

Supporting Information

Fonio et al. 10.1073/pnas.0812513106

SI Materials and Methods

Experimental Animals. Subjects were 40 BALB/C male mice (Harlan Laboratories). Mice were 10 weeks old at the onset of the observations. From the age of 8 weeks and on they were group housed, three per cage. Animals were maintained under standard conditions of 22 °C, a 12:12 light/dark cycle with lights on at 8:00 PM, water and food available ad libitum. This protocol was followed until the observation period.

Walled Open Field. Walled open field consisted of a 2.2-m-diameter arena with a gray Formica floor and a 40-cm-high wall. The arena was elevated 45 cm above the floor and was lit by two fluorescent lamps (350 lux) placed over its center. A sealed box was placed inside the arena near the wall. To create a relatively homogenous environment the arena was surrounded by white curtains.

Elevated (Wall-Less) Open Field. Same as above except for the walls that were moved 20 cm away from the edge and placed around the platform, to transform the arena's edge into a cliff and to prevent the animal from jumping off the platform and escaping during the observation.

Walled Open Field Mice. Eleven mice were used in this test. Each mouse was exposed to the arena for three successive 1-h daily sessions.

Elevated (Wall-Less) Open Field Mice. Twenty-nine mice were used; each mouse was exposed to the arena for three successive 1-h daily sessions. The large number of animals was necessary because some of the animals jumped off the platform. An animal that jumped off the platform, did not participate in the rest of the experiment. Of the 29 tested mice we presented here only the first session of the first 11 mice, to match the number of animals tested in the other groups.

To elevate the level of the animals' activity, experiments were conducted during the first part of the nocturnal phase, when the level of activity is at its highest (1). Before the beginning of the test, the mouse was brought to the arena inside a small non-transparent plastic container and placed near the sealed box. The test started after the plastic box was lifted. Mouse behavior was recorded throughout the session by a stationary video camera whose lens covered the whole arena. Immediately afterward, the mouse was returned to its cage. The arena was washed with water and dried after each session.

Data Acquisition and Processing. The mouse's path was recorded by using the Noldus Ethovision Tracking System (2) and software developed in our lab. The spatial sensitivity of the system was 0.5–2 cm², with a temporal resolution of 25 frames per s. The output of a 1-h session is a file containing 90,000 records of location.

Algorithms of Types of Motion. (motion 1) "Peep-and-hide" detects partial penetration into the arena (the animal enters with only snout/head/part of body into the arena before withdrawing into home-cage). This algorithm provides pairs of indexes marking the start and end of Peep-and-hide episodes. The algorithm also counts the number of pixels of the mouse's image that protrude into the arena through doorway and calculates the percentage of the mouse's body within the arena, providing the timing and build up of peep-and-hide behavior.

(Motion 2) "Cross-and-retreat" detects instances of full body area of the animal in the arena (i.e., when the animal fully crossed the doorway) followed by retreating backwards into the home-cage. Backward progression is identified by the heading value calculated on the basis of the tracked direction of the animal's midsagittal plane. The algorithm provides the pairs of indexes that mark the start and end of this motion.

(Motion 3) "Circle-in-place" detects instances of full body area of the animal in arena followed by rotation of midsagittal body axis in the garden, until the animal's snout is directed toward the doorway. The algorithm provides the pairs of indexes bounding this motion.

(Motion 4) "Depart-head-on" detects the initial performance of departure head-on into the home-cage. Forward progression is identified by the heading value calculated on the basis of the tracked direction of the animal's midsagittal plane. The algorithm provides the indexes that bound the first occurrence of this motion.

(Motion 4') "Extended-garden-roundtrip." The garden is an area near the doorway characterized by high dwell time. The algorithm detects roundtrips whose angular extension from doorway on arena periphery is not larger than the garden extension, and whose ratio between radial extension and angular extension is larger than 1. Garden extension is obtained by plotting a density plot of dwell time in arena throughout the session and obtaining the boundary of the two-dimensional Gaussian surrounding the doorway.

