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2 **Machine Learning Reveals Modules of Economic Behavior from Foraging Mice**

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26 **HIGHLIGHTS**

27 • Identification of 59 economic behavior modules underlying foraging

28 • Discrete modules are linked to memory, reward, risk and effort responses

29 • Genetic and parental effects shape foraging by changing module expression

30 • *Magel2,* a Prader-Willi Syndrome gene, affects specific modules at specific ages

31

32 **SUMMARY**

33 **Complex ethological behaviors could be constructed from modules that are**

34 **discrete functional units of behavior with a genetic basis. Here, we test this idea**

35 **for foraging, and develop a paradigm to dissect foraging patterns in mice. We**

36 **uncover discrete behavioral modules linked to round trip excursions from the**

37 **home. Machine learning reveals 59 modules across different genetic**

38 **backgrounds and ages. Different modules develop at different ages and are**

39 **linked to different aspects of economic behavior, including memory, reward, risk**

40 **and effort responses. Crosses of distant mouse strains reveal that parental and**

41 **genetic effects shape foraging differently, and parental effects grow stronger with**

42 **age. Specific behavioral modules, genes and pathways are found to be sensitive**

43 **to parental effects. One candidate gene, *Magel2*, is linked to Prader-Willi**

44 **Syndrome and shaped the expression of discrete modules in an age-dependent**

45 **manner. Our results reveal building blocks for normal and abnormal economic**

46 **behavior patterns.**

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49 **INTRODUCTION**

50 Most genomic elements influencing mammalian brain development and function shape

51 instinctive and learned behavior patterns in ways that are unknown (Bakken et al., 2016;

52 Kang et al., 2011; Miller et al., 2014; Silbereis et al., 2016; Thompson et al., 2014).

53 Behavior could be constructed from discrete "modules" that are independent units of

54 behavior (Vogelstein et al., 2014; Wiltschko et al., 2015; Yang and Shah, 2014). From

55 this perspective, specific genomic elements could have evolved to shape specific

56 behavioral modules, which in turn are building blocks for more complex patterns.

57 Changes to the genome, and brain structure and function, would thereby shape new

58 behavior patterns by either altering the expression of existing modules and/or by

59 creating novel modules. However, for most complex ethological behaviors, methods are

60 lacking to test this hypothesis. It is generally unclear how to define modules. We do not

61 know which behavioral patterns are comprised of modules, the identity of the modules

62 involved, how they are expressed or the mechanisms involved. Module expression

63 potentially encompasses frequency, timing and sequential order. There are three main

64 problems to solve. First, improved methods are needed to study complex, ethological

65 behavior patterns. Second, methods are needed that dissect apart these patterns to

66 identify underlying modules. Finally, approaches are needed to investigate how module

67 structure, module expression and behavior patterns change in response to targeted

68 genetic/environmental manipulations.

69

70 Here, we devise methods to investigate potential modular architecture in foraging,

71 a rich and deeply conserved repertoire of economic behavioral responses (Stephens et

72 al., 2007). Many neural systems are involved in foraging, including novelty-seeking,

73 anxiety, reward, perseveration, sensorimotor integration, hunger, satiety, attention,

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74   activity, navigation, as well as learning and memory (Stephens et al., 2007). Natural

75   selection shapes foraging patterns through these systems to minimize predation risk

76   and energy expenditure, while maximizing caloric intake (Brown and Laundré, 1999;

77   Pulliam and Charnov, 1977; Stephens et al., 2007). Understanding the basis of different

78   foraging patterns and tradeoffs is considered important for understanding mammalian

79   economic behaviors (Brown and Laundré, 1999; Pulliam and Charnov, 1977; Stephens

80   et al., 2007), which have roles in human obesity, addiction, fear, anxiety and psychiatric

81   disorders (Monterosso et al., 2012; Rowland et al., 2008; Sharp et al., 2012a).

