Exploring exploration: A multidimensional analysis of exploration across standardised and semi-natural contexts in rats

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Abstract

Exploration is a major personality trait in animals that reflects individual differences in gathering information about environmental features relevant to survival and reproduction. Yet, exploration is commonly assessed using brief standardised tests, which provide a convenient but artificial experimental setting. Whether such measures reflect how individuals explore complex and changing environments under more natural conditions remains unclear. Here, we aimed to provide a multidimensional assessment of exploration across a standardised and a semi-natural context in female laboratory rats (*Rattus norvegicus*). We combined three repeated Open Field tests with six weeks of continuous RFID tracking in a semi-natural environment. We additionally introduced foraging and simulated risk events to examine exploration under more dynamic environmental conditions. On the group level, distance travelled in the Open Field declined after the first exposure. Movement and space use in the semi-natural environment likewise varied across event-free nights but without following a uniform temporal pattern. Foraging and risk events both increased movement but affected visits to the manipulated cages differently. Rats entered food-reward cages more frequently and remained there longer, whereas risk events were characterised by more frequent but shorter visits to the manipulated cages. Individual differences in exploration were repeatable within both contexts, although their strength depended on the behavioural measure. Despite this within-context consistency, Open Field distance was not associated with initial exploration or longer-term movement and space use in the semi-natural environment while associations with foraging and risk events were weak and restricted to specific events. Overall, our findings show that exploration comprises partly distinct components captured by different behavioural measures and influenced by environmental context. Standardised tests and semi-natural observations should therefore be considered complementary rather than interchangeable, emphasising the need for multidimensional assessments when characterising exploration as a personality trait.

Keywords: animal personality, continuous-time Markov chain, exploration, foraging, open field, rats, repeatability, risk-taking, roaming entropy, semi-natural environment

Introduction

Individuals within the same population often differ markedly in their behaviour. Importantly, this variation does not merely represent random noise but may reflect consistent between-individual differences that persist over time and/or across contexts. Such consistent behavioural differences are commonly conceptualised as animal personality and have become a central focus of animal behaviour research and behavioural ecology (Sih et al., 2004; Dingemanse & Réale, 2005; Réale et al., 2007; Stamps & Groothuis, 2010; Wolf & Weissing, 2012; Kaiser & Müller, 2021). Five core behavioural axes have been identified as major personality traits, with exploration being commonly studied alongside boldness, activity, sociability, and aggressiveness (Réale et al., 2007; Kaiser & Müller, 2021). Exploration is widely conceptualised as an individual's behavioural responses to novel situations, such as unfamiliar habitats, foods, or objects and involves the acquisition of information about environmental features relevant to survival and reproduction (Dall et al., 2005; Réale et al., 2007; Moretti et al., 2015; Renner, 1990; Rojas-Ferrer et al., 2020). Consistent individual differences in exploration have been reported across a broad range of taxa, including birds, fish, mammals, amphibians, reptiles, and insects (e.g., Hall et al., 2015; Sroka et al., 2024; De Meester et al., 2022; Dochtermann & Nelson, 2014; Baker et al., 2018; Kelleher et al., 2017).

Exploration can be assessed using a range of behavioural assays that expose animals to novel environments or objects and quantify responses such as movement, spatial use, approach or interaction (Hughes, 1997). Among these approaches, the Open Field test is one of the most widely used standardised assays to quantify exploration in a wide range of vertebrate and invertebrate taxa, ranging from mammals, especially rodents (e.g., Kalueff et al., 2006; Montiglio et al., 2010; Rudolfová et al., 2022) to birds, amphibians, fish and insects (e.g., Dingemanse et al., 2002; Carlson & Langkilde, 2013; d'Ettorre et al., 2017; Villegas-Ríos et al., 2018). In this test, an individual is placed in an unfamiliar, enclosed arena for a defined period, during which its locomotion and spatial use are recorded using measures such as the distance travelled or time spent in predefined areas of the arena (Seibenhener & Wooten, 2015). However, the test also involves illumination of the test arena and therefore creates an approach–avoidance conflict between the motivation to explore a novel environment and the tendency to avoid open and brightly illuminated spaces (Walsh & Cummins, 1976). Therefore, individual differences in Open Field behaviour cannot be attributed solely to exploration. Instead, they may also reflect aspects of general locomotor activity, anxiety-like behaviour, or a combination of these behavioural components (Walsh & Cummins, 1976; Carter et al., 2013; Stanford, 2007). Furthermore, the

Open Field test assesses exploration in an artificial setting and therefore omits important features of natural environments.

Natural environments expose animals to changing opportunities and threats that influence exploration behaviour in diverse ways. During foraging, animals must balance the benefits of gathering information about potentially profitable resources against the costs of leaving known options and investing time and energy in uncertain alternatives, thereby facing an exploration-exploitation trade-off (Dall et al., 2005; Mehlhorn et al., 2015). At the same time, predation risk can strongly influence when, where, and how extensively animals move and forage, as the potential benefits of resource acquisition must be weighed against the risk of encountering predators. Consequently, exploration under naturalistic conditions is likely to depend not only on novelty itself but also on the distribution of resources and perceived risk (Lima & Dill, 1990; Brown & Kotler, 2004). Against the background of animal personality, individuals may differ consistently in how they resolve these trade-offs, as shown by repeatable individual variation in foraging under perceived predation risk (Dammhahn & Almeling, 2012; Eccard et al., 2020; Bračić et al., 2025).

Thus, examining exploration under more complex, naturalistic conditions can reveal whether consistent individual differences extend beyond standardised tests. Semi-natural environments (SNEs) provide a comparatively uncommon but complementary approach. They can offer larger and more structurally complex spaces, maintain animals in social groups, and allow individuals to choose where and when to move over extended periods (Lewejohann et al., 2009; Krebs et al., 2019). In combination with automated tracking technologies such as radio-frequency identification (RFID), SNEs enable the continuous recording of individual movement and space use in group-housed animals without repeated handling during data collection (Howerton et al., 2012; Freund et al., 2013). Krebs and colleagues (2019) compared activity- and exploration-related measures from standardised behavioural tests with movement and space use during the initial colonisation of a semi-natural enclosure in wild house mice. The largely negative relationships between both contexts suggested that exploration behaviour in standardised tests may not directly translate to exploration under more naturalistic conditions, underscoring the value of a multidimensional investigation of exploration.

Against this background, we here aimed to provide a multidimensional assessment of exploration as a personality trait across standardised and semi-natural contexts in laboratory rats. First, we characterised exploration behaviour in the Open Field and the SNE using different behavioural measures assessed across different temporal scales. In the SNE, we also examined exploration under stable as well as dynamic conditions by simulating foraging and risk scenarios. For a thorough analysis of exploration under more natural conditions, we used established measures while also introducing continuous-time Markov models to capture more complex patterns of

exploration. We hypothesised that foraging and risk events reorganise exploration within the SNE, with different movement patterns depending on the presence of a perceived risk. Second, because temporal consistency is required to interpret between-individual variation as animal personality, we assessed whether individuals differed consistently in their behaviour across repeated Open Field tests and over time in the SNE. Based on previous evidence of repeatable individual differences in exploration, we expected exploration to be repeatable within both contexts. Third, we tested whether individual differences observed in the Open Field were associated with exploration in the SNE. Based on the limited and partly negative relationships between behaviour in standardised tests and a semi-natural environment as reported by Krebs and colleagues (2019), we hypothesised that exploration assessed in the Open Field and the SNE reflects different aspects of exploration, shown by weak or absent relationships across the two settings. Such a pattern would support a context-dependent interpretation of exploration.

Material and methods

Animals and housing conditions

Nineteen female Long Evans rats (Rj:LE) were obtained from a commercial breeder (Janvier Labs, Le Genest-Saint-Isle, France) at four weeks of age. During the following 6 weeks, animals were housed in groups of 6-7 individuals to facilitate habituation to handling. In the first two weeks, each group was kept in only one cage (model "Furat", ferplast S.p.A., Castelgamberto, Italy; 78 × 48 × 70 cm) since the animals were still learning to climb. Then, a second cage was connected via a transparent plastic tube consisting of four bent and one straight tunnel module ("Tunnel FPI 4844", Ø 10.5 × 25 cm and "Tunnel FPI 4840", Ø 10.5 × 29 cm; ferplast S.p.A., Castelgamberto, Italy), situated beneath the first cage. Each cage contained wood shavings as bedding material (Espe Classic 2-5mm LtE E-001, ssniff Spezialdiäten GmbH, Soest, Germany) and two additional levels to add structure for climbing and jumping. To provide shelter, a transparent red house (ZOONLAB GmbH Animal Husbandry Experts, Germany; 20.5 cm × 15.7 cm and 11.5 cm high) and tunnel (ZOONLAB GmbH Animal Husbandry Experts, Germany; 15 cm × 9 cm and 9.5 cm high), a cardboard tunnel (ZOONLAB GmbH Animal Husbandry Experts, Germany; length: 12.5 cm, Ø 9 cm) and two plastic hammocks (Sputnik, SAVIC, Belgium; 29 cm × 26 cm and 19 cm high) attached to the cage top were used. Cardboard shreds (Sizzlenest, ssniff Spezialdiäten GmbH, Soest, Germany) were added as nesting material and two wooden balls were provided as a gnawing opportunity. Housing rooms were maintained at a temperature of 21 °C ± 1 °C and a relative humidity of 55 –10/+20%. A reversed 12:12 h light-dark cycle was used, with lights gradually switching off from 9:30 a.m. to 10:00 a.m. and switching on from 9:30 p.m. to 10 p.m. We refer to the dark phase hereafter as "night" and the light phase as "day". Food (R/M-H extr V1534, ssniff Spezialdiäten GmbH, Soest, Germany) and water were available *ad libitum*.

Experimental Design

To investigate exploration across different methodological contexts, we combined repeated Open Field testing with continuous RFID-based tracking in a semi-natural environment (SNE). The Open Field test was used to assess exploration in a standardised unfamiliar environment. RFID tracking in the SNE allowed us to quantify spontaneous space use and movement patterns in a familiar, structurally complex social setting under relatively stable environmental conditions. Finally, foraging and risk events were introduced into selected compartments of the SNE to examine the context-dependent expression of exploration under dynamic environmental conditions. We further assessed whether exploration measured in the standardised Open Field

test was associated with RFID-derived measures of exploration and behavioural responses within the SNE.

Rats arrived at the age of 4 weeks and were housed in three separate subgroups during an initial handling period lasting approximately 6 weeks. At postnatal day (PND) 69, rats were implanted with RFID transponders. One individual lost its transponder and was re-injected on PND 70. On PND 71, the three subgroups were merged and released into the full SNE enclosure. From this point onward, movements within the enclosure were continuously monitored using the RFID system. Exploration in an unfamiliar standardised setting was assessed using the Open Field test. The test was repeated three times on PND 77, 84, and 91 to investigate the consistency of individual differences in exploration. In parallel, continuous RFID tracking was used to assess exploration in the SNE under relatively stable conditions. Subsequently, environmental changes were introduced into the SNE at irregular intervals to assess exploration under more dynamic conditions. Three foraging events took place on PND 94, 97, and 98, followed by four risk events on PND 100, 104, 107, and 112 (see "Exploration in the semi-natural environment under dynamic conditions"). During these events, food rewards were added to certain parts of the SNE either without or in combination with risk-related cues. Rats' responses to the respective event cages were then also assessed in relation to their explorative behaviour in the Open Field test (Fig. 1).

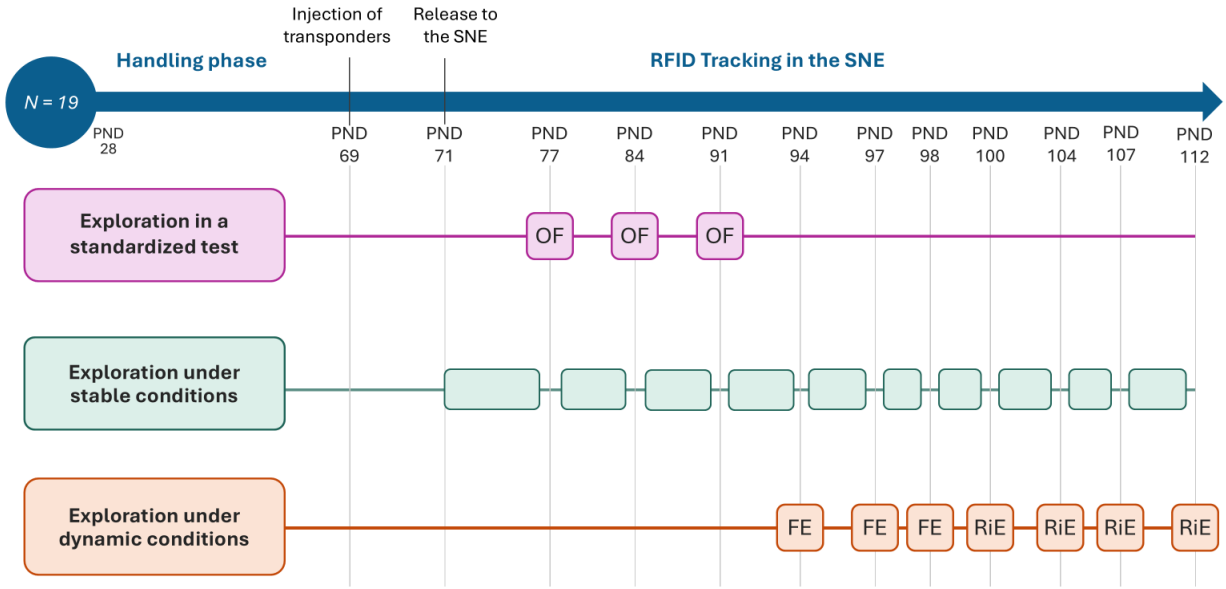


Figure 1: Experimental Design. After an initial handling phase, rats received transponders and were released into the semi-natural environment (SNE). From this point onward, movements within the SNE were continuously tracked using RFID technology. However, only event-free nights were used to assess exploration under stable conditions. Simultaneously, three Open Field tests (OF) were conducted to assess consistent individual differences in exploration in a standardised test setting. Subsequently, three foraging events (FE) and four risk events (RiE) were applied in selected compartments of the SNE to simulate a dynamic, changing environment. The timeline is shown in postnatal days (PND).