(Motion 5) "Borderline-roundtrip" detects roundtrips that are exclusively constructed of wall-cursions and include a single outbound and a single inbound portion. The inbound segment consists of a monotonic increase in angular position, and the outbound segment consists of a monotonic decrease in angular position, culminating with a departure into the home-cage. The algorithm provides a list of the pairs of indexes of all of the Borderline-roundtrip.

(Motion 6) "Home-related-shuttle" detects changes of direction within the inbound segment of Borderline-roundtrip. The change of direction is based on the angular position. This motion consists of two variants. In one, the change of direction occurs in the garden (cage skip), and in the other the change of direction (shuttle) occurs outside the garden. This algorithm provides the pairs of indexes bounding home-related shuttles and cage skips.

(Motion 7) "Borderline-roundtrip-in-other-direction" detects borderline roundtrips to the other, opposite, side. Positive values of angular position represent Borderline-roundtrip to one side, whereas negative values stand for borderline roundtrips to the other side. This algorithm provides the pairs of indexes bounding Borderline-roundtrip to the opposite side.

(Motion 8) "Full-circle" detects complete full circles; that is, a continuous Borderline-roundtrip that covers at least 95% of arena circumference. This algorithm provides the indexes bounding full circles and almost full circles.

(Motion 9) "Incursions" detects simple incursions away from the wall and into the center of the arena. The algorithm defines for each mouse separately a statistically significant cutoff point that distinguishes the incursions from the background noise associated with movement along the wall. The algorithm provides a list of pairs of indexes bounding Incursions.

(Motion 10) "Border-related-shuttle" detects changes of direction in the border-bound portion of Incursions. The change of direction is based on the radial position of the animal.

(Motion 11) "Reaching-the-center" detects the incursion that

reaches or exceeds the quantile 0.95 of the radius of the arena and provides the two indexes bounding this Incursion.

(Motion 12) “Jumping” detects jumping on the wall. During jumping the mouse traces a path that appears to cross the boundary of the arena when the path is viewed from the point of view of the camera attached to the ceiling. The protrusions of the boundary by the path traced during jumping are captured by the algorithm.

Access Instructions to Public Database. The database includes data of all mouse sessions used in the present study: free BALB/c and

C57BL/6 mice, forced-walled and forced-elevated open field sessions of BALB/c mice. The database consists of raw location data, SEE files (for explanations see <http://www.tau.ac.il/~ilan99/see/help/>) and entry indexes that segment the session into entries into the arena. The database is available via the following web address: <http://oes.tau.ac.il/NetStorage> (user name: `ilang_public`; password: `year03b`). After getting through enter the folder `DriveH@LIFESTORE`, then `Zoology`, and finally to `ilang_public`, which contains the database. Please use the Logout button to exit the database rather than just closing the web page.

1. Lassalle JM, Halley H, Rouillet P (1994) Analysis of behavioral and hippocampal variation in congenic albino and pigmented BALB mice. *Behav Genet* 24:161–169.

2. Spink AJ, Tegelenbosch RAJ, Buma MOS, Noldus LPJJ (2001) The EthoVision video tracking system—A tool for behavioral phenotyping of transgenic mice. *Physiol Behav* 73:731–744.