82

83   Learning how complex foraging patterns are constructed at different ages could

84   also advance our understanding of the principles of behavioral development. Selective

85   pressures on foraging shaped mammalian life histories, including brain and behavioral

86   developmental processes (Barton, 2012; Jones, 2011; Melin et al., 2014; Reader and

87   Laland, 2002; Sol et al., 2005; Walker et al., 2006). Indeed, major mammalian

88   developmental milestones are linked to foraging, such as the elimination of suckling

89   during nursing and emergence of independent foraging after weaning (Galef, 1981;

90   Hewlett and Lamb, 2010).

91

92   Our study tests the hypothesis that foraging is comprised of discrete behavioral

93   modules differentially expressed at different ages and in response to genetic and/or

94   parental effects. We further test for the existence of distinct modules shaping memory,

95   reward, risk and effort responses. To achieve this, we introduce a rich foraging behavior

96   paradigm, machine-learning approaches to study foraging architecture in mice and an

97   approach to detect modular architecture from round trip foraging excursions from a

98   home base. Parental and genetic effects on foraging, module formation and module

99   expression are investigated at different ages using reciprocal F1 hybrid offspring

100 derived from crosses of distantly inbred subspecies of *Mus musculus*. The expression of

101 specific modules changes due to parental and genetic effects in an age-dependent

102 manner, and for parental effects, we perform genomics and functional studies to

103 uncover genes involved. Top candidates are genes linked to Prader-Willi Syndrome

104 (PWS) and autism (Cassidy et al., 2012; Schaaf et al., 2013). We perform functional

105 validation studies for one of these genes, *Magel2,* to test for age-dependent effects on

106 specific modules and gain deeper insights into how loss of *Magel2* function shapes

107 behavior. Overall, our study uncovers an atlas of 59 core modules underlying mouse

108 foraging patterns across different ages, helping to elucidate behavioral development,

109 the basis of normal and abnormal economic behavior patterns and how evolution

110 shapes new patterns.

111

112 **RESULTS**

113 **An Approach to Uncover Modular Architecture in Mouse Foraging**

114 Behavioral patterns encompass where, when and what an animal does, while

115 behavioral modules are the building blocks for *how* it constructs the pattern. To test for

116 foraging modules, we required a rich assay in which mice exhibit diverse, ethological

117 foraging patterns and methods to identify underlying behavioral modules. It is

118 challenging to recapitulate all ecological elements of foraging in the lab. One element

119 frequently overlooked is the home base (Eilam and Golani, 1989). For animals in the

120 wild, the home base is a refuge from predation, shelter from the environment, and a

121 safe haven for rest, mating, offspring rearing and food storage. Selective pressures

122   related to the home can shape behavior patterns (Eilam and Golani, 1989; Gorny et al.,

123   2002; Weber et al., 2013). Most lab tests miss this dimension of behavior and lift the

124   animal from the home cage into a testing apparatus (Wahlsten, 2010). Others monitor

125   home cage behaviors, but in the absence of access to an outside environment (Loos et

126   al., 2014). Therefore, existing assays are unsuitable for our objectives.

127

128   The central role of the home base in rodent and human ecology potentially

129   makes it a key anchor point to discover modular structure in complex foraging patterns.

130   In previous studies rodents performed variable types of round trip excursions when a

131   home base was available during an open field test (Eilam and Golani, 1989). We

132   hypothesized that a richer assay, designed to allow the expression of diverse and

133   complex foraging patterns, would reveal a deeper variety of home base excursion

134   subtypes. Our rationale was that subtypes of foraging excursions from the home base

135   would be discrete modules of economic behavior, involving different motivations,

136   functions and underlying mechanisms. The expression frequency, timing and sequence

137   of these modules would then shape more complex foraging patterns over time.

138   Alternatively, there may be near-infinite types of excursions composed of different

139   behavioral responses in a random order.

