Identifying reliable traits across laboratory mouse exploration arenas: A meta-analysis.

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Abstract

This study is a meta-analysis of 367 mice from a collection of behaviour neuroscience and behaviour genetic studies run in the same lab in Zurich, Switzerland. We employed correlation-based statistics to confirm and quantify consistencies in behaviour across the testing environments. All 367 mice ran exactly the same behavioural arenas: the light/dark box, the null maze, the open field arena, an emergence task and finally an object exploration task. We analysed consistency of three movement types across those arenas (resting, scanning, progressing), and their relative preference for three zones of the arenas (home, transition, exploration). Results were that 5/6 measures showed strong individual-differences consistency across the tests. Mean inter-arena correlations for these five measures ranged from +.12 to +.53. Unrotated principal component factor analysis (UPCFA) and Cronbach's alpha measures showed these traits to be reliable and substantial (32-63% of variance across the five arenas). UPCFA loadings then indicate which tasks give the best information about these cross-task traits. One measure (that of time spent in "intermediate" zones) was not reliable across arenas. Conclusions centre on the use of individual differences research and behavioural batteries to revise understandings of what measures in one task predict for behaviour in others. Developing better behaviour measures also makes sound scientific and ethical sense.

1. Introduction

Although a field that could perhaps be best described as 'mouse psychometrics' has barely existed to date, the use of correlation-based research across individuals or strains has recently received much interest from mouse behaviourists (Galsworthy et al., 2002, 2005; Wahlsten et al., 2003; Locurto et al., 2003; Matzel et al., 2003, 2006). Not only does the psychometric approach clarify task reliability and measurement structure, but when applied across diverse measures, the methodology also allows the delineation of broad and specific traits. The classic way to quantify the influence of traits across a set of measures is factor analysis. Factor analytic approaches have been used in laboratory mouse behaviour analysis to examine the variable relationships within cognitive tasks (Lipp & Wolfer, 1998), between cognitive tasks (Galsworthy et al., 2002; Locurto et al., 2003; Matzel et al., 2003), within exploratory tasks (Rodgers & Johnson, 1995; Wall & Messier, 2000), and between different exploratory/ activity tasks (Mill et al., 2002).

Factors such as cognitive performance show low correlations across tasks, particularly so when the motivations and demands are diverse (Galsworthy et al., 2005). At the other end of the scale, the same measure taken repeatedly within the same arena may vary from very low to very high trial-to-trial correlations, depending on many factors (Mill et al., 2002; Galsworthy 2003). However, very few studies have explored the same essential measure taken across a variety of different arenas. This has been explored for escape latencies in water navigation tasks (Locurto & Scanlon, 1998), but has not, to our knowledge, been explored for the many standard exploration tasks that are commonly used in mouse behaviour testing. The presumption that certain traits such as "anxiety",

"activity" or "neophobia" can be shown by a variety of tasks needs to be qualified and quantified by studies that show that anxious or explorative behaviour on one task is associated with similar behaviour on another task (Lad et al., 2010).

This study explores the consistency in individual differences for same set of behavioural measures across five different exploratory arenas. The aim was to validate the movement type measures nominated by Drai and Golani (2001) and the zone divisions that are employed as standard in our lab to measure exploratory behaviour. With regard to the latter, it should be noted that the exploration of exposed areas is widely used as a measure of anxiety, although there is little data in the literature indicating that this behaviour in the light/dark box, null maze and open field do indeed represent the same underlying behavioural trait.