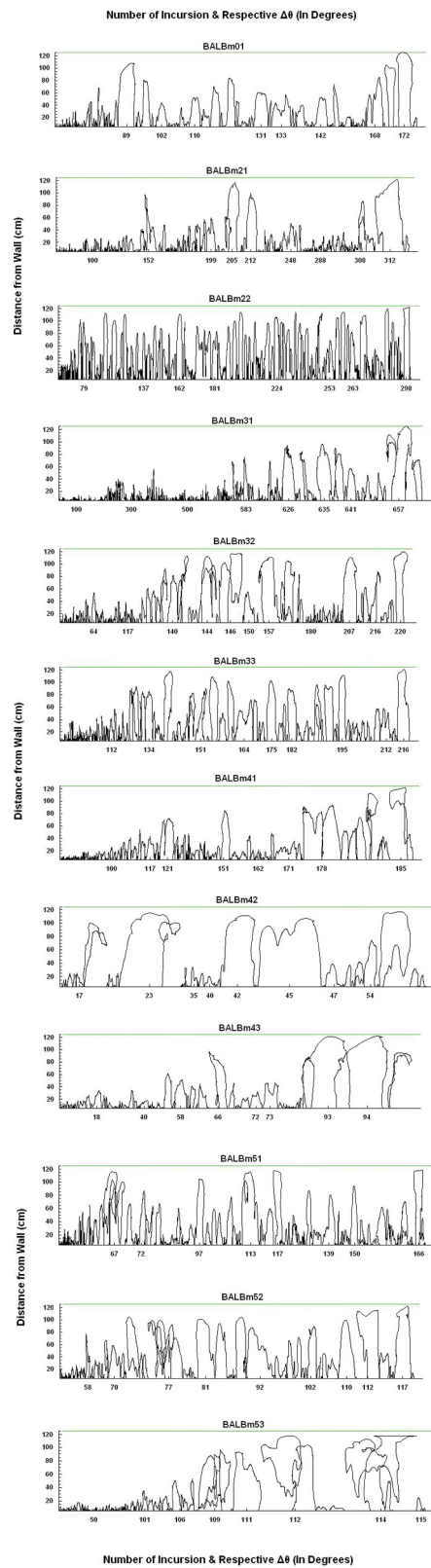


Fig. S2. The build up of amplitude and complexity of incursions, from the initial incursion to the incursion that reached the center, in all of the examined free BALB/c mice. Incursions are plotted side-by-side in the order of their performance. For each incursion, the x-axis demarcates the angular (border) section it covers, and the y axis demarcates arena radius. Note incremental nonmonotonical growth in distance from wall, in the section of the circle covered by incursions, and in the number and complexity of Border-related-shuttles.