140

141   Based on this rationale, we designed a foraging paradigm that examines a rich

142   behavioral landscape, while permitting free access to a home base cage (Fig. 1A, see

143   Supplemental Information). The paradigm integrates concepts from behavioral ecology,

144   including sand and food patches containing seeds (Brown and Kotler, 1994; Stephens

145   et al., 2007). We constructed foraging arenas with 4 pots filled with sand, one of which

6

146   also contains seeds (the food patch) (Fig. 1A-C). For the home base, mouse cages are

147   fitted with a 3D printed tunnel that is "plugged" into a connecting tunnel to the foraging

148   arena (Fig. 1A). The experimenter starts a trial by removing a door in the tunnel,

149   allowing access to the arena. This reduces handling and promotes innate, ethological

150   behavior sequences. Behavior testing encompasses two phases: a novel Exploration

151   phase and a familiar Foraging phase (Fig. 1B-D), as described below. A protocol to print,

152   assemble and run the assay is provided (Supplemental Information).

153

154   During the Exploration phase, mice are given 25 minutes to spontaneously enter

155   the arena, explore and discover seeds placed on top of the sand in the food patch at

156   Pot#2 (Fig. 1B,C). During this phase, the mouse expresses behavioral sequences

157   related to the exploration of a novel environment and the discovery and consumption of

158   food in a novel food patch (Movie S1). Four hours later, the mice are again given 25

159   minutes of access to the arena in the Foraging Phase. However, the seeds are no

160   longer atop Pot#2 but are buried in the sand in Pot#4 (Fig. 1B,D). During this more

161   familiar and goal-directed phase, mice express behavioral sequences related to their

162   expectation of the former food patch in the now familiar environment and the discovery

163   of a new, hidden food source (Pot#4) (Movie S2). We food deprived the mice prior to

164   the assay such that the animals experience the same degree of body weight loss (~10%

165   of starting body weight), setting a common baseline for comparison across ages (see

166   Supplemental Information and Experimental Procedures). Food deprivation increases

167   learning and stereotyped patterns of behavior (Makowiecki et al., 2012) (Fig. 1B).

168

169   ***Resolving Foraging Patterns in Mice***

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170   To analyze behavior patterns, we defined features that describe where, when and what

171   an animal does during testing. The features systematically atomize foraging into the

172   spatiotemporal components that show where the animals go in the arena during the

173   Exploration and Foraging phases, quantifying their visits to different areas (Fig. 1-

174   S1A,B). Spatiotemporal features are further broken into 5 minute time bins that capture

175   patterns from five different early, mid and late stages temporal windows. Finally, we

176   measure features germane to foraging, which include total food consumption, digging

177   and distance traveled (Fig. 1-S1B). Locomotor features related to velocity, numbers of

178   excursions performed and gait patterns (slow, medium, fast) are also measured. In total,

179   foraging patterns are deconstructed into 338 features that are each assigned a unique

180   ID (Fig. 1-S1B and Table S1 and S2).

181

182   We tested whether this feature space captures diverse and complex (high

183   dimensional) foraging patterns in our paradigm. In the study detailed below, we collect

184   foraging profiles for 383 mice of different ages, genotypes and crosses. Principal

185   component analysis of the feature data from all 383 mice revealed high dimensionality

186   (Fig. 1-S1D,E). A parallel analysis found that twenty-four principal components are

187   retained to explain the variance in the data (Fig. 1-S1E). Therefore, features and assay

188   reveal complex behavioral patterns, as intended. Below, we build on these foundations

189   to apply supervised machine-learning and data visualization strategies to uncover

190   foraging patterns for different ages, genotypes and crosses.

191

192   ***Discovering the Modules Underlying Foraging Patterns***

193   Our final major methodological challenge was to devise an approach to uncover

194   modules that are discrete units of behavior underlying more complex foraging patterns.

195   As described above, we hypothesize that modules could be defined as home base

196   anchored excursions (Fig. 1E). To potentially identify these modules, we developed a

197   data analysis pipeline in R, called "DeepFeat", which takes XY tracking data from

198   commercially available tracking software as an input. DeepFeat detects discrete

199   modules from excursions relative to a user-defined anchor point (the home base) (Fig.

200   1E and Supplemental Information). For each excursion, DeepFeat extracts a vector of

201   statistical features describing XY movements over time. We refer to these as

202   "phenovectors". For all excursions performed by a cohort of mice, agglomerative

203   hierarchical clustering is performed on the phenovectors and a dynamic tree cutting

204   algorithm (Langfelder et al., 2008) is used to define discrete clusters of related

205   excursions in a dendrogram. A cut branch in the dendrogram thereby defines

206   excursions of a similar type, which collectively comprise a discrete module of behavior.