The semi-natural environment

Enclosure

To investigate exploration in a more natural setting, the rats were introduced to a semi-natural environment (SNE). Such enclosures aim to approximate natural social and physical characteristics of the animals' environment, for example by providing large multi-level spaces with several compartments, multiple food and water sources, nesting materials and shelters in large groups (reviewed by Hernández-Arteaga & Ågmo, 2023). Our SNE was a modular system which consisted of eight highly enriched cages as described before (see "Animals and housing conditions"; Fig. 2). Thus, each cage contained food and water *ad libitum*. Vertical cage connections consisted of four bent and one straight tunnel module. Horizontal cage connections were designed to avoid rats nesting in the tunnels and confounding RFID readouts (see "RFID Tracking"). Therefore, horizontal cage connections were angled and consisted of two bent and one straight tunnel module in between. The junctions between tunnel and cage could be closed by sliding doors made from transparent plastic (11 cm × 11 cm) to facilitate handling of the rats as well as cage changes for cleaning or experimental procedures.

RFID Tracking

The SNE was equipped with an RFID-based tracking system (Euro I.D. Identifikationssysteme GmbH & Co. KG, Frechen, Germany) to continuously monitor individual space use and movements. Each rat was implanted with a sterile subcutaneous RFID transponder (IID 100C trovan animal transponder, 2.2 mm × 12 mm) carrying a unique identification code. During the 6 weeks prior to transponder implantation, animals were habituated to handling and the restraint required for the procedure through positive reinforcement with sweetened condensed milk. Transponders were then implanted in the neck region on PND 69 using an injector pistol for 30-mm cannulae (Euro I.D. Identifikationssysteme GmbH & Co. KG, Frechen, Germany) under veterinary supervision. Following implantation, animals were monitored and allowed to recover for two days before being released into the full SNE. One rat had lost its transponder when scanned one day after implantation and was re-injected with another transponder. However, neither bleeding nor infections at the injection site, nor changes in the animals' behaviour after transponder implantation were observed.

In total, 20 RFID antennas were installed at the tunnels connecting the cages of the SNE. Each tunnel was equipped with two ring antennas (air-coils, Ø120-mm). To avoid interference with transponder detection, a minimum distance of 10 cm from metal components and 20 cm between antennas was maintained. Accordingly, one antenna was mounted at each end of the

central tunnel module, ensuring sufficient distance from metal shelves, cage materials, and adjacent antennas. The antennas were connected to RFID reader units operating at 125 kHz. The reader units were connected to a computer via an RS-485 interface and a USB–RS-485 converter. Detection events, including transponder ID, antenna number, and timestamp, were recorded using ID2File multicom software and stored as .csv files.

This setup allowed movements between cages to be detected based on the order of antenna readings. RFID tracking was conducted continuously throughout the experimental period, except during behavioural testing, cleaning, or technical maintenance. Raw data were processed using custom-written scripts (see “Statistical analysis”). The processed data were used to calculate individual movement and space-use measures (see “SNE-derived measures of exploration”).

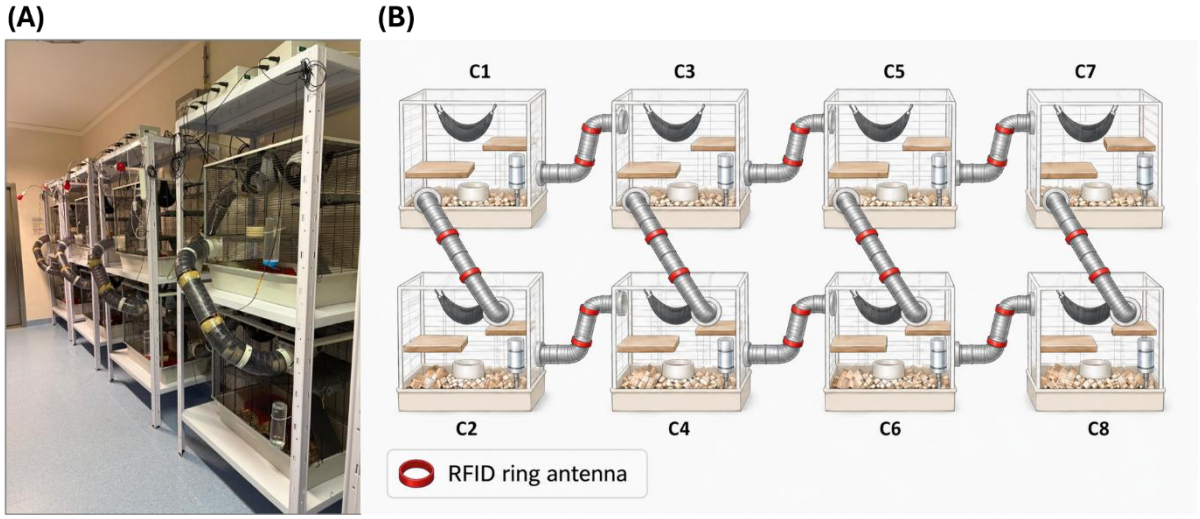


Figure 2: Semi-natural environment and RFID system. (A) Photograph of the semi-natural environment, comprising eight cages connected by tunnels across two levels. Rats’ movements were continuously tracked using RFID antennas positioned around the tunnels. The RFID system units were placed on top of the shelves. (B) Schematic overview of the semi-natural environment showing the spatial arrangement of cages (C1-C8), tunnel connections and the location of the RFID ring antennas (depicted in red). Panel B was generated using ChatGPT-6 Sol (OpenAI), based on an original illustration by the first author and reference photographs.

Experimental contexts for assessing exploration

Exploration in a standardised test

The Open Field test was used to characterise the rats’ level of exploration. It was conducted three times at PNDs 77, 84 and 91. The Open Field apparatus consisted of a square arena made of grey plastic (104 cm × 104 cm) with walls 40 cm high and transparent flexible barriers mounted on top to prevent climbing. The area within 26 cm of the walls was defined as the peripheral zone, whereas the central 52 cm × 52 cm area was designated as the centre zone. Illumination from above was adjusted to approximately 35 lux. Testing was conducted during the dark phase from 12:30 p.m. to 5:30 p.m., when the rats were active, in a room separate from the housing room. Before each trial, the apparatus was cleaned with 70% ethanol and paper towels. To facilitate

retrieval of the rats from the cages, all rats were guided into the upper row of cages in the SNE and tunnel junctions were closed using sliding doors. At least three rats were kept in each of the four cages to distribute the animals across the cages throughout testing of all rats. The order of animals tested was semi-randomized as the experimenter only identified each rat after capture by scanning its transponder. After each rat had been tested, another cage was chosen to retrieve the next rat for the following trial. However, when only one rat remained in a cage, this rat was tested next to avoid prolonged separation from physical contact with conspecifics. Nevertheless, rats remained in olfactory and auditory proximity to their group during the waiting period.

Rats were transported in a semi-transparent red plastic box (floor space: 22 cm × 22 cm; height: 15 cm), which was cleaned between individuals using water and paper towels. Prior to the start of the test, each rat remained in the transport box for a 1-min acclimatization period to ensure similar arousal levels across individuals (Izídio et al., 2005). The rat was then placed in the front left corner of the arena facing the wall, and behaviour was recorded for 5 min using a camera (Logitech HD Pro C920 Full HD-Webcam, 1920 × 1080 pixels, Logitech, Apples, Switzerland) and analysed with tracking software (ANY-maze Video Tracking Software, version 6.32, Stoelting, Wood Dale, United States). To minimize potential experimenter effects, the experimenter left the room during testing.

After completion of the test, the animal was returned to the SNE using the transport box. Tested animals were placed into the lower row of cages to separate them from animals that had not yet been tested. The junctions between cages in the lower row remained open, allowing rats to distribute freely across the four cages after testing. As soon as the last rat had been tested, all junctions were opened and RFID tracking was resumed.

Exploration in the semi-natural environment under stable conditions

On PND 71, the rats were released into the full SNE and the subgroups were merged during the dark phase at 5:30 p.m., when the rats were active. Prior to release, all cages were replaced with clean cages to remove pre-existing odour marks that could have influenced the distribution of the subgroups across the enclosure or the establishment of dominance hierarchies. Each subgroup was initially placed into one cage in the lower cage row. The junctions to the tunnel system were then opened, allowing the rats to access and explore the entire SNE. The movements of the rats during the first 4 hours after release until the beginning of the light phase (i.e., the rats' inactive phase) were analysed to assess exploration speed in the new environment. Apart from that, movements during all days and nights without experimental changes (i.e., Open Field testing, cage cleaning, foraging and risk events) were assessed as exploration under stable conditions.

Exploration in the semi-natural environment under dynamic conditions

To assess exploration under changing environmental conditions, different events were simulated within specific parts of the SNE, i.e. three foraging events and four risk events. The three foraging events were conducted before the risk events. This order allowed to assess how different personality types responded to foraging opportunities in the absence of risk while also habituating the rats to the procedure and to the possibility of foraging for rewards. This was intended to ensure that subsequent responses to cages containing risk cues reflected the risk manipulation rather than a lack of experience with reward availability.

Foraging events

The foraging events were conducted on PND 94, 97, and 98. Events were scheduled at irregular intervals to increase unpredictability for the rats. Accordingly, the first foraging event was conducted at 1:30 p.m., the second at 6:15 p.m., and the third at 9:00 a.m. On each event day, two cages were selected at pseudo-random positions within the SNE. Rats were guided out of the selected cages, and the corresponding tunnel junctions were temporarily closed to prepare the foraging event. For the first two events, 20 g of oat flakes were used as food rewards. During the third event, 10 g of yogurt drops were added to the oat flakes to maintain novelty and motivation to forage. Food rewards were distributed across the cage and mixed with the bedding. The tunnel junctions to the respective cages were then reopened, and the experimenter left the housing room. After 1.5 h, the SNE was checked by the experimenter. As no rats were observed foraging at that point, this time window was used during later analysis to assess exploration behaviour during foraging events.

Risk events

Following the foraging events, risk events were conducted to assess food-motivated exploration under perceived risk. In total, four risk events were presented between PNDs 100 and 112. To reduce potential effects of pre-existing cage preferences or odour marks, each simulated event was prepared in a clean cage, which then replaced one of the existing SNE cages at a pseudo-randomly selected position. In preparation of each event, 40 g of oat flakes and 20 g of either silvered almonds or yogurt drops were mixed with double the amount of bedding (approximately 5 cm deep) to increase the time the rats needed to find all rewards. The same amount was added again after half of the scheduled time for the risk event passed. This was intended to avoid presenting perceived risks without rewards. The tunnel junctions connected to the respective cage were temporarily closed during cage exchange and reopened once the event cage had been attached to the tunnel system. The timing of cage exchange, access to the event cage, and removal of the event cage was recorded for later analysis.

The first risk event was conducted on PND 100 from 7:00 to 10:00 a.m. It was expected to represent the mildest risk manipulation. It consisted of a clean, empty cage containing food rewards but no enrichment or shelter structures and was presented during the light phase. Thus, rats had to enter an exposed and unfamiliar cage environment without access to cover while foraging. This manipulation was based on the tendency of rodents to avoid exposed and brightly lit areas, which are commonly used as anxiety- or risk-related contexts in behavioural assays (La-Vu et al., 2020), and on evidence that wild Norway rats prefer areas with cover (Lambert et al., 2008). Thus, the first condition was used as a mild risk-related context before introducing potentially more salient risk cues in the subsequent events. Following risk events therefore did not include any enrichment, shelters or additional levels in the respective cages as well.

The second risk event took place on PND 104 from 1:45 p.m. to 6:45 p.m. during the dark phase. For this event, an electric rotating disco ball with LED lights (height: 12 cm; IAN 304011, LED party light, OWIM GmbH & Co. KG, Neckarsulm, Germany) was mounted to the underside of the intermediate shelf board directly above one cage of the SNE, projecting moving light patterns into the cage below. The putative risk-related character of the event was further increased by adding novel reflective objects (three metal tablespoons, two metal food bowls, and three metal bottle caps). This event introduced artificial illumination during the rats' dark phase, simulating artificial light at night (ALAN) (Gaston et al., 2013; Longcore & Rich, 2004). In rodents, artificial illumination has been shown to reduce the use of foraging sites which is consistent with light acting as an indirect cue of predation risk (Farnworth et al., 2016; 2019). Moreover, the moving light patterns and novel reflective objects increased the novelty, complexity, and unpredictability of the cage environment because novel stimuli can evoke both exploratory approach and neophobic or anxiety-related avoidance in rats, particularly when the environment is difficult to predict or control (Chrzanowska et al., 2022; Kiyokawa et al., 2024). To prevent light from spilling into adjacent cages, the event cage was shielded with cardboard walls attached to the shelves, leaving openings only for the adjacent tunnels.

The third risk event used a life-sized eagle owl decoy as a putative predator-related risk cue. The event was conducted on PND 107 from 8:00 a.m. to 12:00 p.m. and therefore started during the light phase and extended into the beginning of the dark phase. To ensure visual exposure to the decoy, the eagle owl was attached to a tripod and positioned so that its feet were approximately level with the floor of the event cage. Thus, the decoy towered above the rats while they were foraging on the cage floor. This manipulation was based on evidence that rodents can detect and respond to visual predator cues and that overhead visual stimuli can elicit shelter-seeking behaviour in rats (Bedoya-Pérez et al., 2019; Wallace et al., 2013). Therefore, the elevated eagle

owl decoy was used as a putative predator-related risk cue while still allowing voluntary approach and foraging.

The fourth risk event was again based on ALAN but at a higher light intensity than previous events to tap on the tendency of rats to avoid brightly illuminated, exposed spaces (La-Vu et al., 2020; Farnworth et al., 2019). It was conducted on PND 112 from 5:00 p.m. to 9:00 p.m. A rectangular LED frame (56 cm × 56 cm) was attached to the underside of the intermediate shelf board directly above one cage of the SNE. The LED frame was set to a soft flashing mode with an illumination level of approximately 40 lux inside the cage.

Behavioural measures of exploration

Open Field-derived measure

The movements of the animal in the Open Field arena were tracked automatically. Total distance travelled was used as an activity-based measure of exploration, reflecting the extent to which rats moved through the Open Field arena (cf. Quante et al., 2026).

SNE-derived measures

We derived three complementary measures from the RFID data to characterise exploration: roaming entropy, cage-switching frequency, and transition measures derived from a continuous-time Markov chain (CTMC).