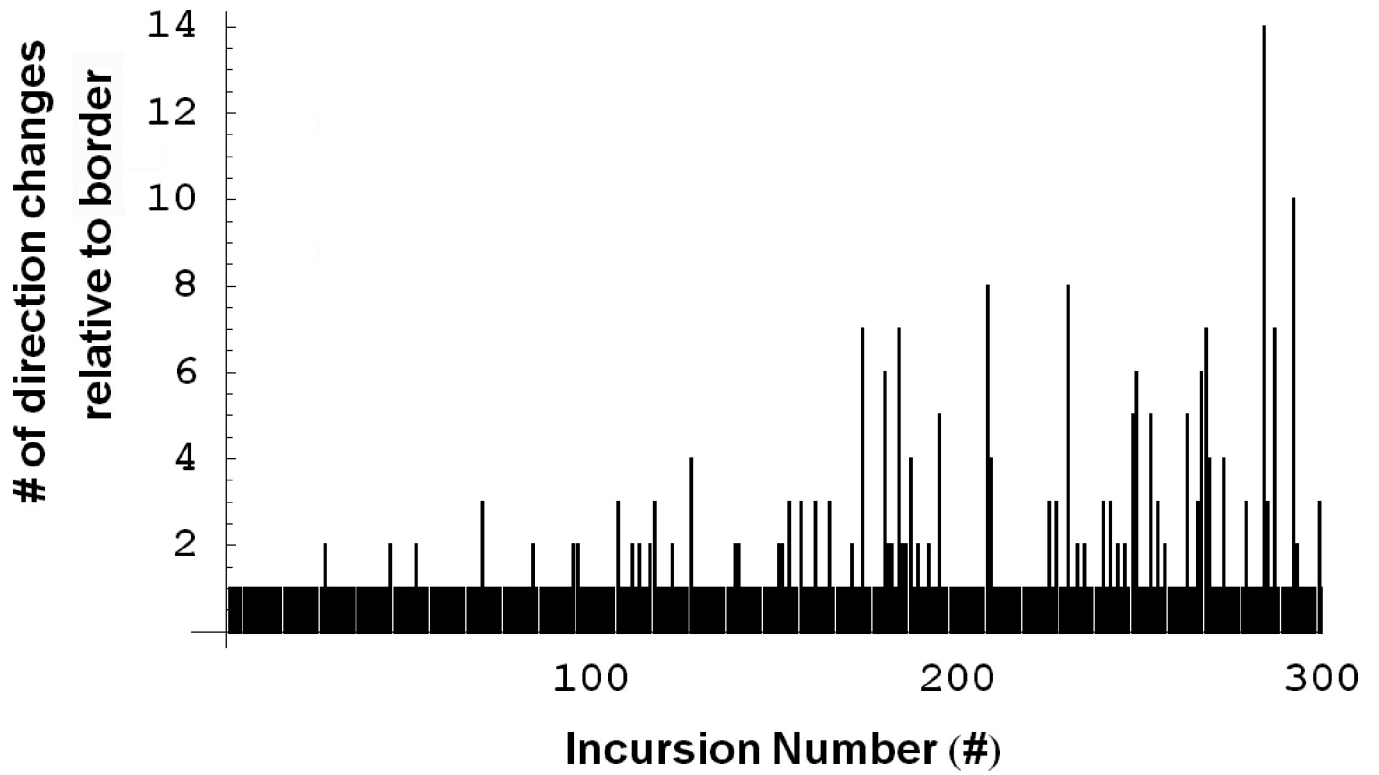


Fig. S3. The frequency of changes of direction relative to borderline per incursion in a selected free mouse-session (BALB/c #41). Incursions are represented in the order of their performance. Simple incursions that include a single change of direction are followed by incursions that include an increasing number of direction changes (Border-related-shuttles). In this way incursions become progressively more tortuous.