207   Individual excursions are assigned a module identification key that we call a Concise

208   Idiosyncratic Module Alignment Report (CIMAR) string. For each excursion, the module

209   assignment, underlying raw data and metadata (eg. mouse of origin, time of expression,

210   phase and other details), are stored in a list with CIMAR string keys, allowing simple

211   data retrieval, quantification and analysis of the expression of different modules.

212

213   To test whether particular phenovectors and clustering conditions identify bona

214   fide, biologically relevant modules, we use random forest machine learning to determine

215   whether mice in a cohort can be classified from the expression of the identified modules

216   according to a factor of interest (eg. age, genotype, cross, etc). Therefore, similar to

217 recursive methods for feature selection in the machine-learning field (Miao and Niu,

218 2016), our approach can be tuned to optimize module detection to maximize

219 classification accuracy (Fig. 1-S2). The generalization error of the classifier is

220 conservatively estimated using out-of-bag (OOB) error (Breiman, 1996). This process

221 detects bona fide, biological modules of behavior, as shown below. We now turn to the

222 application of all these methods to first study foraging patterns.

223

224 **Parental Effects in Mice Shape Foraging Patterns at Later Ages, While Genetic**

225 **Effects Are Robust from Weanling to Adulthood**

226 We hypothesized that parental and genetic effects shape different foraging patterns in

227 offspring. Independent foraging emerges at weaning, and we therefore profiled early

228 weaning (postnatal day (P) 15), weaning (P20), post-weaning (P25), and adult (N=45-60

229 mice per age) mice to investigate foraging pattern development, as well as parental and

230 genetic effects. Our design uses reciprocal crosses of *Mus musculus castaneous* (Cast)

231 and C57BL6/J (B6) mice, which are wild- versus lab-derived subspecies of mice

232 separated by ~500,000 years (Zheng et al., 2014). They are phenotypically distinct (see

233 Table S2). By comparing the F1cb (Cast mother x B6 father) and F1bc (B6 mother X

234 Cast father) hybrid offspring from these crosses, we test how parental effects influence

235 foraging (Fig. 2A). The parental effects, as previously described (Lacey, 1998),

236 potentially encompass *environmental parental effects* related to the in utero

237 environment or parental behaviors, and *genetic parental effects* involving changes to

238 imprinted genes, X-linked genes or mitochondrial genes that are preferentially inherited

239 from one parent. Genetic effects due to differences between Cast and B6 alleles are

240 uncovered by comparing F1bc offspring to B6 offspring, or F1cb offspring to Cast

10

241   offspring (Fig. 2A, N= 15-20). This allows us to enrich for genetic effects because the

242   mice share the same *maternal effects*, but are genetically different (Fig. 2A), though

243   possible paternal influences are not eliminated.

244

245   At P15, only ~20% of B6 offspring entered the arena from the home base, while

246   over 80% of F1cb, F1bc and Cast offspring entered during both phases of the assay

247   (Fig. 2-S1A,B). However, only ~20% of F1cb, F1bc and Cast P15 animals consumed

248   seeds in the assay. Thus, the full repertoire of foraging behavior in our assay has not

249   developed at P15, but early stage patterns are captured from Cast, F1cb and F1bc

250   offspring (Fig. 2-S1B). By P20, all mice of all crosses enter the arena, consume seeds

251   and display independent foraging.

252

253   We profiled foraging for 191 mice across the different ages (P15, P20, P25,

254   Adult), genetic backgrounds and crosses (B6, F1cb, F1bc and Cast), and captured

255   measures for the feature set introduced above (Fig. 1-S1, Table S3). Distinguishing

256   patterns shaped by parental or genetic effects at each age were revealed using

257   Random Forest machine learning to trained classifiers that detect mice according to

258   parental cross (F1cb vs F1bc) or genetic background (Cast vs F1cb; B6 vs F1bc). The

259   results revealed F1cb and F1bc mice are not significantly distinguishable at P15 or P20,