2. Methods

2.1. Animals

Of the >4,200 mice of inbred, hybrid, mutant and outbred genotypes which ran the exploration tasks available in the Zürich laboratory (data archive up to the year 2004), there were 1,966 individuals that ran more than one procedure. A total of 764 ran the open field, null maze and light-dark box, and 1,285 ran the emergence test and novel object test. There were 367 mice which ran all five exploration tests available in the lab, and these are the mice detailed here. The mice were run under eight independent studies, but as all mice were run within the same lab following identical procedure, these studies were compiled for this internal meta-analysis. The 367 mice (192 females and 175 males) were either heterozygous or homozygous for one of nine gene

manipulation types, or were wildtype controls, as shown in Figure 1. The genetic backgrounds for the groups varied, with all but 30 FVB inbred and knockout mice having some form of cross or mix with C57Bl/6 in their background. This group can be subdivided into hybrid backgrounds (F1s of C57Bl/6 with 129/SvEv or FVB/N, n=116) and outbred backgrounds (mixes of C57Bl/6 with 129/SvEv or CBA, or backcrosses of 129/Ola and FVB/N onto C57Bl/6, n=221). The preponderance of outbred backgrounds in this study, plus the variety of genetic manipulations makes the population ideal for individual differences investigation.

Insert Figure 1 near here

2.2. Housing and handling

All behavioural procedures were approved by Swiss animal welfare authorities. One week before the experimental period, animals were transferred to standard single mouse cages and maintained at a 12:12h inverted cycle with lights on between 20:00 and 08:00. Standard mouse chow, water and nesting material were available ad libitum.

2.3. Apparatuses and procedures

The mice were tested in sets of approximately 30 individuals between 08:00 and 20:00. Only one type of experiment was run on the same day. The home cage rack was brought to the test room at least 30 min before each experiment. Dry surfaces of

apparatus were thoroughly cleaned with 70% ethanol before releasing the animal. Behavioural analysis began at the age of 12 weeks and tests were run in the following order for all mice: Open field, null maze, dark/light box, emergence test, novel object test.

Open-field: The round open-field arena had a diameter of 150 cm, a white plastic floor, and a 35 cm high circular wall made of white polypropylene. Illumination was by indirect low-level room light (four 40W bulbs, 12 lux). Each subject was released near the wall and observed for 10 min. The same procedure was repeated the following day, resulting in a total observation time of 20 min (Wolfer, 2001).

Null maze: The maze (also called the "O-maze") is a ring-shaped runway constructed from grey plastic and elevated 40cm above the floor. The runway width is 5.5 cm and the total maze diameter is 46 cm. Two opposing 90° sectors were protected by 16 cm high inner and outer walls of opaque grey plastic (Shepherd, 1994; Konig, 1996). Animals were released in one of the protected sectors and observed for 10 min.

Light/dark box: The arena consists of 20 x 30 x 20 cm high transparent Perspex "light" box (500 lux direct room light) connected by a 7.5 x 7.5 cm aperture to a covered 20 x 15 x 20 cm high polyvinyl-chloride "dark" box. Each subject was released in the middle of the lit compartment and observed for 5 min (Crawley, 1980).

Emergence test: Procedure modified after Dulawa (1999). Frames of non-reflective aluminium 37 cm high were used to partition the above open field into four 50 x 50 cm square arenas, allowing for concurrent observation of 4 animals. Each arena had a

12 x 8 x 4 cm plastic home box with an aperture of 8 x 4 cm, positioned in a corner at 5 cm from both walls, with the aperture facing out. 24h prior to testing, a thoroughly cleaned home box was placed in the home cage of each test subject. The next day, test subjects and home boxes were introduced into the arenas and observed for 30 min.

Novel object test: Procedure modified after Dulawa (1999). Arenas were the same as for the emergence test, but without the home box. The novel object was a 12 x 4 cm semi-transparent 50 ml Falcon tube positioned vertically in the centre of the arena. Each subject was observed for 30 min in the empty, cleaned arena. Then, the novel object was introduced and observation continued for another 30 min.

2.4. Data recording

Animals were video-tracked at 4.2 Hz and 256x256 pixel spatial resolution using a Noldus EthoVision 1.96 system (Noldus Information Technology, Wageningen NL, www.noldus.com). For each sample, the system recorded xy position, object area and the status of defined event recorder keys on the keyboard. Rearing and grooming were recorded using the keyboard event-recorder provided by the video-tracking system. Exploratory head dips in the null maze were also recorded using the keyboard event-recorder provided by the video-tracking system. Raw data were then transferred to public domain software Wintrack 2.4 (www.dpwolfer.ch/wintrack) for organisation and the creation of higher-order variables (Wolfer, 2001).