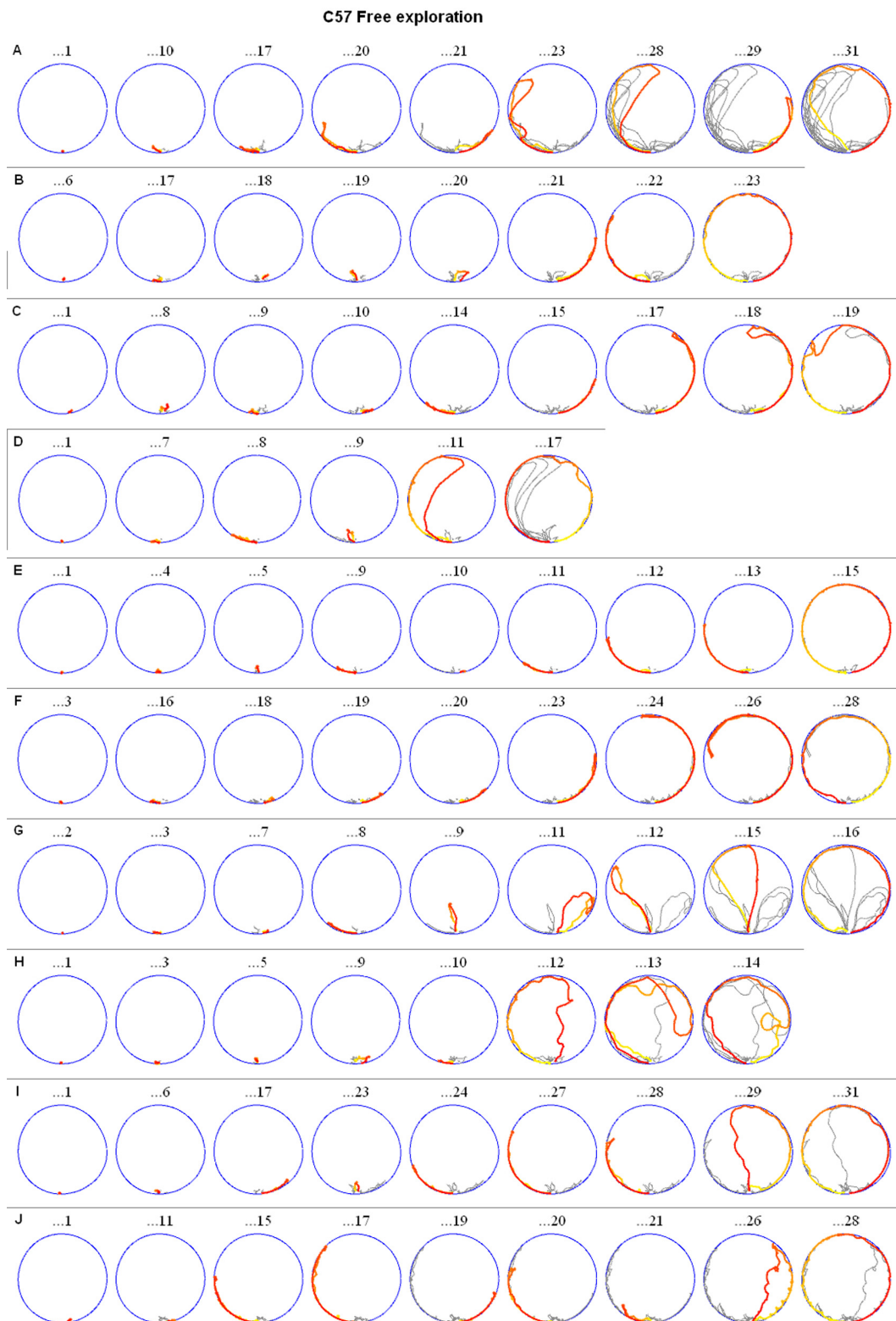


Fig. S4. Path plots of the developmental sequence and build up of the staying-in-place behavior (zero MDF) and borderline (one MDF) stages until the performance of Full-circle (landmark 8) in the C57BL/6 mice tested in the free setup. Note the Extended-garden-roundtrips near the doorway away from wall (zero MDF). These were performed before and immediately after the onset of Borderline-roundtrips. Extended-garden-roundtrips were also present in incipient form in two free BALB/c mice. Also note in the C57BL/6 mice the primacy of borderline movement over radial movement in all mice, the overlap between borderline and radial movement in most but not all mice, and the separate build up of movements in the zero-, one-, and two-dimensions.