260   indicating parental effects at weaning are undetectable from our feature set (Fig. 2B,

261   Parental Effects). This observation is intriguing because weaning is the stage during

262   which the bond between mother and offspring changes and one might have predicted

263   the opposite result (Lee, 1996; Trivers, 1974). Instead, we found that significant parental

264   effects emerge *after* weaning. Both P25 and adult F1cb and F1bc offspring are

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265   differentiated greater than chance based on their foraging patterns (Fig. 2B). For

266   example, F1cb and F1bc adults are distinguished with 67% sensitivity and 88%

267   specificity (P=0.002, Fisher's Test of Confusion Matrix). The OOB error is 23%, showing

268   the classifier generalizes to a majority of cases (77%) (Fig. 2B).

269

270   While parental effects manifested at later ages, genetic effects robustly shaped

271   foraging patterns at all ages beginning from P15, the earliest age tested (Fig. 2B). Cast

272   patterns are distinguished from F1cb (and F1bc) patterns at P15 with 92% specificity

273   and 100% sensitivity (Fig. 2B, Genetic Effects). B6 mice do not forage at P15 and are

274   therefore different from F1bc (and F1cb) offspring due to genetic effects (Fig. 1-S2).

275   Differences persisted across older ages and B6 mice were distinguished from F1bc

276   (and F1cb) offspring with 100% specificity and sensitivity at P20, P25 and in adults (Fig.

277   2B). These data show that parental and genetic effects in Mus musculus have distinct

278   development stages of influence on offspring foraging patterns. The next challenges

279   include determining the nature of these patterns and whether they involve modules.

280

281   **Parental and Genetic Effects Shape Foraging Patterns Differently at Different**

282   **Ages**

283   The nature of the foraging patterns exhibited by the mice above can be extracted from

284   the random forest classifiers we trained to detect significant parental or genetic effects.

285   In one outcome, parental and/or genetic effects shape similar patterns across the

286   different significantly impacted ages. Alternatively, parental and/or genetic effects

287   manifest differently at different ages. To learn which occurs, we defined the informative

288   features for the distinguishing patterns identified by the random forest classifiers for

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289   parental or genetic effects at each age. Informative features were identified, and non-

290   informative features eliminated, using permuted importance analysis, which normalizes

291   random forest feature importance measures, corrects feature importance bias and

292   identifies significantly informative features in a classifier (Altmann et al., 2010). We

293   found that significant features characterizing parental effects on P25 patterns are

294   completely different from those characterizing effects on adult patterns (Fig. 2C,

295   Permutated Importance values, P<0.01). Thus, parental effects shape foraging patterns

296   differently at different ages.

297

298   The patterns of parental effects were visualized using radar plots of the data for

299   significantly informative features (Fig. 2D and E) (see Table S1 for feature definitions,

300   Table S3 for data). At P25, a top ranked feature is total visits to Pot#2 during the

301   Exploration phase (E.TVP2.0), which is greater in F1cb than F1bc offspring (Fig. 2D). In

302   contrast, a top feature distinguishing adult F1cb and F1bc foraging is the total visits to

303   the center zone during the third time bin of the foraging phase (F.TVC.3) (Fig. 2E). F1cb

304   mice perform this behavior more frequently, while F1bc mice spend more total time at

305   the food patch at Pot#4 during the same time period (F.TTP4.3) (Fig. 2E). The results

306   show parental effects shape complex, non-obvious patterns that differ according to age.

307

308   Intriguingly, genetic effects also shape foraging differently at different ages (Fig.

309   2-S2). A Venn diagram of the features with significant importance values in the P20,

310   P25 and adult classifiers for genetic effects shows that none of the features are

311   common to all ages (Fig. 2-S2A). Further, the majority of significant features are age-

312   specific (Fig. 2-S2A). Radar plots show the distinguishing patterns due to genetic effects

313   at each age (Fig. 2-S2B-D). For example, a feature distinguishing B6 from F1bc (and

314   F1cb) mice at P20 is the total time in sector 2 during the last time bin of the Exploration

315   phase (E.TTS2.5), which is highest in B6 (Fig. 2-S2B). A significant feature in adults,

316   but not other ages, is the peak velocity during the Exploration phase (E.PeakVelocity),

317   which is higher in F1bc/F1cb mice compared to B6 (Fig. 2-S2D). The basis of these

318   age-dependent patterns could involve changes to specific modules, which we

319   investigate next.