RFID data processing

To analyse the exploration and space use of the rats in the SNE, the data output from the RFID system first had to be processed. The raw data contained the transponder ID, the antenna ID and a timestamp. These data were processed to reconstruct individual cage use and transitions between cages. First, consecutive multiple detections of the same individual at the same antenna were collapsed by retaining the last detection of each series. Cage entries were then inferred from predefined antenna sequences corresponding to movements through the tunnels into specific cages (e.g., if antenna 2 followed antenna 1, the rat entered cage 1). If intermediate antenna detections were missing but could be inferred from the preceding and following detections, synthetic detections were inserted and flagged as artificial. For these reconstructed transitions, tunnel traversal time was set to the calculated median tunnel time, and an artificial timestamp was generated accordingly. Across the whole experiment, 10,232 of 372,638 antenna reads were reconstructed synthetically, corresponding to an overall error rate of 2.75 %. Errors were strongly concentrated on antenna 20 which was temporarily malfunctioning: 6,499 synthetic reads originated from this antenna alone, accounting for 63.5 % of all synthetic reads.

Excluding antenna 20, the synthetic-read rate across the remaining antennas was 1.06 %. Cage dwell time was calculated as the time from entry into a cage until the next entry into another cage.

Roaming entropy

Roaming entropy (RE) is a commonly used measure to describe exploration behaviour as space use (e.g. Freund et al., 2013; Mieske et al., 2021; Zocher et al., 2020), used here by combining information on how many different cages a rat visits and how evenly it spreads its time between them. Thus, roaming entropy reflects the extent to which an individual's cage use is spread across the available compartments, with higher values indicating a more even use of space and lower values indicating a stronger concentration on a subset of cages. It was calculated as normalized Shannon entropy (Shannon, 1948):

$$RE_{k,\tau} = -\sum_{j=1}^{n} p_{j,k,\tau} \log \left(p_{j,k,\tau}\right)/\log(n)$$

where n is the number of available cages and $p_{j,k,\tau}$ is the proportion of time individual k spent in cage j during the respective observation period τ. Normalization by (\log(n)) scales roaming entropy between 0 and 1, with values close to 0 indicating that an individual spent most of its time in one or only a few cages, and values close to 1 indicating an even distribution of time across all available cages.

Cage-switching frequency

As a complementary measure of exploration, we calculated the frequency of cage changes. This measure captures how often an individual moved between compartments during a certain observation period. While it does not reflect the evenness of space use like the RE, it describes the amount of locomotion within the SNE.

CTMC-based transition measures

A continuous-time Markov chain was used to model the rats' movements between cages based on transition intensities. These intensities describe the instantaneous probability of moving from one cage to another and thus capture both how long a rat is expected to remain in a given cage after entering it and which cage it is most likely to visit next. Thus, it is able to reflect space use as well as locomotion to measure exploration. The transition intensities for the eight cages are summarized in an 8 × 8 transition intensity matrix. As only physically possible direct transitions were estimated, this resulted in 20 unique transitions. The transition intensity matrix allowed us to derive the model-implied dwell time for each cage, that is, the expected time a rat remained in

a cage after entering it. In addition, the stationary distribution of the Markov chain informs about the model-implied proportion of time spent in each cage, which reflects overall cage use. For visualization purposes, transition intensities were transformed into probabilities of direct switching between cages within a minute. All transition intensities were estimated for nighttime and daytime separately, since rats are a nocturnal species with most of their locomotion happening at nighttime (Stephan & Zucker, 1972; Nagano et al., 2003; see Supplementary Material "Methods" for details on CTMC model formulation).

Statistical analysis

For this experiment, we conducted an *a priori* power analysis with a medium effect size (f = 0.6) and a statistical power of 80 % (G*power, version 3.1.9.7) which resulted in a sample size of 19 rats. One animal had to be excluded from the Open Field data because it jumped out of the test arena during each of the three test repetitions, reducing the sample size to *N* = 18 for all Open Field-dependent analyses. Data analysis was conducted in R (R Core Team, 2025, version 4.5.1). All plots were produced with the "ggplot2" package (Wickham, 2016; version 3.5.1).

Exploration in a standardised test

Overall patterns of exploration in the Open Field

To assess group-level differences in exploration over the three Open Field tests, we fitted a linear mixed-effects model with the distance travelled (OFD) as the response variable, "test round" (from 1-3) as a fixed effect and "ID" (for the individual) as a random intercept to account for repeated measures within subjects. Model residuals were checked using the "DHARMa"-package (Hartig, 2024; version 0.4.6). Pairwise comparisons of the three test rounds were conducted using the "emmeans" package (Lenth, 2024; version 1.10.3; adjust = "tukey").

Individual differences in exploration in the Open Field

To assess individual differences in exploration in the Open Field test, we estimated the repeatability (R) of distance travelled in the apparatus across the three test repetitions. Repeatability is defined as the proportion of total phenotypic variance in repeated behavioural measures attributable to between-individual differences within a population (Nakagawa & Schielzeth, 2010). Adjusted repeatabilities (R_{adj}) account for fixed effects and were estimated using the R package "rptR" (Stoffel et al., 2017; version 0.9.22). The uncertainty of the repeatability estimates was calculated by applying parametric bootstrapping (*N* = 1000, confidence level = 95 %) and statistical significance was determined by using likelihood ratio

tests. The model included "OFD" as the response variable, "test round" as fixed effect and "ID" as random effect.

Exploration in the semi-natural environment

Overall patterns of exploration in the SNE

Exploration under stable conditions

To characterise the movement patterns in the SNE under stable conditions, transition probabilities, model-implied dwell times, and overall model-implied cage-use proportions were derived from the CTMC for event-free periods (see Supplementary Material "Methods" for details on derivation of the mentioned measures).

Exploration under dynamic conditions

To assess how exploration changed under dynamic environmental conditions, we compared behavioural measures recorded during event periods with the corresponding time windows on days without events. For roaming entropy, event-related effects were modelled separately for each event type using beta generalized additive mixed models (GAMMs). Because roaming entropy is bounded between 0 and 1, values were assumed to follow a beta distribution, and the models used a logit link following the structure:

$$RE_{ij} \sim Beta(\mu_{ij}, \varphi)$$

$$logit(\mu_{ij}) = \beta_0 + \beta_1 \cdot Event_{ij} + f(Day_{ij}) + u_i + \varepsilon_{ij}$$

where RE_{ij} is the roaming entropy of individual i on day j, μ_{ij} is its expected value, φ is the precision parameter, $Event_{ij}$ indicates whether the event occurred, and $f(Day_{ij})$ accounts for nonlinear temporal trends modeled nonparametrically using penalised splines. The model included a random intercept u_i per individual to handle repeated measurements and a residual term ε_{ij}, with β_1 being our coefficient of interest - the average effect of the event on roaming entropy.

Cage-switching frequency was analysed using GAMMs with a negative-binomial response distribution to account for overdispersion in the counts of cage switches. The models used a log link for the conditional mean and followed the same structure as for roaming entropy (i.e., including event as a fixed effect, individual as a random intercept, and time as a penalised spline). Each event type was again modelled separately. All GAMMs were fitted using the R package "mgcv" (Wood, 2017).

At the level of movement structure, the CTMC model included an overall effect of foraging and an effect of risk events on all transition intensities to test whether the overall rate of movement

between cages changed during each event type relative to event-free periods. Additionally, cage-specific deviations from these overall effects were estimated. For foraging events, one shared pair of effects was fitted for transitions to and from the cages containing food rewards, whereas separate pairs of cage-specific effects were fitted for each risk event to allow responses to differ among manipulations.

Individual differences in exploration in the SNE

Individual exploration speed

The period from the first release of the rats to the semi-natural environment until dawn (i.e., beginning of the light phase) was analysed separately from the remaining event-free period in the SNE. Proportional cage use as well as the exploration speed were descriptively analysed by visualizing the course of the cumulative roaming entropy over the first 4 hours after release.

Repeatability of exploration in the SNE

To assess individual differences in exploration in the SNE, analyses were restricted to event-free nights. To meet the assumptions of the Gaussian model, roaming entropy was logit-transformed. Cage-switching frequency was modelled using a Poisson distribution. Repeatability of roaming entropy and cage-switching frequency was assessed analogously to the Open Field analysis, with the respective SNE-derived measure as the response variable, “day” as a fixed effect, and “ID” as a random effect.

Link between exploration in standardised test and semi-natural environment

To examine whether exploration in the Open Field was associated with exploration in the SNE, distance travelled in the Open Field (OFD) was related to SNE-derived measures under stable and dynamic conditions. Under stable conditions, associations with roaming entropy and cage-switching frequency were assessed using separate GAMMs as described before. Thus, roaming entropy was modelled using a beta distribution with a logit link, whereas cage-switching frequency was modelled using a negative binomial distribution with a log link. Both models included OFD as a predictor, ”day” as a penalised spline to account for nonlinear temporal trends, and “ID” as a random effect. Additionally, the association between the two SNE-derived measures was assessed using a third beta GAMM, with roaming entropy as the response variable, cage-switching frequency as a predictor, ”day” as a penalised spline, and “ID” as a random effect. Moreover, OFD was included in the continuous-time Markov models as a predictor of overall transition intensity. For the initial period following release into the SNE, Pearson correlations were used to relate OFD to roaming-entropy-based exploration speed during the first

30 min and the first 4 h. For these individual-level correlations, OFD represented each rat's mean distance travelled across the three Open Field tests, and one exploration-speed value per rat and time window was calculated as the mean cumulative roaming entropy across the respective period. In addition, a CTMC was fitted to the first 4 h following release into the SNE which included an overall effect of OFD on transition intensities.

Under dynamic conditions, cross-context effects were assessed within the Markov modelling framework by including separate interactions between OFD and the overall foraging and risk event effects, as well as interactions between OFD and the cage-specific foraging- and risk-related transition parameters, to test whether responses to changing environmental conditions differed according to Open Field behaviour.

Ethical note:

All procedures were conducted in compliance with German animal welfare legislation (Animal Welfare Act) and EU regulations governing animal experimentation (Directive 2010/63/EU). Approval was obtained from the relevant local authority (Gesundheits- und Veterinäramt Bielefeld, Nordrhein-Westfalen) and the responsible federal authority (LAVE, Landesamt für Verbraucherschutz und Ernährung, Nordrhein-Westfalen; reference no. 81-02.04.2023.A147). Animal welfare was closely monitored throughout and beyond the experimental period. Besides that, only minimally invasive or non-invasive methods were used. The rats were housed in a spacious, structured and highly enriched enclosure. After the study, the animals were either retained in our housing facility or rehomed.

Results

Exploration in a standardised test

Overall patterns of exploration in the Open Field

First, we fitted a linear mixed model to assess exploration across the three Open Field tests at the group level. We found a significant effect of "test round" on distance travelled in the arena (χ^2 = 56.736 , df = 2, P < 0.001). Subsequent pairwise comparisons revealed significant differences between the first and the second as well as the third test, while the second and third test did not differ significantly (Table S1). The rats travelled significantly more through the arena during the first compared to the following two test rounds, with mean distance travelled decreasing from 27.15 ± 5.18 m in the first test to 19.10 ± 5.11 m in the second and 18.99 ± 3.34 m in the third test (Fig. 3A).

Individual differences in exploration in the Open Field

In the next step, we assessed the consistency of exploration across the three Open Field tests by analysing the repeatability of the distance travelled in the apparatus. We found exploration to be moderately repeatable (R_{adj} = 0.351, CI = [0.038, 0.608], P = 0.007), indicating that the rats behaved consistently in their exploration in an artificial standardised test. Individual trajectories of distance travelled across tests are shown in Fig. 3B.

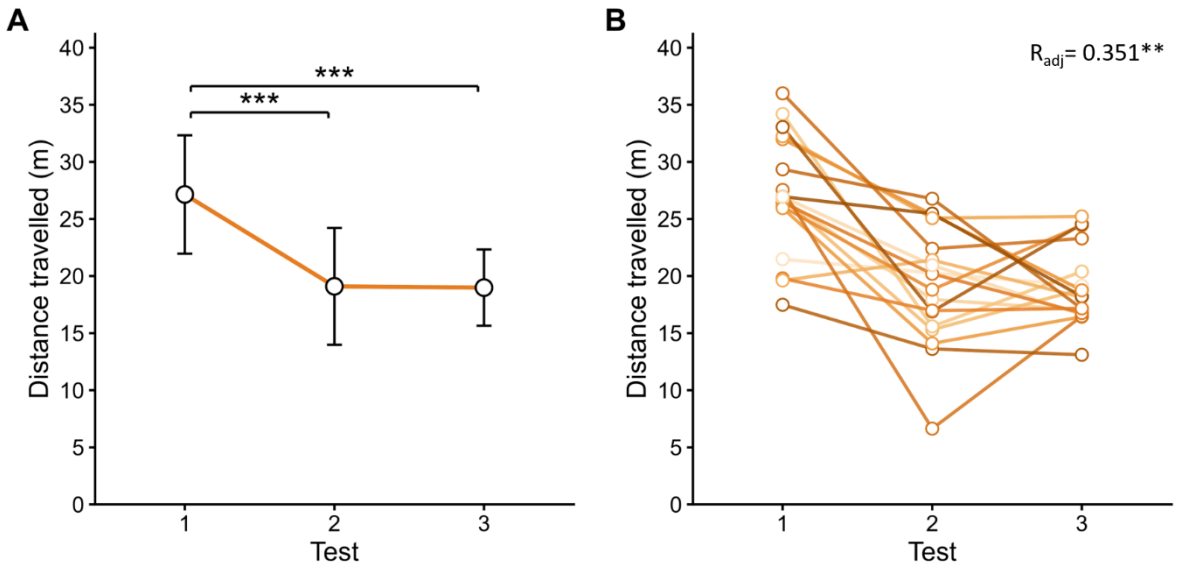


Figure 3: Exploration in the Open Field test. (A) Mean distance travelled (m) ± SD across tests 1–3. Linear mixed effect model with pairwise comparisons, tukey-adjusted. (B) Individual trajectories of all rats across the three repeated tests. Adjusted repeatability with likelihood ratio test. **P ≤ 0.01; ***P ≤ 0.001.

Exploration in a semi-natural environment

Overall patterns of exploration in the SNE

Exploration under stable conditions

Across the 23 event-free nights, both SNE-derived measures varied over time (Fig. 4). Mean roaming entropy across all nights and individuals was 0.92 ± 0.05 and mean cage-switching frequency was 236.6 ± 73.4. Specifically, roaming entropy varied across nights but remained generally high across individuals, indicating that the rats used most of the available space evenly. Cage-switching frequency also varied across nights while showing substantial variation among individuals within nights, suggesting that individuals differed in the amount of movement between cages. Together, neither measure changed uniformly across the observation period. Nevertheless, the GAMs showed a significant effect of "day", detected for roaming entropy (edf = 1.144, $\chi^2 = 7.703$, P = 0.014) and cage-switching frequency (edf = 1.867, $\chi^2 = 8.351$, P = 0.018; Table S2).