BALB/c mice; forced exploration; walled arena

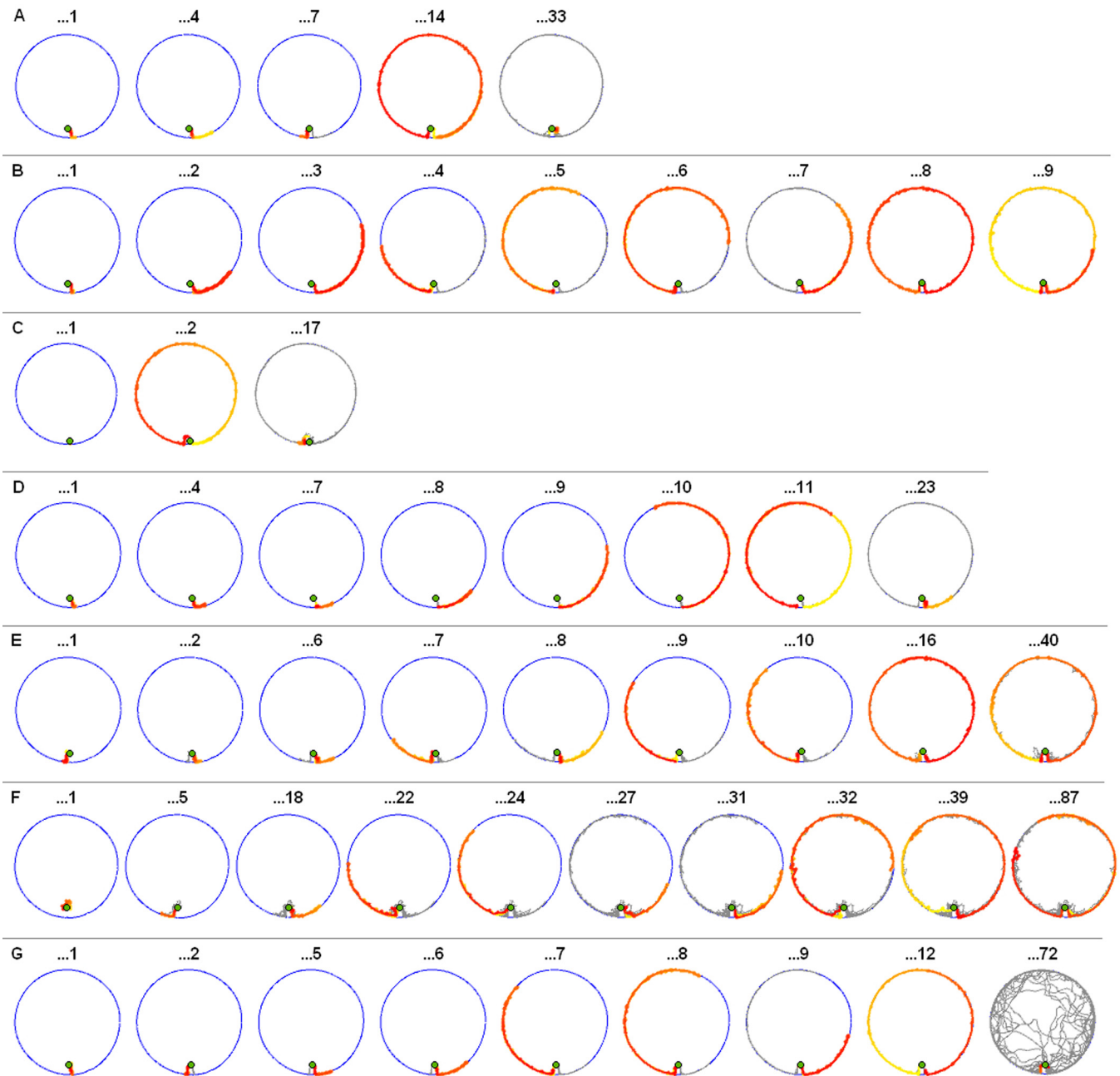


Fig. S5. Legend as in Fig. S4 except that the animals are BALB/c mice tested in a walled, forced setup. A wooden cube is situated near the wall at the place where the mouse is introduced into the arena. Arena diameter equals 2.2 m. Each mouse-session is represented by a horizontal sequence of circles, from left to right. Each circle presents all roundtrips performed that far, from the start of the session (in gray), until the last, "current" roundtrip (colored from yellow to red) enumerated above the circle. For the sake of brevity we present only (i) the roundtrips that brought about a significant build up of the path, (ii) the first full-circle roundtrip along the circumference, and (iii) the last roundtrip in the session. Note that 7 out of the 11 tested mice exhausted the circumference before commencing to the next, radial (two) dimension.

BALB/c mice; forced exploration; cliff (wall-less arena)