320

321   **Discovery of 59 Discrete Modules Underlying Foraging Across Different Ages,**

322   **Crosses and Genetic Backgrounds**

323   To test whether modules underlie foraging patterns, DeepFeat was applied to the XY

324   tracking data for the Cast, F1cb, F1bc and B6 mice in our cohort. We found 6,585 home

325   base excursions, and unsupervised hierarchical clustering and dynamic tree cutting

326   identified 59 distinct behavioral modules from these excursions (Fig. 3A). If the modules

327   detect meaningful behavioral sequences, they are expected to be sensitive to context

328   and age. We quantified the expression frequency of each module for each mouse in

329   each phase of the assay and display the results as a heatmap showing mean

330   expression frequencies according to phase and age (Fig. 3B). Both phase (context) and

331   age dependent differences are apparent from the module expression data and

332   clustering grouped sets of modules with similar patterns (Fig. 3B). Chi-Square tests of

333   independence confirmed that module expression frequencies depend significantly on

334   the phase (P= 2.3×10^{-85}), and on age (P=7.0×10^{-64}). We conclude that the modules are

335   bona fide, biological behavioral sequences. Support for this claim grows further below.

336

337   Our data indicate increased module expression frequency and diversity in the

338 Exploration compared to Foraging phase, revealing contextual effects on module

339 expression (Fig. 3B). One example of a module that is especially enriched in the

340 Exploration phase is Module 37, which involves a direct round trip from the home base

341 to the food patch (Pot#2) (Fig. 3B, C). We found two modules are expressed more

342 frequently in the Foraging phase, including Modules 6 and 1 (Fig. 3B,C). Module 6

343 involves a complex pattern with a visit to the new food patch in the Foraging phase

344 (Pot#4) followed by a looping survey of the former patch (Pot#2) (Fig. 3C). In contrast,

345 Module 1 involves a long, extended survey of the environment without an obvious

346 destination (Fig. 3C). Nine modules are expressed similarly in the Exploration and

347 Foraging phases, suggesting context independence in terms of expression frequency

348 (Modules: 30, 47, 0, 32, 10, 46, 49, 50 and 55) (Fig. 3B).

349

350   Developmental changes to the expression of specific modules are revealed in the

351 data. For example, Module 37 is predominantly expressed by adults (Fig. 3B,C), while

352 Module 34, which involves a sub-second dart into and out of the arena, is predominantly

353 expressed by P20 juveniles (Fig. 3B,D). All 59 modules are expressed by adults and

354 P25 juveniles at some frequency, but 14% and 5% of modules are not expressed by

355 P15 and P20 weanlings, respectively (expression frequency ≤ 1 across all mice) (Fig.

356 3E). Modules 50 and 55, for example, emerge after P20. Thus, our data reveal modules

357 underlying foraging, show changes to module expression in naïve versus more goal

358 orientated contexts and uncover a time course for the development of discrete modules.

359

360 **Genetic Effects in Mice Shape Different Foraging Patterns Through Changes to**

361 **the Expression of Shared Modules, Not the Creation of Novel Modules**

362 The genetic effects that shape different foraging patterns in Cast, B6 and F1bc/F1cb

363 mice (Fig. 2) could involve the creation of distinct modules in one strain versus another

364 and/or changes to the expression of a shared set of core modules. Here, we set out to

365 determine which occurs and uncover a basis for genetic differences in Mus musculus

366 foraging patterns. Our analysis of the DeepFeat output did not find any modules that are

367 solely expressed by one genetic strain, but not another (eg. B6, but not F1bc). This

368 indicates the genetic differences do not give rise to different modules, and the 59

369 modules we uncovered are shared across all strains. Instead, genetic differences in

370 foraging might involve changes to the expression of specific modules.