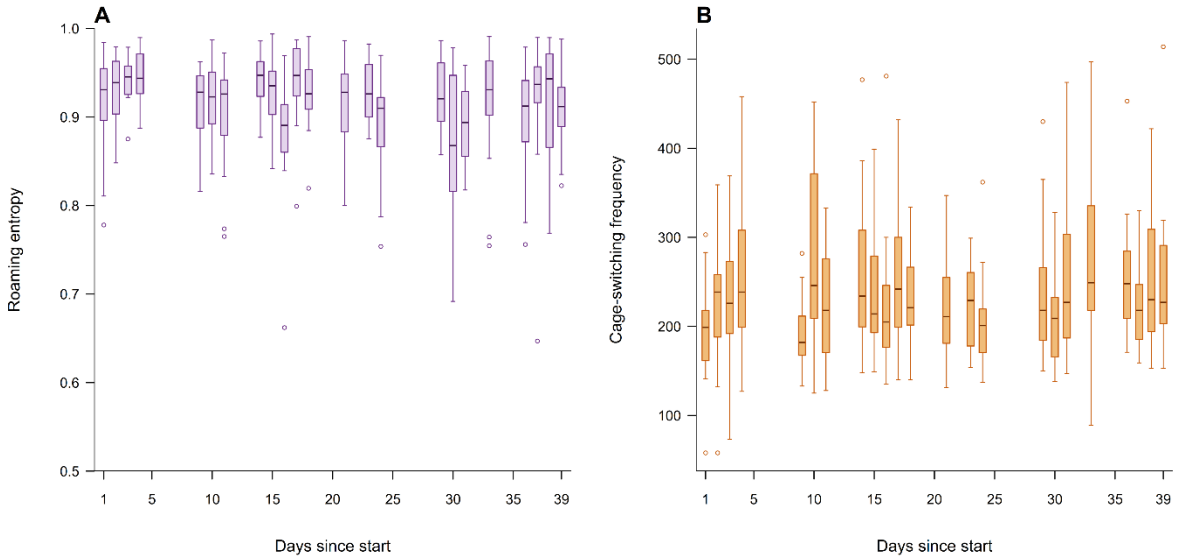


Figure 4: Roaming entropy and cage-switching frequency across event-free nights. Each boxplot represents one of 23 event-free nights and shows the distribution across individuals. The x-axis indicates the number of days since the first included night. Panel A shows roaming entropy, and panel B shows cage-switching frequency. Horizontal lines indicate medians, boxes represent the interquartile range, whiskers extend to 1.5 times the interquartile range, and open circles indicate outliers. Nights containing experimental events were excluded and therefore appear as gaps on the x-axis.

At the group level, movement patterns across all rats during event-free *nights* as implied by the CTMC indicated a slight preference for cages at the outer edges of the SNE (Fig. 5A; Fig. 6C). Overall, model-implied dwell times, cage switching probabilities and model-implied proportions of time spent in a cage seemed to be rather evenly distributed across cages (Fig. 5A; Fig. 6C). During event-free *days*, model-implied dwell times and cage-use proportions were higher in C1, C3, C5, C7, and C8 than in C2, C4, and C6, whereas cage-switching probabilities remained

relatively low across transitions (Fig. 6A). Thus, the rats moved less between cages and stayed longer in some preferred cages during the light phase than during the dark phase.

Exploration under dynamic conditions

Events generally increased movement but their effects on space use depended on event type. The CTMC revealed positive overall effects of both event types on transition intensities (foraging events: $\beta = 0.44$ [0.37, 0.50], corresponding to a 55.0% increase (95% CI [45.1%, 65.7%]); risk events: $\beta = 0.62$ [0.58, 0.65], corresponding to an 85.1% increase (95% CI [79.1%, 91.2%])). Consistent with these overall effects, the GAMMs indicated that cage-switching frequency increased during all foraging events and three risk events, with only the third risk event (i.e., the owl decoy) showing no clear change (Table S3). Roaming entropy changed more selectively, increasing during the second foraging event ($\beta = 0.648$, $P = 0.007$) and the first risk event (empty cage without shelter; $\beta = 0.466$, $P = 0.013$), and decreasing during the second risk event (moving light cues and reflective objects; $\beta = -0.310$, $P = 0.046$) (Table S4; Fig. S1). Thus, events broadly stimulated movement, whereas changes in the evenness of cage use were restricted to specific contexts.

The pattern of this response differed between foraging and risk events. During foraging events, rats remained longer in the food-reward cages than during the corresponding no-event condition (Fig. 5; Table S5). Relative to the no-event condition, transitions into food-reward cages increased (factor = 1.85 [1.70, 2.01]), whereas transitions out of these cages decreased (factor = 0.77 [0.71, 0.83]; Table S6). Thus, foraging events increased time spent in the food-reward cages through both increased entries and reduced leaving.

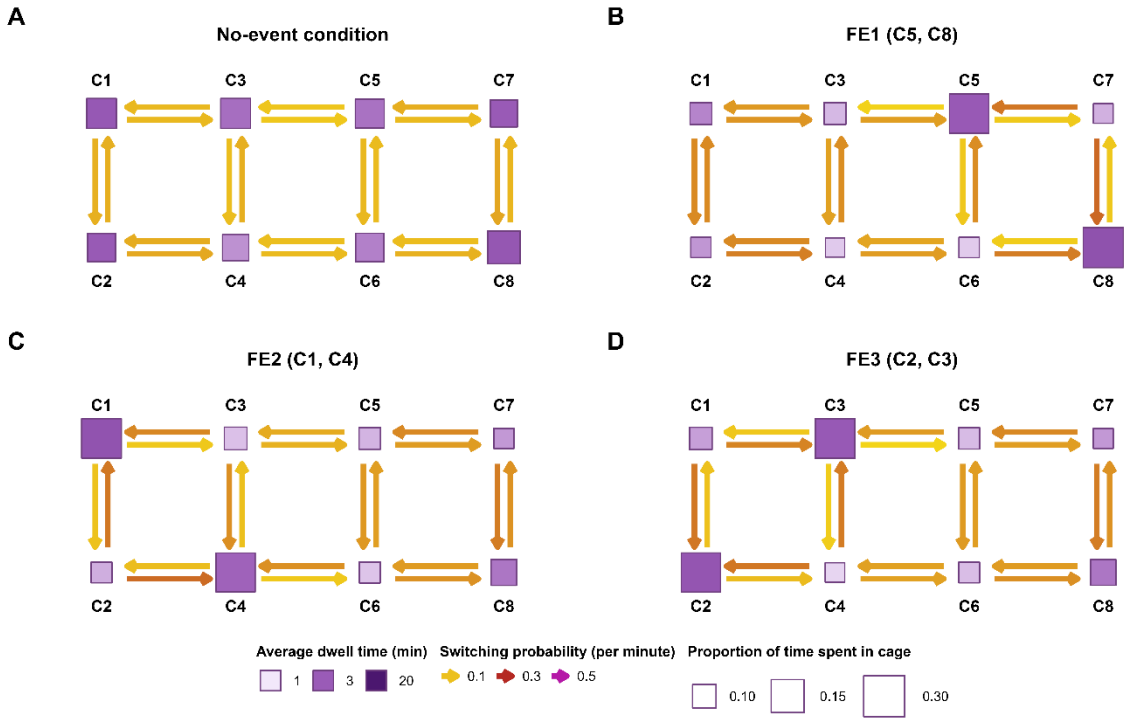


Figure 5: Markov-model-derived movement patterns during foraging events compared with the no-event condition. Shown are all food events (FE) which were conducted in different cages (C1-8). Panels show (A) the no-event night condition, (B) FE1 with food rewards in C5 and C8, (C) FE2 with food rewards in C1 and C4, and (D) FE3 with food rewards in C2 and C3. Square shading indicates model-implied dwell time in minutes, arrow colour indicates switching probability per minute, and square size represents the model-implied proportion of time spent in a cage.

Risk events produced a different movement pattern: all four events elicited increases in transitions into the manipulated risk cage (factors = 2.71-5.36) relative to the no-event condition, while event-specific effects on leaving differed among manipulations (Fig. 6). Leaving was especially increased during the first risk event (i.e., the empty cage without shelter), whereas the increase in leaving was less pronounced during the other three risk events (Fig. 6; Table S6). Despite these transition patterns, model-implied dwell times in the risk cages were shorter than during the corresponding no-event condition, with the strongest reduction during the first risk event (i.e., empty cage without shelter; Fig. 6; Table S7). Thus, risk events were characterised by more frequent entries into the manipulated cages but shorter stays within them.

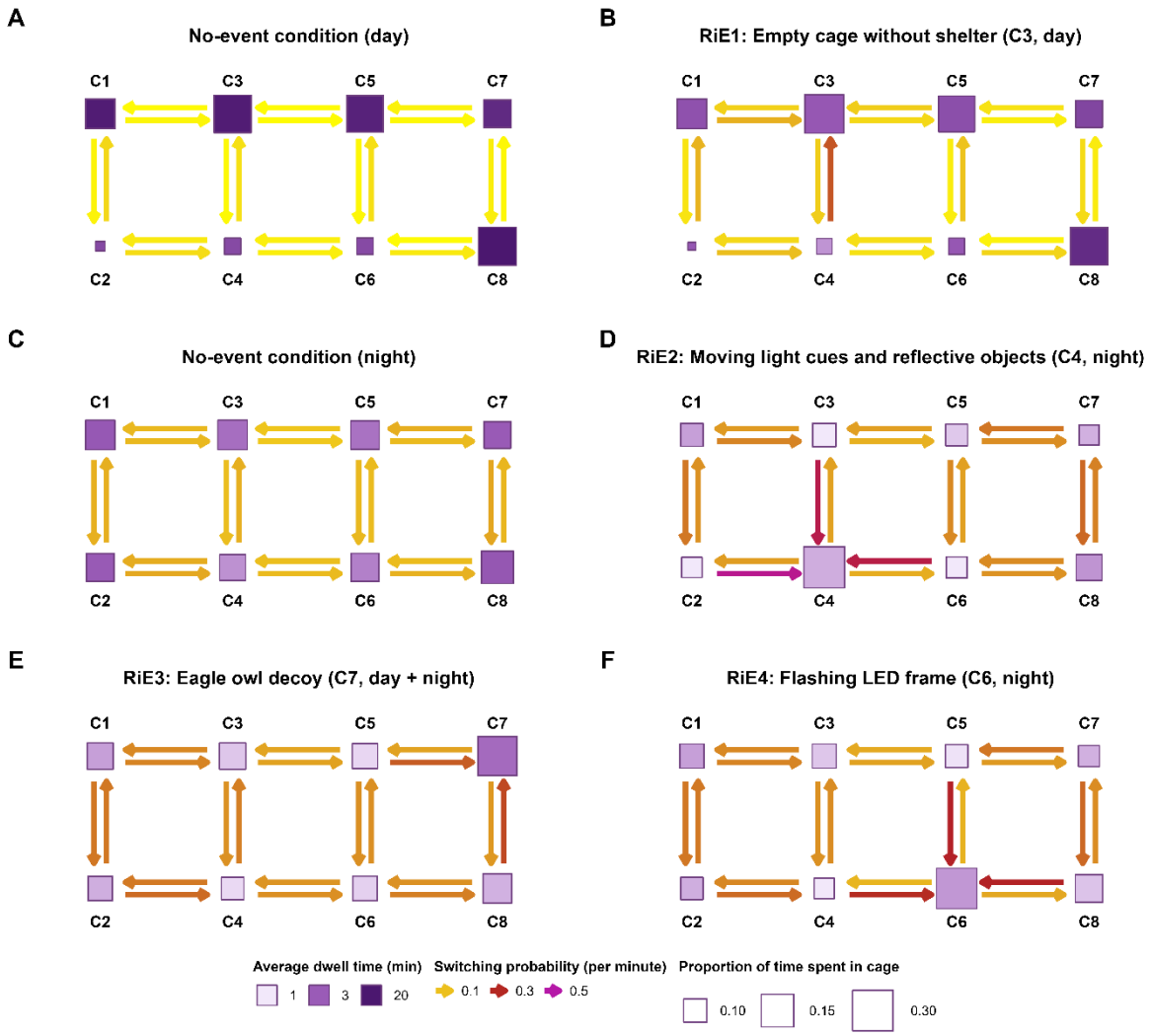


Figure 6: Markov-model-derived movement patterns during day and night risk events compared with the corresponding no-event conditions. Shown are all four risk events which were conducted at different times of day and in different cages (C1-8). Panels show (A) the no-event daytime condition; (B) Risk Event (RiE) 1, an empty cage without shelter (cage 3, daytime); (C) the no-event nighttime condition; (D) RiE2, moving light cues and reflective objects (cage 4, nighttime); (E) RiE3, eagle owl decoy (cage 7, spreading from day to nighttime); and (F) RiE4, a flashing LED frame (cage 6, nighttime). Square shading indicates model-implied dwell time in minutes, arrow colour indicates switching probability per minute, and square size represents the model-implied proportion of time spent in a cage.

Individual differences in exploration in the SNE

Individual exploration speed

Descriptive analyses of the first 4 h after release revealed individual variation in both proportional cage use (Fig. S2) and cumulative roaming-entropy trajectories (Fig. 7). Across individuals, mean cumulative roaming entropy increased from 0.87 ± 0.11 after 30 min to 0.93 ± 0.07 by the end of the 4-h observation period. Within the first 30 min, 11 of 19 rats had visited all eight cages, and all individuals had done so within the first 4 h. Differences in the trajectories were particularly apparent during the first 30 min, with some rats reaching high roaming entropy rapidly, whereas others increased more slowly or remained at lower values. These patterns indicate descriptive differences in the timing and extent of initial exploration.