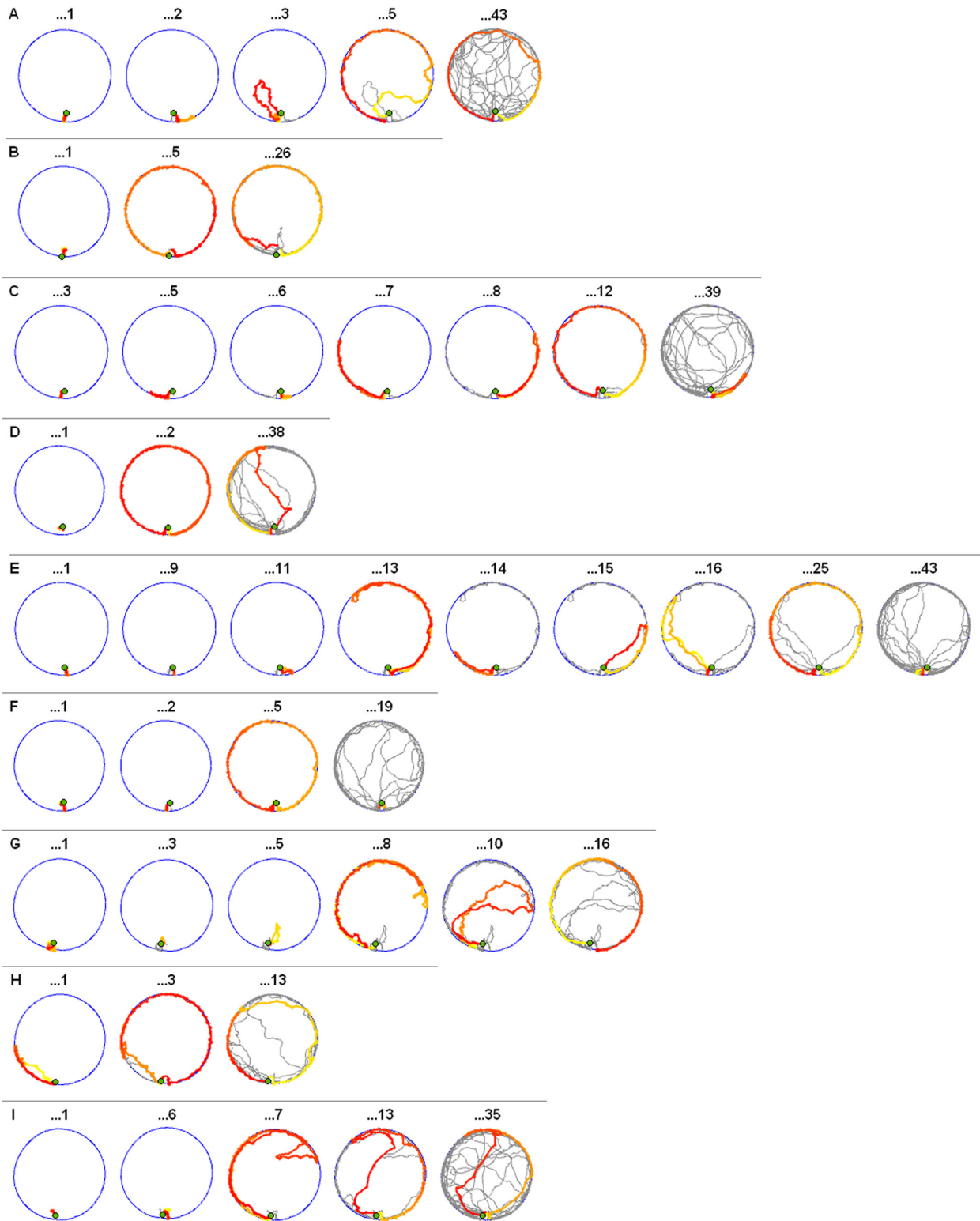
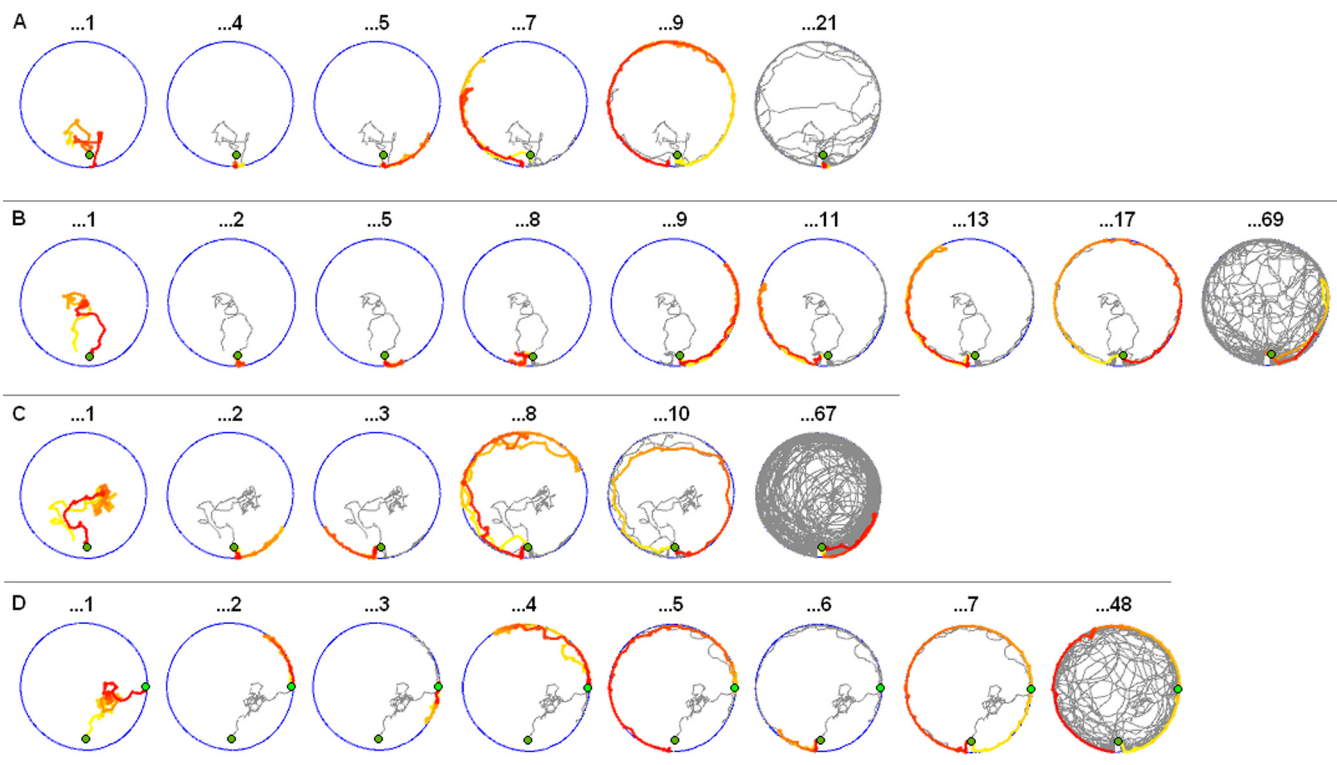