For the classification of exploratory style, recorded tracks were segmented into three motion states according to criteria modified from Drai (2001):

(i) "Progressing" episodes corresponded to bouts of long-distance locomotion and were defined by a threshold for average velocity (8.5 cm/s in the open-field, emergence and novel object tests; 4.0 cm/s on the null maze and the light/dark box) and total distance moved (5 and 3 cm, respectively). Rapid decelerations (deeper than 15 and 8 cm/s, respectively) were subtracted and classified as scanning (see below).

(ii) "Resting" episodes were periods lasting 2 s or longer with smoothed speed values (averaging frame 0.5 s) below the system noise level of 2.5 cm/s. Resting episodes included periods of immobility as well as grooming which caused cursor movements at or near the system noise level.

(iii) "Scanning" episodes constituted the remaining time and were associated with exploratory behaviours such as brief stopping, sniffing, establishing snout contact with the substrate or an object, looking around, stretch attend postures, rearing or leaning against the wall. Because the tracking system also monitored apparent subject area, vertical activity could estimated by counting reductions of subject area deeper than 250 mm^2 while the animal was not progressing.

To assess approach-avoidance behaviours, recordings of the arena space were divided into three "zones" for each arena. These zones are shown in Figure 2 and described below:

(i) The "exploration" zone was defined as the most avoided and hence most aversive parts of the arena.

(ii) The "home" zone was defined as the most preferred part of the arena, usually where the animal was started.

(iii) The "intermediate" zone was defined as the remainder and usually constituted the transition area between the home and exploration zones.

Insert Figure 2 near here

The specific implementation of these three zones in each test was based on the retrospective quantification of preferences in a large number of subjects (1000-3500 mice depending on the paradigm). To allow comparison of zones irrespective of their size, an index of zone preference was calculated using the formula 100%*x(100-C)/ [x(100-C)+C(100-x)], where x = % time spent in the zone and C = % of arena surface occupied by the zone. According to this formula, an index value of 0 indicated complete avoidance of the zone and a value of 100 maximal preference. By this method a score of 50% would be obtained by a randomly moving animal for each zone, irrespective of zone size. With the *open field* arena, the exploration zone was a circular centre field comprising 50% of the arena surface and a 7 cm wide wall zone constituted the home zone. In the *light-dark box*, the home and intermediate zones were 10 cm wide and located next to the aperture of the dark compartment and at the opposite end, respectively. The remaining central segment was the least attractive area and thus defined as exploration zone. Zone geometry in the *null maze* was defined as follows: an intermediate zone comprising four 30° segments at the ends of the protection walls separated the two 50° wide home zones (= protected sectors) and the two 70° wide exploration zones (= unprotected sectors). With these boundaries, the system detected entries to the unprotected sectors only when the animal moved into it with all four paws. Head dips that occurred while the animal was registered to the

transition zone with all or part of its body between the protection walls were classified as protected dips, all others as unprotected dips. Within the visible area of the *emergence test*, the 40x40 cm centre field constituted the exploration zone and a 18x22 cm home zone surrounded the home box, including the arena corner located next to it. In the *novel object test*, a 5 cm wide corridor along the wall formed the home zone. The circular exploration zone of 18 cm diameter was located in the arena centre where the object was introduced, while the surrounding space was defined as intermediate zone.

2.5. Statistical analyses

All data for the five exploratory tasks were initially stored independently as Wintrack 2.4 files (www.dpwolfer.ch/wintrack). They were then moved via tab-delimited text files into Microsoft Access 97, where they were held as separate tables within the same database. An Access "query" table within the database then identified, by a common coding system, the 367 mice that had run all five exploratory tasks. StatTransfer (Circle Systems, Seattle, WA, USA) was then used to transfer this table of 367 mice (and tables representing each arena independently) into Microsoft XL or Stata 8.0 (Stata Corporation, TX, USA) for analysis. All analyses reported below are generated by Microsoft XL for simple descriptives or Stata 8.0 for correlation-based results.