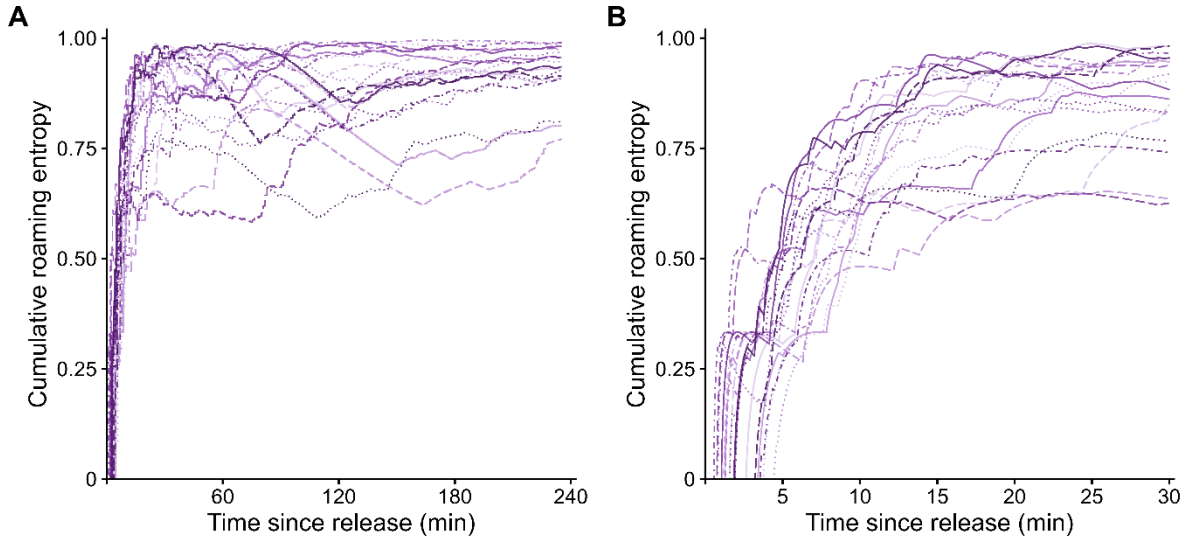


Figure 7: Individual exploration during the first 4 h after release into the SNE. Each line represents one rat and shows the development of cumulative roaming entropy over time following release. (A) Individual trajectories during the first hours until dawn. (B) Individual trajectories during the first 30 min after release.

Repeatability of exploration in the SNE

To assess the consistency of exploration in the SNE, we calculated the repeatability of roaming entropies and the overall cage-switching frequency across nights without events. We found low but significant repeatability estimates for roaming entropy (R_{adj} = 0.054, CI = [0.003, 0.118], P = 0.002), indicating that there were consistent individual differences in the rats' distribution over the cages. Moreover, the cage-switching frequency was moderately repeatable (R_{adj} = 0.267, CI = [0.125, 0.417], P < 0.001). Thus, individual differences appeared to be more pronounced for the frequency of movement between cages than for the evenness of space use, with a comparably higher proportion of variation attributable to between-individual differences (Fig. 4).

Link between exploration in standardised test and semi-natural environment

Under stable conditions, Open Field distance was not associated with either roaming entropy (β = -0.0171, SE = 0.0130, z = -1.315, P = 0.188) or nightly cage-switching frequency (β = -0.0003, SE = 0.0111, z = -0.029, P = 0.977). In contrast, nightly cage-switching frequency was significantly positively associated with roaming entropy (β = 0.0046, SE = 0.0004, z = 10.30, P < 0.001), indicating that the two SNE-derived measures captured related aspects of exploration, whereas exploration in the Open Field was unrelated to either measure (Figs. S3-S4; Table S2).

During initial exploration following release into the SNE, roaming-entropy-based exploration speed was not associated with OFD during either the first 30 min (r = -0.145, 95% CI [-0.573, 0.346], P = 0.567) or the first 4 h after release (r = -0.074, 95% CI [-0.523, 0.407], P = 0.771; N = 18

for both correlations), suggesting exploration in the Open Field to be unrelated to exploration of a novel environment under semi-natural conditions.

The CTMC analyses supported the same general conclusion: OFD showed no clear association with overall transition intensity under stable conditions or during the first 4 h after release (Table S8). Together, these results provided no evidence for a link between exploration in the Open Field and in the SNE under stable conditions. Under dynamic conditions, associations between OFD and CTMC-derived event responses were generally small to modest and varied in direction between foraging and risk events and among event-specific entry and exit processes (Figs. S5-S6; Tables S8-S11). Because these interactions were estimated alongside several related parameters, they should be interpreted cautiously.

Discussion

In the present study, we investigated exploration across a standardised and a semi-natural context in female laboratory rats. At the group level, distance travelled in the Open Field decreased across repeated tests, while exploration in the semi-natural environment varied over time and changed in response to simulated foraging and risk scenarios. During foraging events, rats entered food-reward cages more often and remained there longer, whereas risk events were characterised by more frequent but brief visits to the manipulated cages. Despite these flexible behavioural responses, individual differences were moderately repeatable for the distance travelled in the Open Field and cage-switching frequency in the semi-natural environment, whereas repeatability was lower for roaming entropy. Yet, this within-context consistency did not translate into a relationship across contexts. Open Field exploration was not associated with initial exploration or exploration under stable conditions in the SNE, while associations with exploration under dynamic conditions were small and specific to particular event types and transitions. Together, these findings suggest that the two approaches capture different aspects of exploration. Observations from standardised tests and semi-natural environments should therefore be considered complementary rather than interchangeable, supporting a multidimensional and context-dependent view of exploration.

Group-level changes in exploration across time and environmental conditions

To address our first aim, we examined how exploration at the group level changed over time and in response to altered environmental conditions. The declining distance travelled in the Open Field across tests is consistent with habituation to the initially novel arena, as exploratory locomotion typically decreases with increasing familiarity of the environment (Leussis & Bolivar, 2006; Rudeck et al., 2020; Chen et al., 2023). In the SNE, both roaming entropy and cage-switching frequency varied from night to night throughout the six-week observation period which may reflect shifts in spatial preferences as well as in social interactions. More specifically, rats can develop shared resting locations and segregate multi-compartment housing into functionally distinct areas (Weiss et al., 2018; Amendola et al., 2023). Similarly, our rats used cages relatively evenly at night but preferred particular cages during the day. Because they also moved less and remained in the cages longer during the day, these preferences likely reflected the use of particular cages as resting locations during the inactive phase. Moreover, exploration over longer periods of time might capture different aspects of this behaviour: Once the rats had become familiar with the SNE, exploration was not primarily driven by novelty, a defining feature of exploration (Hughes, 1997; Réale et al., 2007). Instead, the rats may have continued to monitor

familiar surroundings to update existing spatial representations (cf. Poucet et al., 2015; Thompson et al., 2018). Together, measuring movement and space use over longer periods of time may capture exploration-related behaviour extending beyond initial responses to novelty, including monitoring of a familiar environment but also spatial preferences and social interactions.

Beyond this temporal variation, foraging events showed that rats adjusted their movement patterns when food rewards were introduced at specific locations in the SNE. More precisely, the CTMC models revealed an overall increase in transition intensities during foraging events, indicating increased movement through the SNE. Within this overall increase, rats entered food-reward cages more frequently but were less likely to leave them, resulting in longer stays. From an ecological perspective, this movement pattern may result from both ongoing exploration and the local exploitation of a profitable food patch (Dall et al., 2005; Mehlhorn et al., 2015). In rats, the balance between these strategies has been shown to depend on the predictability of resource locations (Jackson et al., 2020). Because food rewards appeared unpredictably within the otherwise familiar SNE, their introduction may have promoted a broad search for newly available resources. Consistent with this interpretation, roaming entropy was maintained or increased during these events, indicating that the rats continued to use the whole SNE rather than staying near the food-reward cages. At the same time, once the rats encountered the food rewards, the reduced exit intensity suggests that they spent more time exploiting the available resource before moving elsewhere. Because the rats experienced these events as a social group, their movements may additionally have been influenced by social information, competition or the behaviour of conspecifics (cf. Amorim et al., 2022). Accordingly, the observed patterns may partly reflect the dynamics of this particular group, whereas replication across independent groups could clarify whether these findings generalize to other social contexts (cf. Lazic et al., 2018).

Consistent with our initial hypothesis, risk events elicited a movement pattern distinct from that observed during foraging events. While they also increased transition intensities across the whole SNE and promoted entries into the risk cages, leaving was additionally increased compared to the no-event condition. Thus, risk events were characterised by frequent but brief visits to the manipulated cages. In line with this, studies involving food and predator-related cues indicate that perceived predation risk does not suppress food approaches but increases hesitation, retreat or escape responses and risk assessment, ultimately leading to lower foraging success (Choi & Kim, 2010; Amir et al., 2015; Engelke et al., 2021). The frequent but brief visits observed here may therefore reflect repeated information gathering or a conflict between approaching food and avoiding a putative threat. By combining simulated foraging and risk scenarios with continuous tracking, our study revealed that these conditions produced distinct patterns of entry,

exit and residence in manipulated cages. However, RFID tracking could not determine whether brief visits included vigilance behaviours or other forms of risk assessment. Complementary video observations could clarify the underlying behaviour, as illustrated by a study in which white noise exposure in a rat SNE increased locomotor activity while video observations revealed fear-specific behaviours such as hiding, huddling, and heightened alertness (Le Moëne et al., 2020). Although entries into the manipulated cages increased across all four risk events, the magnitude of this response and the increase in exit intensity varied among events, with the strongest reduction in dwell time occurring during the first risk event (i.e., the empty cage without shelter). The rats' behaviour in this exposed foraging situation during the light phase is consistent with the preference of wild Norway rats for areas with cover (Lambert et al., 2008). During the second and fourth risk events, the use of localized ALAN was based on rats' aversion towards open, bright spaces (La-Vu et al., 2020) and on the role of light as an indirect cue of predation risk (Farnworth et al., 2016; 2019). In the second event, the moving light cues and novel reflective objects were chosen based on the conflict between neophilic and neophobic behaviour (cf. Hughes, 2007). Against this background, moving cues can additionally introduce uncertainty about the environment and increase avoidance (cf. Chrzanowska et al., 2022). The eagle owl decoy used for the third risk event resembled a natural predator of Norway rats (Hadad et al., 2022) and rodents respond to visual predator cues (Bedoya-Perez et al., 2019). During the last risk event, the flashing LED frame provided artificial illumination at a higher intensity. However, a recent meta-analysis showed that antipredator responses depend on interactions between the environmental context and the type and sensory characteristics of the cues presented, rather than following a uniform pattern across cues (Jones et al., 2024). The manipulations used here differed in their sensory characteristics and were presented at different times and locations. For example, the particularly short dwell time during the first risk event (i.e., the empty cage without shelter) may reflect the greater exposure, but this was also the only risk event conducted exclusively during the day. Because each manipulation was presented only once, these factors cannot be separated and differences between events are best interpreted as responses to the specific scenarios rather than as general reactions to the respective cue types. Moreover, novelty and perceived risk cannot be readily separated in the present study because each manipulation introduced unfamiliar and unexpected stimuli into the otherwise familiar SNE. Novel cues provide uncertain information and may elicit both investigation and precautionary avoidance, meaning that responses to harmless unfamiliar stimuli can resemble responses to potential threats (Hughes, 2007; Crane et al., 2020; Crane et al., 2024; Chrzanowska et al., 2022). Consequently, we cannot determine whether the observed responses were driven by novelty, perceived threat or a combination of both. We therefore interpret the manipulations more cautiously as putative risk

scenarios rather than as validated risk simulations. To assess their actual risk character more directly, future studies could verify the risk events directly through repeated presentations, control conditions containing novel but putatively nonthreatening stimuli, and complementary behavioural and physiological measures of fear and stress.

Consistency of individual differences in exploration depends on the behavioural measure

As hypothesised, exploration was significantly repeatable in both contexts, supporting extensive evidence that exploration represents a consistent personality trait (Réale et al., 2007; Bell et al., 2009; Kaiser & Müller, 2021). Nevertheless, the proportion of repeatable variation differed markedly among measures. Approximately 35% of the variance in Open Field distance and 27% of the variance in cage-switching frequency, but only 5% of the variance in roaming entropy were attributable to between-individual differences. The moderate repeatability of Open Field distance is consistent with previous evidence for temporally stable exploration in rats (Améndola et al., 2022; Quante et al., 2026). Open Field distance and cage-switching frequency mostly quantify movement within an arena and between SNE compartments, respectively. Both measures therefore primarily reflect locomotory exploration. Roaming entropy, by contrast, captures how evenly residence time is distributed across the compartments and thus reflects a spatial component of exploration (cf. Freund et al., 2013). Although positively associated, roaming entropy was less stable over time than cage-switching frequency. This lower stability may reflect shifts in spatial preferences, the use of resources or resting sites and social interactions (Weiss et al., 2018; Freund et al., 2015; Amendola et al., 2023). Moreover, similar entropy values can result from different cage-use patterns (cf. Kempermann et al., 2022), and the generally high roaming entropies observed here left comparatively little variation between individuals. Studies in mice housed in large RFID environments suggest that individual roaming entropy trajectories and consistent individual differences in roaming entropy may become more pronounced over several months (Freund et al., 2013; Zocher et al., 2020; Mieske et al., 2021). The lower repeatability in the present study may partly reflect the shorter period available for stable spatial differences to become apparent, although differences in species, age, housing systems and analytical approaches limit direct comparisons among studies.

Finally, individual roaming entropy trajectories following the first release suggested differences in the timing and extent of initial exploration. Related studies reported variation in how evenly rats explored a novel environment and in their typical excursion extent, although rates of spatial expansion were similar among individuals (Tchernichovski et al., 1998; Štolhoferová et al., 2024). The cumulative roaming entropy measure used here combines the pace and extent of initial

exploration. However, repeated releases into unfamiliar or altered environments would be needed to test whether the same individuals consistently explore new spaces more rapidly.

Standardised and semi-natural measures capture different dimensions of exploration

Despite repeatability within contexts, we found no cross-context consistency between Open Field and SNE measures, consistent with our prediction of weak or absent relationships across the two settings. Thus, Open Field distance did not predict roaming entropy, cage-switching frequency or transition intensities under stable SNE conditions. Moreover, event-related associations did not show a uniform pattern across events or transition types and need to be interpreted cautiously due to repeated statistical testing.

The weak cross-context associations observed here are consistent with the study by Krebs and colleagues (2019) who found limited and partly negative relationships between behaviour in standardised tests and subsequent space use in a semi-natural environment. Similarly, repeatable exploratory behaviour did not covary across differently structured environments in great tits (Arvidsson et al., 2017) or across three standardised tasks in rats (Rudolfová et al., 2022). Conversely, partial or positive correspondence between responses to novel spaces and objects was found in common mynas, different small mammals and island songbirds (Perals et al., 2017; Mazza et al., 2021; García-Loor et al., 2025). Together, these findings suggest that cross-context consistency is possible but may be more apparent when the compared variables capture responses to related environmental challenges.