Fig. S6. Legend as in Fig. S4, except that the wall is replaced by a cliff. Note that the tendency to exhaust the borderline dimension before commencing into the radial dimension prevails also in this setup. Note that in this setup only four out of 11 mice exhausted the circumference before commencing to the next dimension. There is, nevertheless, also in this setup, a primacy of the borderline dimension.

The coercive effect at the beginning of forced exploration

BALB/c mice; forced exploration; walled arena



BALB/c mice; forced exploration; cliff (wall-less arena)

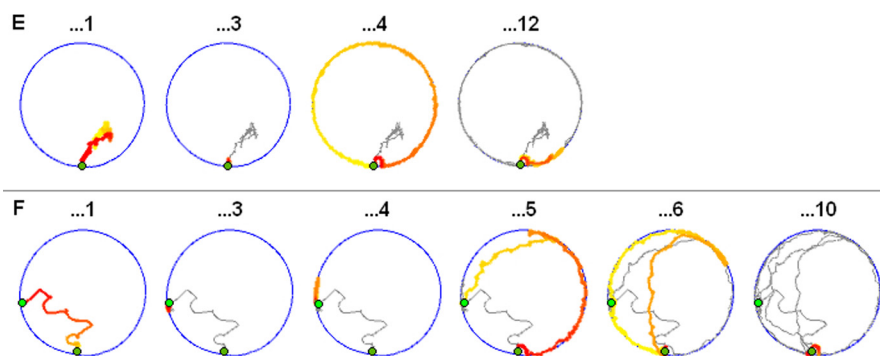


Fig. S7. Legend as in Fig. S4, except that these mouse sessions illustrate the distorting effect of coercive introduction of the mouse into the arena (mouse's point of introduction marked by a dark green dot). Upon its introduction near the wooden cube, the mouse flees away from the wall, slows down and even freezes, then moves around, usually returning back to the release point (mice A, B, C, E from top), but sometimes reaching the wall at a different location (denoted by light green dot; mice D, F) creating a false impression of advanced two-dimension movements. This transient disappears once the mouse hits the border; the border-contact location then becomes the origin of axes for the performance of increasingly larger Borderline-roundtrips.