3. Results

Table 1 shows the percentages of time spent engaged in the three movement behaviours in the five exploratory tests. Note that with the light/dark box and emergence test, only time in the illuminated areas was visible and recorded. Sample sizes range from 940 mice

to 3,717 mice depending on the task. Note that the results for the subset of 367 mice that ran all five tasks show very similar numbers, varying only ±2.0% from the full set for each task.

Insert Table 1 near here

Table 2 shows the correlation matrices for i) resting measures, ii) scanning measures and iii) progressing measures across the five arenas. Mean correlations are .40 between resting measures, .12 between scanning measures and .53 between progressing measures. Of the 30 inter-correlations reported in Table 2, only one was negative (but non-significantly negative, p= .20). Of the remaining 29 positive correlations, 23 were significantly so beyond the 5% level - of which 21 were also significant beyond the 1% level (two-tailed probability).

Insert Table 2 near here

Table 3 shows the correlation matrices for time spent in the i) home, ii) intermediate and iii) exploration zones in the five arenas. Mean cross-arena correlations are .23 for time spent in the home zones, .09 for time spent in intermediate areas and .32 for time spent in the exploration zones. Of the 30 inter-correlations reported in Table 3, five were negative,

and all of those were intermediate zone inter-correlations (one of those five negative values being significant). Of the remaining 25 positive correlations, 22 were significantly so beyond the 5% level - of which 21 were also significant beyond the 1% level (two-tailed probability).

Insert Table 3 near here

Table 4 shows first factor loadings from three different principal components factor analyses: resting measures across the test, scanning measures across the tests, and progressing measures across the tests. In all three analyses, loadings are all positive indicating that the first factor is a trait representing a similarity of measurement. The resting trait accounts for 53% of the variance in the resting measures. Similarly, first factors for scanning and progressive movement account for 32% and 63% of their measurement variables respectively. There also appears to be strong individual differences in exploratory style as measured by areas visited. Both home and exploration zones showed co-alignment of loadings in the first factors with 40% and 46% of variance (respectively) accounted for by those primary factors. However, the intermediate zone measure was not consistent across arenas. Two arenas (light/dark box and emergence task) showed negative loadings on the first factor. These two arenas showed the only strong positive loadings on the second factor. Rotation made very little difference to the pattern of loadings.

Insert Table 4 near here

Cronbach's alpha was also run for measures. Cronbach's alpha is a reliability coefficient based on mean inter-trial correlation and number of component trials. The range is from 0 to 1 where over .6 is generally regarded as representing a good ratio of information to error in the whole task. Values for progressing, scanning and resting were .77, .40 and .85 respectively. Values for time spent in the exploratory and home zone were .59 and .71 respectively. The analysis confirmed the UPCFA finding that the intermediate zone measures did not inform in the same direction. However, when the five arenas were forced to inform in the same direction for the time spent in the intermediate zone (as is possible in this version of Cronbach's alpha in Stata 8.0), then the Cr alpha value was .34.

4. Discussion

This paper explored the potential existence of stable individual differences in exploratory traits across differing arenas. Conclusions are that very consistent individual differences can be seen both in the movement type of the mouse when exploring a novel arena, and also in their willingness to venture from the safest parts of the arena. Specifically, the three movement types; resting, scanning, and progressing, were seen to be consistent over all five exploratory tests supporting the proposition by Drai and Golani (2001) that these

represent valid exploratory traits in the mouse. Similarly, mice were consistent in their bias to either home or exploration zones of each of the five arenas. Unrotated principal component factor analysis and Cronbach's alpha measures showed these traits to be reliable and substantial (32-63% of variance) across the five arenas. UPCFA loadings derived from the confirmatory analyses identified the tests that provided the best measures of these three traits. The null maze, open field and novel object were seen to provide the best measures of resting; the null maze and novel object provided the best measures of scanning behaviour, and all measures were seen to provide high information content about progressing movement. With regard to area explored, the light/dark box was seen to give less information about home versus exploratory style, with all other arenas being similar. This is quite possibly because the mice have the choice of spending much of their time in the untracked dark compartment.

