to pup (right) approaches per hour and SEM, across the three developmental periods. **g** Cumulative Bhattacharyya distance (colors) for computing the largest circadian separation based on pup time in the food hopper region (see also Results).

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