In the present study, however, the two settings differed substantially. The Open Field is a short, brightly illuminated, unfamiliar, and socially isolated test, in which locomotion is recorded within a single empty arena. Distance travelled under these conditions may reflect exploration but can also be influenced by general activity and anxiety-like behaviour in response to isolation and exposure to an open space (Walsh & Cummins, 1976; Carter et al., 2013; Stanford, 2007). The SNE instead allows long-term movement within a complex social environment. Moreover, both the Open Field and the initial release into the SNE involved novelty, whereas it was largely absent during later stable conditions. Rodents initially make short home-base excursions that expand with familiarisation (Thompson et al., 2018), while early studies described systematic revisits to familiar living spaces as patrolling or re-exploration (Cowan, 1977; Barnett et al., 1978). The analyses may therefore have compared not only different aspects of exploration in distinct environments but also different phases of exploration, ranging from an initial response to novelty to longer-term monitoring and use of a familiar environment. Although the SNE provided a more naturalistic setting than standardised tests, responses to novelty can differ between laboratory and wild rats (Stryjek et al., 2012; Kiyokawa et al., 2024). While this may limit the direct

transferability of findings to free-living rats, SNEs allow to combine a socially and spatially complex environment with controlled changes.

CTMCs as tools for disentangling movement processes in semi-natural environments

Beyond the biological findings on exploration, our study illustrates the value of CTMCs for analysing movement in complex environments. Whereas aggregate measures describe how frequently an animal moves or how it distributes its time across space, CTMCs decompose these patterns into direction-specific transition intensities, thereby distinguishing entries into and exits from particular locations and their resulting dwell times (cf. Wilson et al., 2018). This process-based perspective revealed that foraging and risk events both increased mobility but reorganised movements into and out of the manipulated cages differently. Previous rodent studies used discrete-time Markov chains to characterise spatial transitions in an elevated plus maze (Tejada et al., 2010) or sequences of scored behaviours (Natarajan et al., 2009; Lee et al., 2019). These approaches described the order and probabilities of transitions without incorporating the time spent in each state. In movement ecology, CTMCs have been applied to telemetry data to examine environmental features affecting how quickly animals move through particular areas and where they move next, and to estimate broader patterns of space use (Hanks et al., 2015; Wilson et al., 2018; Perony et al., 2012). To our knowledge, however, CTMCs have not previously been applied to long-term movement derived from RFID data in semi-natural environments. Future studies could apply this approach to repeated environmental changes to assess the consistency of individual transition dynamics and combine it with video or pose estimation to connect movements between cages with fine-scale behaviour. Models incorporating the movements of group members could additionally reveal social dependencies and link individual movement strategies to social networks.

Conclusion

In the present study, we investigated exploration across a standardised test and a semi-natural environment. Group-level analyses of both settings revealed that responses to short-term novelty and longer-term use of a complex environment follow different temporal patterns. Moreover, foraging events promoted prolonged use of food-reward cages, whereas putative risks elicited frequent but brief visits to the manipulated cages, reflecting trade-offs between exploration and exploitation as well as risk and reward. The repeatability of individual differences in exploration was more pronounced for locomotor measures of exploration than for the evenness of space use. However, we found no clear association between Open Field and SNE exploration. Together, the results suggest that exploration may comprise distinct components and depend on the

environmental context. Both short standardised tests and SNE observations therefore capture aspects of exploration but neither can characterise exploration comprehensively on its own. Consequently, a multidimensional assessment is needed to understand what constitutes exploration and to determine which of its components represent repeatable traits. From a broader perspective, consistent variation in exploration has important ecological consequences because it contributes to how individuals acquire resources, respond to danger and interact with conspecifics (Mazza et al., 2019; Wauters et al., 2021; Hakataya et al., 2023; Wirowska et al., 2024). These differences can affect individual fitness and the capacity of populations to cope with environmental change (Réale et al., 2007; Smith & Blumstein, 2008; Sih et al., 2012; Wolf & Weissing, 2012; Quante et al., 2026). In future studies, repeated and validated manipulations across forms of novelty and risk, combined with social movement analyses, could clarify when exploration generalises across contexts and when it remains specific to particular measures or challenges.

Generative AI statement

During the preparation of this work, the authors used ChatGPT-6 Sol (OpenAI) to generate Fig. 2B based on an original illustration by the first author and reference photographs, and to assist with language editing. The authors reviewed and edited the output as needed and take full responsibility for the content of the published article.

Author statement:

S.H.R. conceived the study. S.H.R. and L.B. designed the experiments. S.H.R., S.K. and R.R. supervised the project. L.B. and R.R. carried out the experiments. C.C.F., S.M. and L.B. conducted the statistical analysis of the data. L.B. wrote the initial draft of the manuscript. All authors critically revised the manuscript and gave final approval for publication.

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Declaration of competing interest:

The authors declare to have no competing interests.

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Supplementary Material

Methods

CTMC – model formulation

The rats' movement between the eight cages was modelled as a continuous-time Markov chain (CTMC) with generator (intensity) matrix

$$Q = \begin{pmatrix} q_{11} & q_{12} & q_{13} & 0 & 0 & 0 & 0 & 0 \\ q_{21} & q_{22} & 0 & q_{24} & 0 & 0 & 0 & 0 \\ q_{31} & 0 & q_{33} & q_{34} & q_{35} & 0 & 0 & 0 \\ 0 & q_{42} & q_{43} & q_{44} & 0 & q_{46} & 0 & 0 \\ 0 & 0 & q_{53} & 0 & q_{55} & q_{56} & q_{57} & 0 \\ 0 & 0 & 0 & q_{64} & q_{65} & q_{66} & 0 & q_{68} \\ 0 & 0 & 0 & 0 & q_{75} & 0 & q_{77} & q_{78} \\ 0 & 0 & 0 & 0 & 0 & q_{86} & q_{87} & q_{88} \end{pmatrix}$$

where $q_{ij;i\neq j}$ is the instantaneous rate of switching from cage i to cage j, restricted to zero for pairs of non-adjacent cages, and the diagonal entries are $q_{ii} = -\sum_{j\neq i} q_{ij}$, the exit rate out of cage i.

To account for experimental events, individual differences in open-field distance and diel rhythms, each transition rate of individual k at time t was modelled as a function of covariates via a log link function. Separate interactions of OFD with the overall foraging and risk indicators and with the corresponding cage-specific transition terms were also included:

$$\log(q_{ij,k,t}) =$$

$$\beta_{ij} + \beta_{day,ij}\mathbb{1}(\mathrm{day_t}) + \beta_{OF}OF_k +$$

$$\beta_F\mathbb{1}_{F(t)} + \beta_R\mathbb{1}_{R(t)} +$$

$$\beta_{treat_{from}}\mathbb{1}(\mathrm{treat_t}\ in\ cage\ i) + \beta_{treat_{to}}\mathbb{1}(\mathrm{treat_t}\ in\ cage\ j) +$$

$$\sum_r \beta_{risk\ r_{from}}\mathbb{1}(\mathrm{risk\ r_t}\ in\ cage\ i) + \beta_{risk\ r_{to}}\mathbb{1}(\mathrm{risk\ r_t}\ in\ cage\ j) +$$

$$\beta_{OF,F}\mathbb{1}_{F(t)}OF_k + \beta_{OF,R}\mathbb{1}_{R(t)}OF_k +$$

$$\beta_{OF,treat_{from}}\mathbb{1}(\mathrm{treat_t}\ in\ cage\ i)OF_k + \beta_{OF,treat_{to}}\mathbb{1}(\mathrm{treat_t}\ in\ cage\ j)OF_k +$$

$$\sum_r \beta_{OF,risk\ r_{from}}\mathbb{1}(\mathrm{risk\ r_t}\ in\ cage\ i)OF_k + \beta_{OF,risk\ r_{to}}\mathbb{1}(\mathrm{risk\ r_t}\ in\ cage\ j)\ OF_k$$

The likelihood was evaluated using the package RTMB[1], which provides exact gradients via automatic differentiation, and the negative log-likelihood was minimised with `nlminb`. Standard errors were derived from the Hessian at the optimum.

[1] Kristensen K (2026). RTMB: 'R' Bindings for 'TMB'. doi:10.32614/CRAN.package.RTMB

CTMC-derived measures

All derived measures were computed from the fitted generator matrix Q evaluated at fixed covariate values k and t, i.e. for a given combination of event condition, time of day (day or night) and open field distance. The model-implied mean dwell time in cage i was calculated as $-\frac{1}{q_{ii}}$. The stationary distribution π was obtained by solving $\pi Q = 0$, subject to the elements of π summing to one, and represents the long-run proportion of time spent in each cage if the respective covariate combination persisted. For visualisation, the one-minute transition probabilities were obtained from the fitted generator matrix with $p_{ij} = \left(\frac{q_{ij}}{-q_{ii}}\right)(1 - e^{60q_{ii}})$ and rates q_{ij} given per second. The element p_{ij} in row i and column j therefore gives the probability of switching directly to cage j within one minute after starting in cage i.

Tables & Figures

Table S1: Pairwise comparisons of Open Field distance travelled between the three repeated test rounds. Pairwise comparisons were calculated using the emmeans package.

Comparison	Estimate	SE	df	t-ratio	p
Test 1 vs. Test 2	8.046	1.24	34	6.477	<0.0001
Test 1 vs. Test 3	8.159	1.24	34	6.568	<0.0001
Test 2 vs. Test 3	0.113	1.24	34	0.091	0.9955

Table S2: Generalised additive mixed model results for relationships among OFD, roaming entropy, and cage switching under stable conditions. Link-scale parametric terms are reported as β (SE) and z; smooth terms as edf (reference df) and approximate χ^2.

Model	Response	Term	β (SE) / edf	z / χ^2	P	N
RE-OFD	Roaming entropy	OFD	-0.0171 (0.0130)	-1.315	0.188	18
RE-OFD	Roaming entropy	day	1.144 (1.272)	7.703	0.014	18
Cage switching-OFD	Cage switching	OFD	-0.0003 (0.0111)	-0.029	0.977	18
Cage switching-OFD	Cage switching	day	1.867 (2.284)	8.351	0.018	18
Cage switching-RE	Roaming entropy	Cage switching	0.0046 (0.0004)	10.300	<0.001	19
Cage switching-RE	Roaming entropy	day	1.002 (1.004)	19.421	<0.001	19

Table S3: Negative-binomial mixed-effects model results for cage-switching frequency during foraging and risk events. Event coefficients are reported on the log scale; temporal variation across experimental days was modelled nonlinearly using penalised splines. P-values are unadjusted across the seven event-specific models.

Event	β	SE	P
FE1 (C5, C8)	0.262	0.117	0.02443
FE2 (C1, C4)	1.298	0.186	<0.001
FE3 (C2, C3)	0.408	0.100	<0.001
RiE1 (C3)	0.628	0.109	<0.001
RiE2 (C4)	0.633	0.102	<0.001
RiE3 (C7)	-0.024	0.086	0.77904
RiE4 (C6)	0.941	0.170	<0.001

Table S4: Beta mixed-effects model results for roaming entropy during foraging and risk events. Event coefficients are reported on the logit scale; temporal variation across experimental days was modelled nonlinearly using penalised splines. P-values are unadjusted across the seven event-specific models.

Event	β	SE	P
FE1 (C5, C8)	0.075	0.195	0.69942
FE2 (C1, C4)	0.648	0.240	0.00700
FE3 (C2, C3)	0.211	0.189	0.26307
RiE1 (C3)	0.466	0.188	0.01298
RiE2 (C4)	-0.310	0.155	0.04628
RiE3 (C7)	-0.249	0.147	0.09106
RiE4 (C6)	0.331	0.236	0.16071

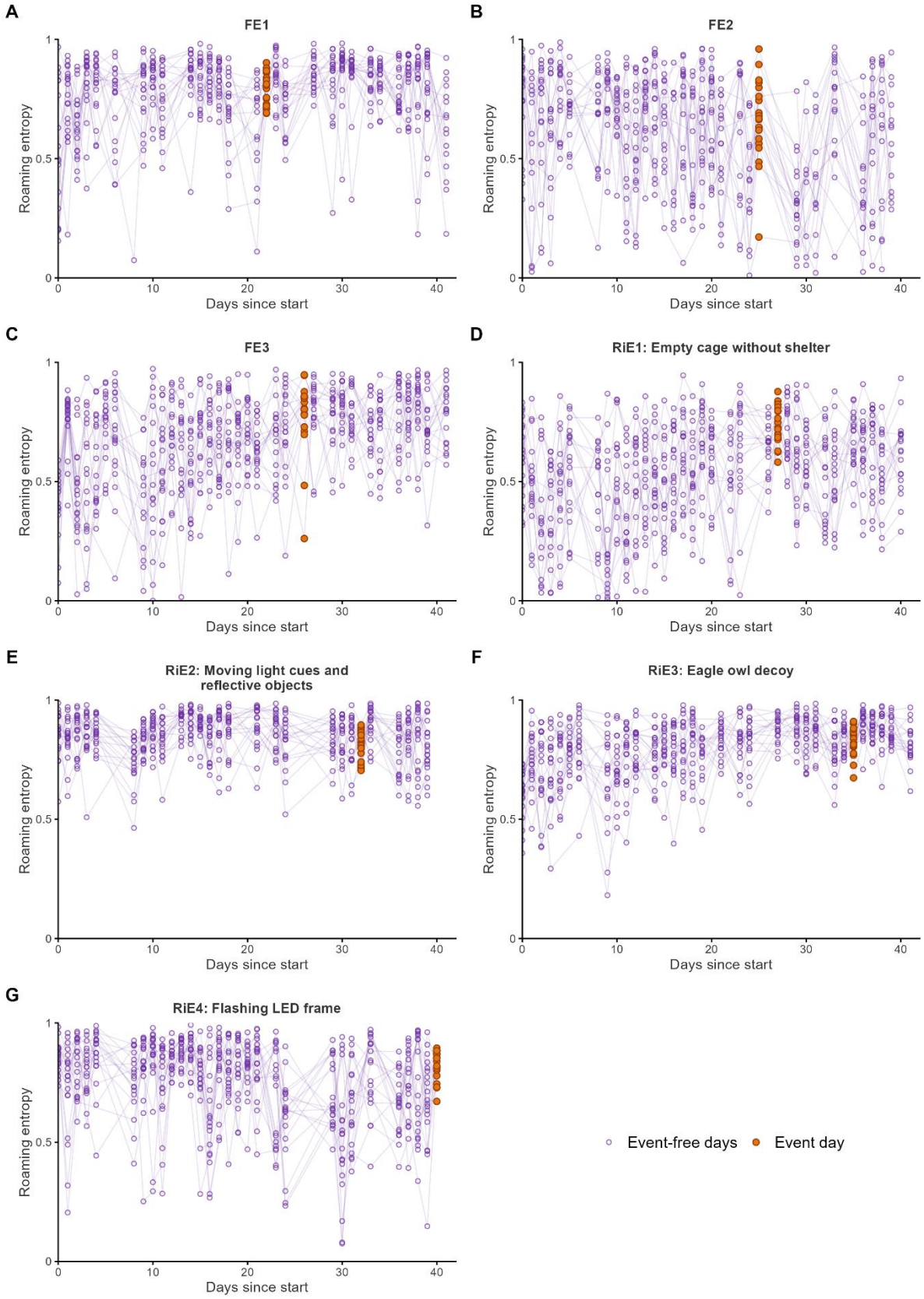


Figure S1: Roaming entropy during foraging and risk events compared with event-free days at the corresponding time of day. Each panel represents one foraging or risk event. Violet circles show individual values during the corresponding time window on all available days, and orange circles show values recorded on the event day. Event-specific model results are reported in Table S4.