There are implications in these findings not only for measures of activity and anxiety, but also for land-based (as opposed to water-based) cognitive tasks. The willingness to explore and engage in experimental activity will certainly be a factor involved in individual differences in learning. In fact, the object exploration task that correlates so well with other exploratory tasks here is also known to associate with cognitive performance in rats (Anderson, 1993) and mice (Galsworthy et al., 2005). In fact, even exploring the centre area of the open field arena has been shown to correlate with cognitive performance in mice (Matzel et al., 2003, 2006) and species differences in initial exploration also appear to associate with better learning (Galsworthy et al., 2005b). Conversely, cognitive aspects will almost certainly also feed back into exploratory behaviour, whether driving the curiosity as has been suggested (Galsworthy et al., 2005) or in other elements of exploratory behaviour such as habituation rate.

Either way, all tasks from the seemingly simple to the seemingly complex will evoke behaviour from individuals that is a complex mixture of baseline (un-elicited) activity, elicited activity, anxieties in response to a variety of stimuli, and associative learning and memory processes. Utilising an individual differences approach to study variance influencing batteries of tasks not only helps to find traits that are consistent across tasks, but also allows variance within one task to be decomposed into elements of behaviour – as shown by associations with other tasks. As such, the authors hope that the psychometric method as applied to laboratory mouse tasks will provide better understanding of the traits that guide mouse behaviour, and with it the development of cleaner tests of those traits of interest. This in turn has clear scientific and ethical value for studies of behaviour neuroscience and behaviour genetics as fewer test animals will be needed to return more reliable, stable and informative data.