371

372 To test our prediction, random forest machine learning was used to train

373 classifiers to detect Cast, B6 and F1 hybrid (F1bc/F1bc) mice at each age based on

374 module expression data (Fig. 4A). We found that each genotype was detected at rates

375 significantly better than chance at each age, with an average of 35% sensitivity and 99-

376 100% specificity (Fig. 4A). The OOB error was low in most cases (≤10%) (Fig. 4A),

377 indicating highly generalizable models. From the classifiers, we uncovered significantly

378 informative modules from permuted importance values ($P<0.05$). The results reveal

379 discrete modules with distinguishing expression patterns for each genetic strain at each

380 age.

381

382 For example, adult F1 hybrids (F1bc and F1cb) are distinguished from all other

383 strains and ages by the expression of 9 modules (Fig. 4B). Module 10 is one example

16

384   and is most frequently expressed by F1cb and F1bc adults, though others also express

385   it (Fig. 4C,D). This module is relatively insensitive to context and equally expressed in

386   the Exploration and Foraging phases (Fig. 4C,D). B6 adults are distinguished from all

387   other strains and ages by differences in the expression of 5 modules. Module 7 is one

388   example and involves a brief entry and pause on the platform (Fig. 4E,F). It is most

389   frequently expressed by B6 adults and shows context dependent expression with

390   enrichment in the Exploration phase (Fig. 4E,F). Thus, genetic differences in mice

391   cause changes to the expression of specific modules underlying foraging patterns.

392

393   Finally, in our analysis we found that some modules appear visually similar,

394   raising the concern that they are not truly distinct module subtypes. We therefore

395   performed a more detailed analysis. For instance, Modules 7 (Fig. 4F), 34 (Fig. 3D), 54

396   and 30 (Fig. 4-S1A), are short, similar looking sequences expressed near the tunnel to

397   the home base cage. However, when we compared descriptive statistics for the

398   phenovectors of excursions classified under these four module types, differences

399   became apparent. Module 34, for example, is expressed more frequently during the

400   middle of the Foraging phase, while the others are expressed more frequently at the

401   beginning (Fig. 4-S1B). Modules 7, 30, 34 and 54 have differences in the kurtosis of the

402   XY movement patterns and/or different lengths (number of frames) (Fig. 4-S1C-E).

403   Modules 7 and 54 differ in terms of the average range of movement in the Y axis (Fig.

404   4-S1H,I). Further, all four modules have different expression patterns by phase, age and

405   genotype (Fig. 4-S1J-L). Module 7 is uniquely highly expressed during the Exploration

406   phase and by adults of the B6 genotype (Fig. 4-S1J-L). Module 34 is expressed

407   predominantly by F1cb and F1bc P20 juveniles in the Exploration phase (Fig. 4-S1J-L).

17

408   We conclude that these modules are in fact different, and that brief, ~1 second

409   behavioral sequences carry phenotypic information reflecting genotype, age and/or

410   context. Overall, these insights, and the observed genetic effects from distant

411   subspecies of mice, help reveal how evolution shapes foraging through modules.

412

413   **Different Modules Link to Memory, Reward, Risk and Effort Responses**

414   Different modules are expected to have different functions. If this is true, specific

415   modules should relate to specific keystone features of foraging that have clear

416   ecological functions. Food consumption is one keystone feature that is an ethologically

417   relevant measure of reward and motivation. Thus, finding modules significantly

418   associated with the total amount of seeds eaten in our assay (TFC.0, see Table S1)

419   could reveal putative modules for reward, feeding and food patch exploitation. A

420   keystone feature for risk-related behavior is thigmotaxis, which is an evolutionarily

421   conserved strategy for predator and risk avoidance that involves aversion to exposure

422   and increased proximity to walls (Prut and Belzung, 2003; Walz et al., 2016). Modules

423   significantly associated with the percentage of time spent in the exposed center zone of

424   the arena (PTC.0) are therefore putative "risk" behavior modules (Fig. 1-S1A and Table

425   S1). Two keystone features for effort and energy expenditure are the total amount of

426   sand dug from the pots (TSD.0) and the total distance traveled (TD, see Table S1),

427   potentially allowing the identification of putative foraging "effort" modules. Finally, our

428   assay includes reversal learning and perseveration, providing an enticing opportunity to

429   uncover modules for memory and food patch abandonment responses. The reversal

430   learning and perseveration involve the following.