Table S5: Markov-model-derived dwell times during event-free nights and the three foraging events (FE).

Cage	No event (night)	FE1 (Cage 5+8)	FE2 (Cage 1+4)	FE3 (Cage 2+3)
C1	3.74[3.68-3.8]	2.41[2.26-2.58]	**4.87[4.46-5.27]**	2.02[1.85-2.2]
C2	3.34[3.29-3.39]	2.16[2.02-2.31]	1.81[1.66-1.96]	**4.36[4-4.71]**
C3	2.73[2.7-2.77]	1.67[1.58-1.76]	1.55[1.45-1.65]	**3.56[3.27-3.87]**
C4	2.22[2.19-2.25]	1.43[1.34-1.53]	**2.9[2.66-3.12]**	1.26[1.18-1.35]
C5	2.67[2.63-2.71]	**3.48[3.2-3.76]**	1.72[1.61-1.84]	1.63[1.54-1.72]
C6	2.47[2.44-2.5]	1.41[1.32-1.5]	1.5[1.43-1.59]	1.59[1.49-1.7]
C7	3.28[3.24-3.34]	1.78[1.63-1.93]	2.12[1.99-2.26]	2.12[1.99-2.26]
C8	4.04[3.98-4.1]	**5.27[4.84-5.71]**	2.61[2.44-2.78]	2.61[2.44-2.78]

Table S6: CTMC effects on transitions to and from food-reward and risk cages. Each cell reports the additional cage-specific deviation from the corresponding overall event-type effect and the combined event effect (overall plus cage-specific). Overall effects were β = 0.439 [0.372, 0.505] for foraging events and β = 0.615 [0.583, 0.648] for risk events. Factor changes are exp(β); confidence intervals for combined effects account for covariance between parameters.

Event	Transition	Additional cage-specific / combined β (95% CI)	Additional cage-specific / combined factor (95% CI)
Foraging events	from food-reward cage	Additional: -0.703 [-0.809, -0.597] Combined: -0.265 [-0.347, -0.182]	Additional: 0.495 [0.445, 0.550] Combined: 0.767 [0.707, 0.834]
Foraging events	to food-reward cage	Additional: 0.177 [0.071, 0.282] Combined: 0.615 [0.533, 0.697]	Additional: 1.193 [1.074, 1.326] Combined: 1.850 [1.703, 2.009]
RiE1 (C3)	from risk cage	Additional: 0.942 [0.802, 1.082] Combined: 1.557 [1.422, 1.693]	Additional: 2.565 [2.231, 2.949] Combined: 4.747 [4.144, 5.437]
RiE1 (C3)	to risk cage	Additional: 1.063 [0.924, 1.202] Combined: 1.678 [1.543, 1.814]	Additional: 2.895 [2.520, 3.326] Combined: 5.357 [4.681, 6.132]
RiE2 (C4)	from risk cage	Additional: -0.420 [-0.492, -0.348] Combined: 0.196 [0.131, 0.260]	Additional: 0.657 [0.612, 0.706] Combined: 1.216 [1.140, 1.297]

Event	Transition	Additional cage-specific / combined β (95% CI)	Additional cage-specific / combined factor (95% CI)
RiE2 (C4)	to risk cage	Additional: 0.935 [0.863, 1.007] Combined: 1.550 [1.486, 1.615]	Additional: 2.546 [2.370, 2.736] Combined: 4.712 [4.419, 5.025]
RiE4 (C6)	from risk cage	Additional: -0.471 [-0.562, -0.380] Combined: 0.145 [0.059, 0.230]	Additional: 0.624 [0.570, 0.684] Combined: 1.156 [1.061, 1.258]
RiE4 (C6)	to risk cage	Additional: 0.652 [0.561, 0.743] Combined: 1.268 [1.182, 1.353]	Additional: 1.920 [1.753, 2.102] Combined: 3.552 [3.262, 3.868]
RiE3 (C7)	from risk cage	Additional: -0.453 [-0.588, -0.318] Combined: 0.163 [0.031, 0.294]	Additional: 0.636 [0.555, 0.728] Combined: 1.176 [1.032, 1.342]
RiE3 (C7)	to risk cage	Additional: 0.380 [0.245, 0.515] Combined: 0.995 [0.864, 1.127]	Additional: 1.462 [1.277, 1.673] Combined: 2.706 [2.373, 3.085]

Table S7: Markov-model-derived dwell times during event-free periods and the four risk events (RiE1-RiE4).

Cage	No event (day)	RiE1 (C3, day)	No event (night)	RiE2 (C4, night)	RiE3 (C7, day+night)	RiE4 (C6, night)
C1	18.67[18.08-19.32]	5.33[4.83-5.89]	3.74[3.68-3.8]	2.02[1.95-2.1]	2.02[1.95-2.1]	2.02[1.95-2.1]
C2	7.37[7.11-7.62]	3.98[3.81-4.17]	3.34[3.29-3.39]	1[0.96-1.05]	1.81[1.75-1.87]	1.81[1.75-1.87]
C3	18.66[18.14-19.19]	**3.93[3.43-4.48]**	2.73[2.7-2.77]	0.97[0.93-1.01]	1.48[1.43-1.53]	1.48[1.43-1.53]
C4	7.25[7.04-7.47]	2.18[1.98-2.39]	2.22[2.19-2.25]	**1.83[1.72-1.95]**	1.2[1.16-1.25]	0.94[0.9-0.98]
C5	17.24[16.8-17.74]	6.04[5.57-6.48]	2.67[2.63-2.71]	1.44[1.39-1.5]	1.24[1.17-1.32]	1.09[1.03-1.14]
C6	7.28[7.07-7.5]	3.93[3.76-4.1]	2.47[2.44-2.5]	0.9[0.87-0.94]	1.34[1.29-1.38]	**2.14[1.96-2.34]**
C7	15.36[14.89-15.84]	8.3[7.92-8.71]	3.28[3.24-3.34]	1.78[1.71-1.84]	**2.79[2.46-3.19]**	1.78[1.71-1.84]
C8	27.14[26.3-28.07]	14.67[14.08-15.35]	4.04[3.98-4.1]	2.18[2.11-2.27]	1.76[1.62-1.9]	1.52[1.43-1.6]

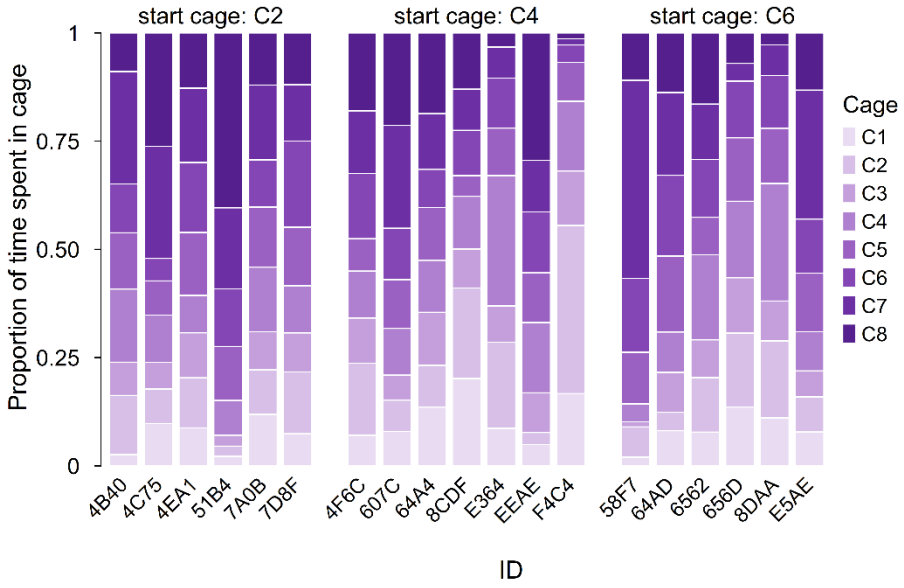


Figure S2: Proportion of time spent per cage during the first 4 h after release into the semi-natural environment. Each stacked bar represents one rat and shows the proportion of the observation period spent in each of the eight cages. Proportions sum to 1 for each individual; rats are grouped by initial release group and starting cage (C2, C4, or C6).

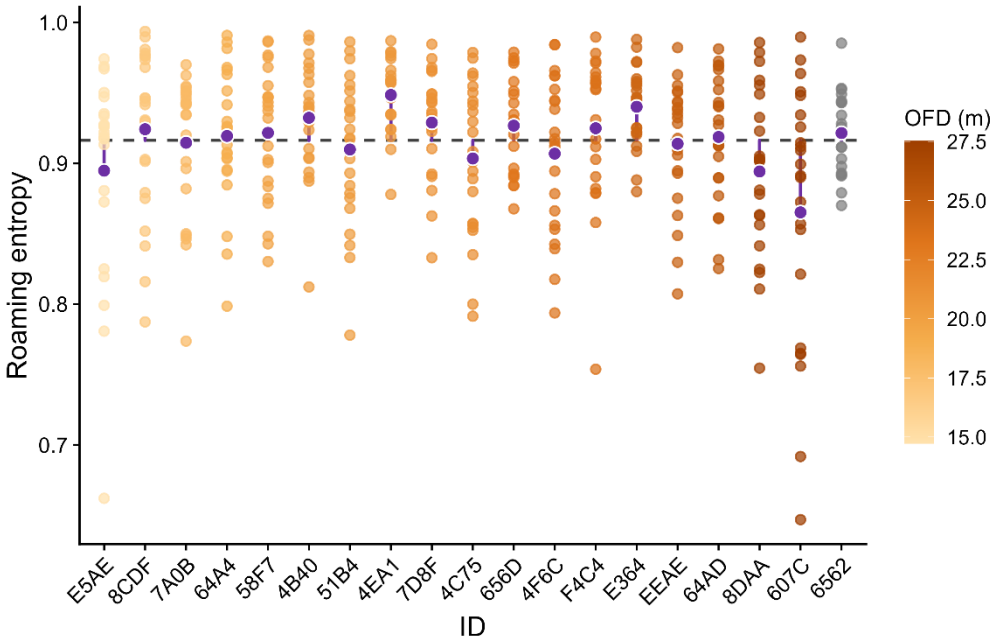


Figure S3: Descriptive distribution of roaming entropy across individuals during event-free nights. Each orange point represents one nightly roaming-entropy value. Individuals on the x-axis are ordered from lower to higher Open Field distance (OFD), which is also represented by the orange colour gradient. ID 6562, for which no OFD was available, is shown at the right in grey. Purple points indicate individual means, and purple vertical segments show the difference between each individual mean and the overall mean across all nightly observations. The dashed horizontal line indicates the overall mean.

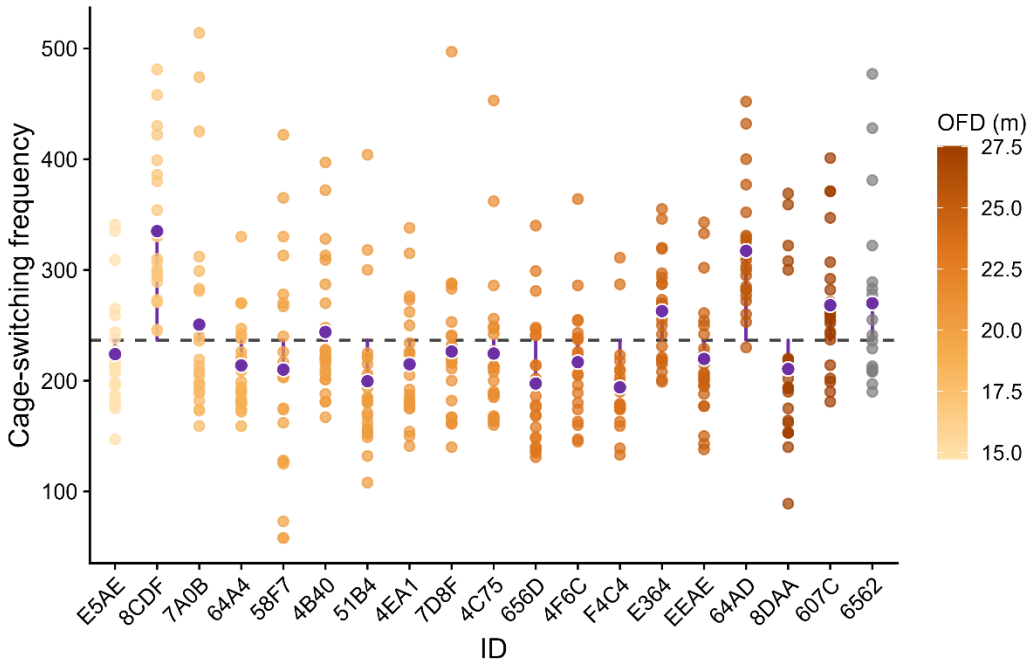


Figure S4: Descriptive distribution of cage-switching frequency across individuals during event-free nights. Each orange point represents one nightly cage-switching-frequency value. Individuals on the x-axis are ordered from lower to higher Open Field distance (OFD), which is also represented by the orange colour gradient. ID 6562, for which no OFD was available, is shown at the right in grey. Purple points indicate individual means, and purple vertical segments show the difference between each individual mean and the overall mean across all nightly observations. The dashed horizontal line indicates the overall mean.

Table S8: CTMC estimates for the association between Open Field distance and overall transition intensity under stable, initial-release, foraging-event, and risk-event conditions. OFD was z-standardised across individuals before model fitting (mean = 21.74 m, SD = 3.49 m). β is the change in log transition intensity per 1-SD increase in OFD; factor changes were calculated as exp(β).