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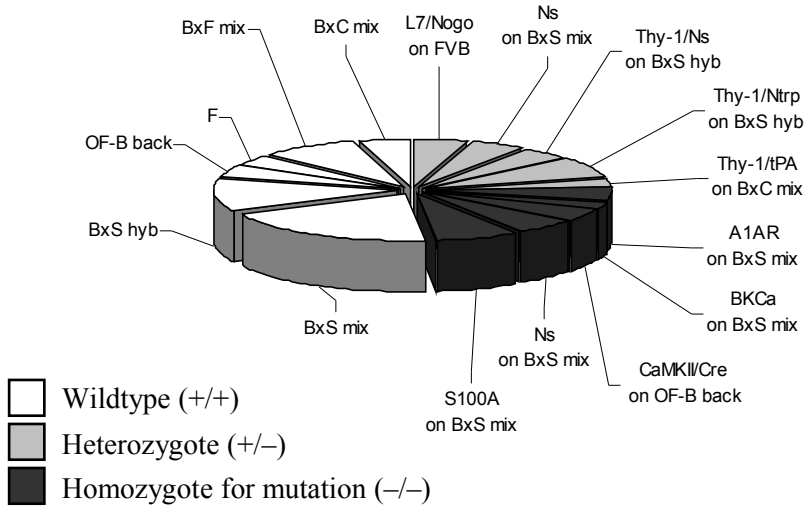
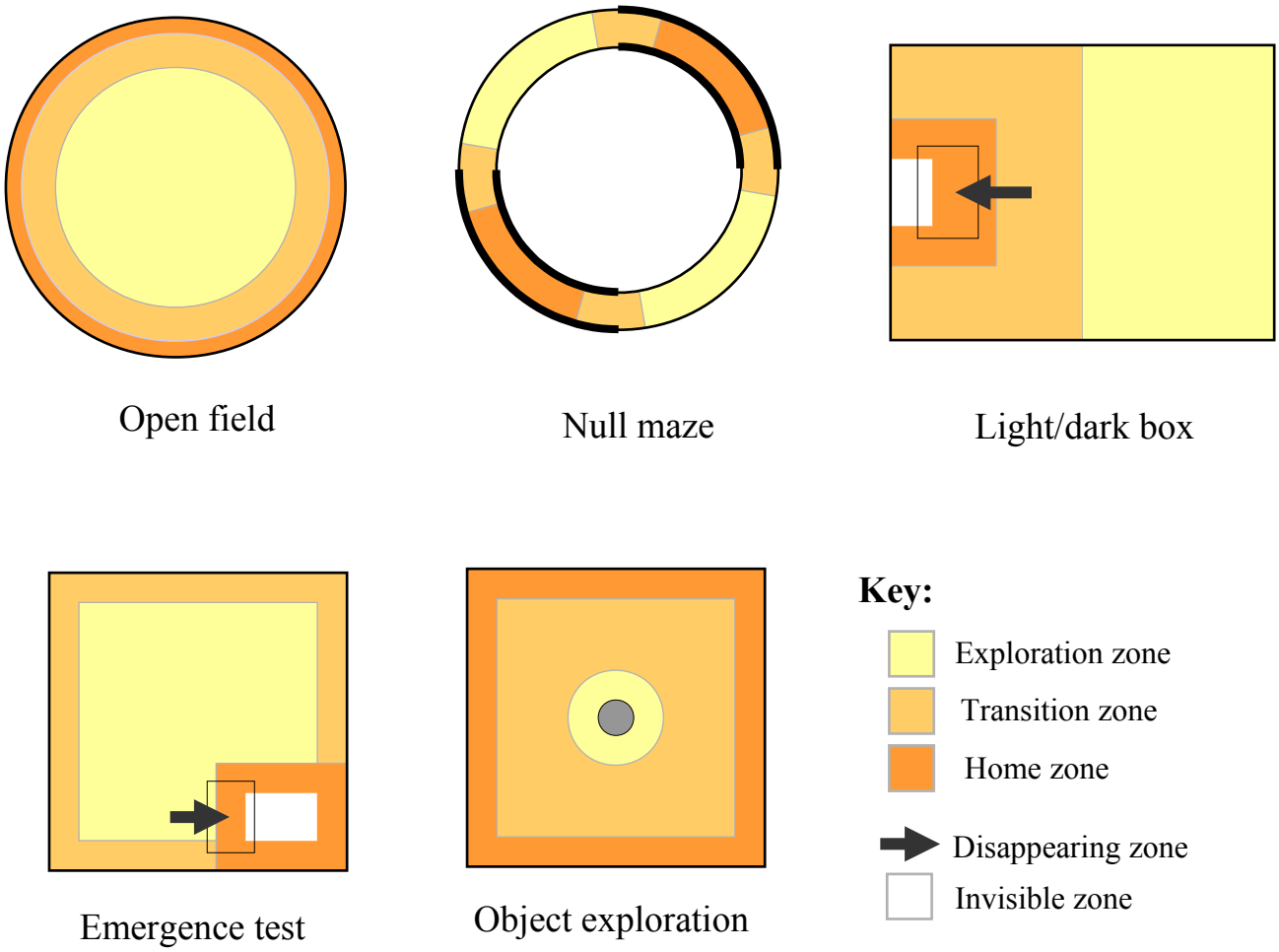
Figure 1. Genotypes for the 367 mice which ran all five exploration tasks.



The meta-analysis population is derived from multiple studies, mostly comparing mice homo- and heterozygote for mutations with their littermate controls. Codes for genetic backgrounds are B = C57Bl/6, C = CBA, F = FVB/N, S = 129/SvEv, O = 129/Ola, "mix" indicates mixture (F_2 or beyond), "hyb" indicates hybrid (F_1), "back" indicates backcross of the first two strains (hybrid?) onto the third. Genetic manipulations are: CaMKII/Cre;MRIx/Ix = postnatal forebrain specific conditional CREB KO, BKCa = Large-conductance Ca^{2+}-activated K^+ channel, A1Ar = Adenosine A1 receptor, Thy-1/tPA = transgenic tissue-type plasminogen activator, Thy-1/NTrp = neuro/neutrypsin truncated, Thy-1/Ns = overexpresses neuroserpin, Ns = neuroserpin constitutive knockout, L7/Nogo = expression of Nogo-A in Purkinje cells under control of L7 promoter.

Figure 2. Activity distribution in the five exploratory arenas and definition of zones.



The areas of the arena that can be tracked are divided into "home", "exploration" and "transition" zones, depending on the frequencies of visits to these areas and the natural progression of exploration within these arenas. "Invisible" zones occur where the mice enter a box or doorway to an untrackable area, with "disappearing" zones occurring around these entrances as the mice sometimes flicker in and out of size-based tracking here.

Table 1. Proportions of time associated with different motion types and areas explored for the five different exploration tests.

Arena	N	Exploration style			Area explored		
		Resting	Scanning	Progressing	Home	Intermediate	Exploration
Light/Dark	940	20% (19)	51% (52)	29% (28)	66% (69)	21% (18)	13% (12)
Null Maze	1,087	25% (27)	58% (57)	17% (16)	47% (49)	45% (44)	7% (7)
Open Field	3,717	14% (13)	44% (43)	43% (44)	64% (74)	23% (15)	13% (10)
Emergence	1,287	26% (27)	56% (56)	18% (18)	47% (50)	36% (32)	17% (18)
Novel Object	1,301	51% (53)	35% (34)	14% (13)	69% (66)	21% (23)	10% (11)

Values in brackets are the respective proportions of time for the subset of 367 mice that ran all 5 exploratory tasks.

Table 2. Correlation matrices for resting, scanning and progressing measures across arenas.

	Arena	Light/Dark	Null maze	Open Field	Emergence
i) Resting	Light/Dark box	-			
	Null Maze	.33**	-		
	Open Field	.39**	.71**	-	
	Emergence	.10	.26**	.37**	-
	Novel Object	.25**	.58**	.57**	.41**
ii) Scanning	Light/Dark box	-			
	Null Maze	.08	-		
	Open Field	.12*	.06	-	
	Emergence	.17*	.01	.04	-
	Novel Object	.04	.53**	-.07	.18**
iii) Progressing	Light/Dark box	-			
	Null Maze	.41**	-		
	Open Field	.52**	.64**	-	
	Emergence	.38**	.44**	.57**	-
	Novel Object	.45**	.50**	.59**	.77**

*p<.05, **p<.01, two-tailed.

Table 3. Correlation matrices for resting, scanning and progressing measures across arenas.

	Arena	Light/Dark	Null maze	Open Field	Emergence
i) Home	Light/Dark box	-			
	Null Maze	.02	-		
	Open Field	.10	.29**	-	
	Emergence	.18**	.25**	.28**	-
	Novel Object	.09	.33**	.52**	.25**
ii) Intermediate	Light/Dark box	-			
	Null Maze	-.15**	-		
	Open Field	-.06	.21**	-	
	Emergence	.19**	.10*	-.03	-
	Novel Object	-.01	.23**	.49**	-.03
iii) Exploration	Light/Dark box	-			
	Null Maze	.39**	-		
	Open Field	.26**	.32**	-	
	Emergence	.31**	.34**	.48**	-
	Novel Object	.14**	.33**	.37**	.30**

*p<.05, **p<.01, two-tailed.

Table 4. First factors from six separate principal component factor analyses.

First factors:	Exploration style			Area explored		
	Resting	Scanning	Progressing	Home	Intermediate	Exploration
Light/Dark	+.52	+.28	+.68*	+.27	–.23	+.59
Null Maze	+.84*	+.81*	+.76*	+.63*	+.58	+.70*
Open Field	+.87*	+.06	+.86*	+.77*	+.80*	+.74*
Emergence	+.50	+.35	+.82*	+.60*	–.04	+.73*
Novel Object	+.80*	+.85*	+.85*	+.77*	+.80*	+.62*
Eigenvalue	*2.7*	*1.6*	*3.1*	*2.0*	*1.7*	*2.3*
Prop^{n} of variance	*53%*	*32%*	*63%*	*40%*	*33%*	*46%*

First factors only with loadings, eigenvalues and proportions of variance are shown for the three exploration style variables and the three area explored variables, to confirm that traits are stable across arenas – as shown by purely positive loadings from all five arenas. * Factor loadings over ± .60.