Condition	Predictor	β (95% CI)	Factor change (95% CI)
Stable SNE	OFD	-0.0037 [-0.0084, 0.0010]	0.9963 [0.9916, 1.0010]
First 4 h after release	OFD	-0.0068 [-0.0433, 0.0296]	0.9932 [0.9576, 1.0301]
Event periods	OFD × foraging events	0.1155 [0.0414, 0.1897]	1.1225 [1.0423, 1.2088]
	OFD × risk events	-0.0437 [-0.0768, -0.0105]	0.9573 [0.9261, 0.9895]

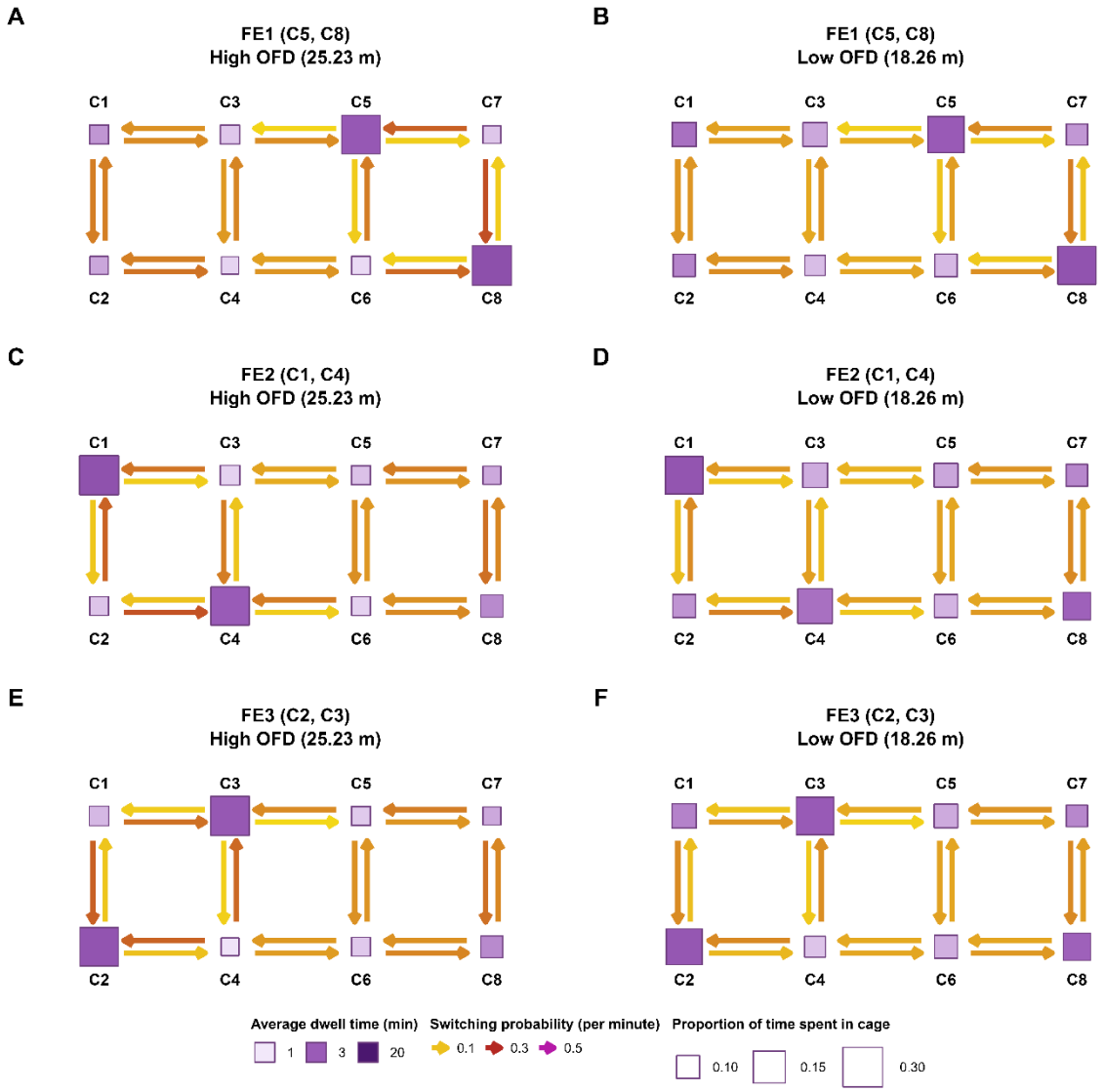


Figure S5: Markov-model-derived movement patterns during foraging events for rats with high and low Open Field distance (OFD). Panels A, C, and E show model predictions at high OFD (25.23 m), and panels B, D, and F show predictions at low OFD (18.26 m). Panels A-B show FE1 (C5 and C8), panels C-D show FE2 (C1 and C4), and panels E-F show FE3 (C2 and C3). Square shading indicates model-implied dwell time in minutes, arrow colour indicates switching probability per minute, and square size represents the model-implied proportion of time spent in a cage.

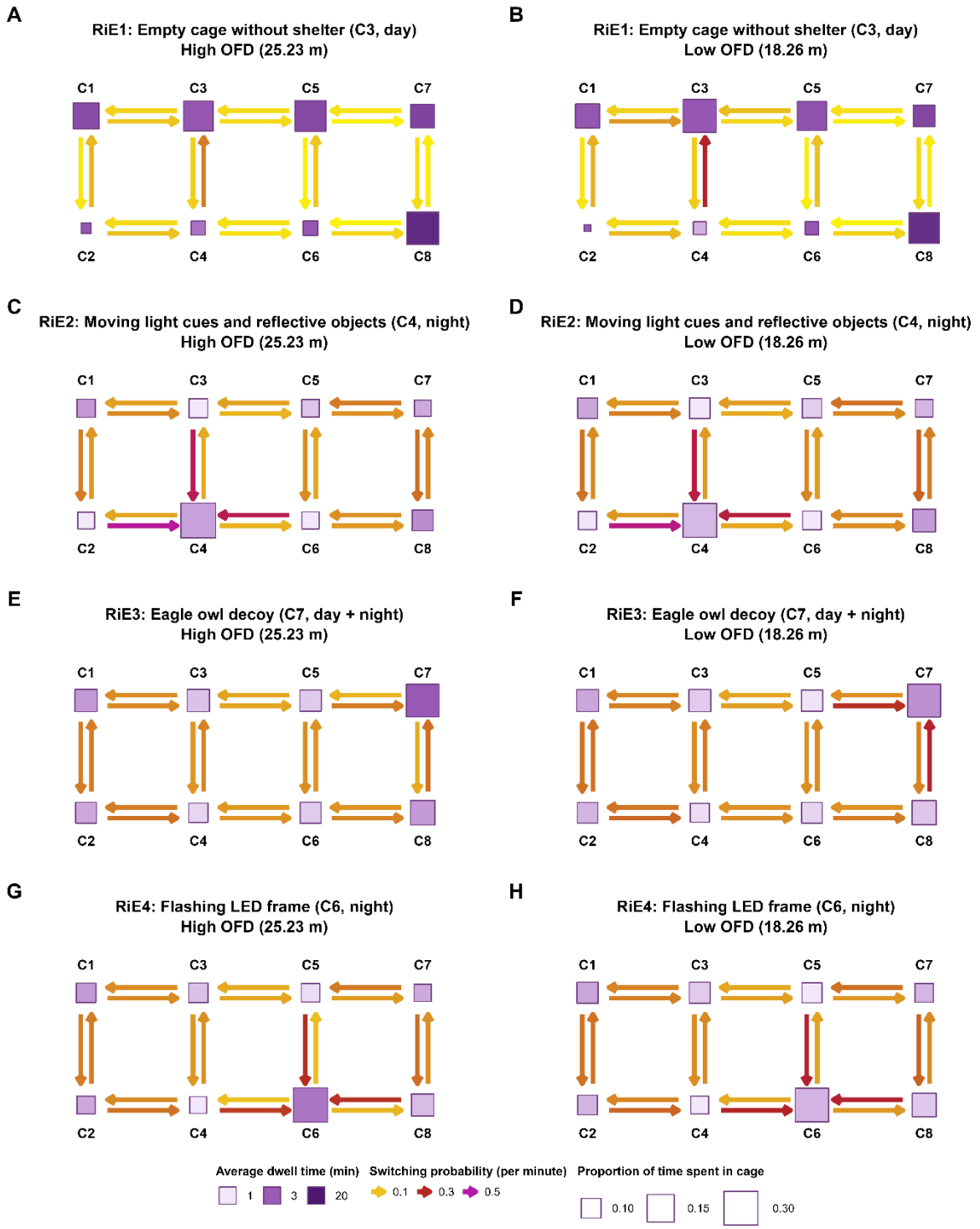


Figure S6: Markov-model-derived movement patterns during risk events with OFD interactions for rats with high and low Open Field distance (OFD). Panels A, C, E, and G show model predictions at high OFD (25.23 m), and panels B, D, F, and H show predictions at low OFD (18.26 m). Panels A-B show RiE1, an empty cage without shelter (C3, day); panels C-D show RiE2, moving light cues and reflective objects (C4, night); panels E-F show RiE3, an eagle owl decoy (C7, day + night); and panels G-H show RiE4, a flashing LED frame (C6, night). Square shading indicates model-implied dwell time in minutes, arrow colour indicates switching probability per minute, and square size represents the model-implied proportion of time spent in a cage.

Table S9: Markov-model interaction effects of Open Field distance on foraging-related transitions. Each cell reports the additional cage-specific interaction and the combined interaction after adding the overall OFD-by-foraging effect per 1-SD increase in OFD (1 SD = 3.49 m). Confidence intervals for combined effects account for covariance between parameters.

Transition	Additional cage-specific / combined β (95% CI)	Additional cage-specific / combined factor (95% CI)
from food-reward cage	Additional: -0.182 [-0.294, -0.070] Combined: -0.066 [-0.151, 0.018]	Additional: 0.834 [0.745, 0.933] Combined: 0.936 [0.860, 1.018]
to food-reward cage	Additional: 0.077 [-0.034, 0.189] Combined: 0.193 [0.109, 0.276]	Additional: 1.080 [0.966, 1.208] Combined: 1.213 [1.115, 1.318]

Table S10: Markov-model interaction effects of Open Field distance on risk-related transitions. Each cell reports the additional cage-specific interaction and the combined interaction after adding the overall OFD-by-risk effect per 1-SD increase in OFD (1 SD = 3.49 m). Confidence intervals for combined effects account for covariance between parameters.

Parameter	Additional cage-specific / combined β (95% CI)	Additional cage-specific / combined factor (95% CI)
from RiE1 (C3)	Additional: 0.011 [-0.130, 0.152] Combined: -0.032 [-0.169, 0.105]	Additional: 1.011 [0.878, 1.164] Combined: 0.968 [0.844, 1.110]
to RiE1 (C3)	Additional: -0.224 [-0.379, -0.068] Combined: -0.267 [-0.420, -0.115]	Additional: 0.800 [0.684, 0.934] Combined: 0.765 [0.657, 0.891]
from RiE2 (C4)	Additional: -0.043 [-0.118, 0.032] Combined: -0.087 [-0.154, -0.019]	Additional: 0.958 [0.889, 1.032] Combined: 0.917 [0.857, 0.981]
to RiE2 (C4)	Additional: 0.095 [0.024, 0.167] Combined: 0.052 [-0.012, 0.116]	Additional: 1.100 [1.024, 1.182] Combined: 1.053 [0.988, 1.122]
from RiE3 (C7)	Additional: -0.167 [-0.313, -0.020] Combined: -0.210 [-0.353, -0.068]	Additional: 0.846 [0.731, 0.980] Combined: 0.810 [0.702, 0.935]
to RiE3 (C7)	Additional: -0.198 [-0.361, -0.035] Combined: -0.242 [-0.401, -0.082]	Additional: 0.820 [0.697, 0.965] Combined: 0.785 [0.670, 0.921]
from RiE4 (C6)	Additional: -0.157 [-0.250, -0.064] Combined: -0.201 [-0.288, -0.114]	Additional: 0.855 [0.779, 0.938] Combined: 0.818 [0.750, 0.893]
to RiE4 (C6)	Additional: -0.030 [-0.126, 0.067] Combined: -0.073 [-0.164, 0.017]	Additional: 0.971 [0.881, 1.069] Combined: 0.929 [0.849, 1.018]

Table S11: Combined event effects on transitions to and from affected cages at low and high Open Field distance values (mean minus or plus one standard deviation: 18.26 m and 25.23 m). Estimates combine overall event-type, cage-specific, and corresponding OFD interaction terms; confidence intervals account for covariance between parameters.

Event	Transition	Low OFD β (95% CI)	High OFD β (95% CI)	Low OFD factor (95% CI)	High OFD factor (95% CI)
RiE1 (C3)	from risk cage	1.59 [1.40, 1.77]	1.53 [1.32, 1.73]	4.90 [4.07, 5.90]	4.60 [3.76, 5.62]
RiE1 (C3)	to risk cage	1.95 [1.75, 2.14]	1.41 [1.20, 1.62]	7.00 [5.76, 8.50]	4.10 [3.31, 5.07]
RiE2 (C4)	from risk cage	0.28 [0.19, 0.37]	0.11 [0.01, 0.20]	1.33 [1.21, 1.45]	1.12 [1.01, 1.23]
RiE2 (C4)	to risk cage	1.50 [1.41, 1.59]	1.60 [1.51, 1.69]	4.47 [4.09, 4.89]	4.96 [4.53, 5.44]
RiE4 (C6)	from risk cage	0.35 [0.23, 0.46]	-0.06 [-0.18, 0.07]	1.41 [1.26, 1.59]	0.95 [0.83, 1.07]
RiE4 (C6)	to risk cage	1.34 [1.22, 1.46]	1.19 [1.06, 1.32]	3.82 [3.40, 4.30]	3.30 [2.90, 3.76]
RiE3 (C7)	from risk cage	0.37 [0.19, 0.55]	-0.05 [-0.25, 0.16]	1.45 [1.21, 1.74]	0.95 [0.78, 1.17]
RiE3 (C7)	to risk cage	1.24 [1.05, 1.43]	0.75 [0.53, 0.97]	3.45 [2.85, 4.17]	2.12 [1.70, 2.65]
FE	from food-reward cage	-0.20 [-0.32, -0.08]	-0.33 [-0.45, -0.22]	0.82 [0.73, 0.93]	0.72 [0.64, 0.81]
FE	to food-reward cage	0.42 [0.30, 0.54]	0.81 [0.69, 0.92]	1.53 [1.35, 1.72]	2.24 [2.00, 2.51]