431

18

432 During the Exploration phase, mice learn that Pot#2 harbors food (Movie S1) and

433 spend significantly more time at this Pot (Fig. 5-S1A). During the Foraging phase, they

434 learn to abandon this now absent food source and discover the hidden food in Pot#4

435 (Movie S2). However, mice spend time searching the former food source, especially

436 during early stages of the Foraging phase (Fig. 5A and 5-S1A) and excavate much of

437 the Pot#2 sand during their search (Movie S2). The amount of Pot#2 sand dug

438 (F.TSDP2) is therefore one keystone feature of the perseverative effects of the learned

439 memory.

440

441 To identify putative functionally distinct modules linked to reward (TFC.0), risk

442 (PTC.0), effort (TSD and TD) and memory (F.TSDP2) responses, we calculated the

443 mean values for each keystone feature across mice in the 12 different ages, genotypes

444 and crosses in our cohort, and tested whether specific modules are significantly

445 correlated to specific keystone features (Spearman rank correlation). For memory and

446 perseverative responses, we identified 15 modules that are significantly correlated to

447 the amount of sand dug from Pot#2 during the Foraging phase (Spearman rho, P<0.05).

448 For example, a plot contrasting the average Foraging phase sand dug from Pot#2 to the

449 average frequency of Module 31 reveals a significant positive correlation (Fig. 5B, rho =

450 +0.8, P = 0.004). Examination of the Module 31 structure during the Foraging phase

451 shows a visit to Pot#2 in the middle of excursion (Fig. 5C). However, other putative

452 memory response modules have different structures, as well as temporal patterns of

453 expression. For example, Module 14 is a relatively short excursion sequence performed

454 at the tunnel to the home base (Fig. 5D). While Module 31 is frequently expressed

455 during early and late stages of the Foraging phase (Fig. 5E), Module 14 is

19

456   predominantly expressed during middle stages (Fig. 5E). Another case, Module 18 (Fig.

457   5E), is highly expressed during the late stages of the phase (Fig. 5C). We conclude that

458   discrete modules are linked to memory and perseverative responses during foraging

459   and show they are expressed at different times, possibly reflecting changes to internal

460   state and adaptive processes for food patch abandonment.

461

462   Using a similar approach, we uncovered 8 modules significantly linked to food

463   consumption (reward) in the Exploration and/or Foraging phase contexts of the assay

464   (Fig. 5F). For example, Module 27 is highly correlated to food consumption (rho = +0.7,

465   P=0.009) and involves extended stops at Pot#4, the Foraging phase food patch (Fig. 5-

466   S1B). We found 35 modules linked to risk (Fig. 5F), including Module 2, which is a high

467   risk module involving many traverses of the exposed center and a relatively long 1-2

468   minute excursion from home (Fig. 5-S1C; rho = +0.9, P= 3×10^{-5}). Finally, 36 and 40

469   putative "effort" modules are significantly linked to the total sand dug from all Pots and

470   the total distance traveled, respectively (Fig. 5F). One example of a top module linked to

471   distance traveled is Module 19 (rho = +0.9, P=2×10^{-5}), which involves a long, looping ~1

472   minute excursion from the home (Fig. 5-S1D). Therefore, distinct modules are linked to

473   distinct ecologically important elements of foraging, suggestive of functional differences.

474

475   **Specific Behavioral Modules, Genes and Pathways are Sensitive to Parental**

476   **Effects in Offspring**

477   Our studies of foraging patterns revealed that parental effects on foraging increase with

478   age (Fig. 2). In particular, we observed the strongest phenotypic effects in adults, but no

479   effects in P15 and P20 weanlings. Here, we seek to test whether parental effects impact

480   the expression of specific modules in adult offspring and identify candidate genetic

481   mechanisms for shaping foraging during later stages of life. To define modules

482   impacted by parental effects in adult offspring, we trained a random forest classifier to

483   detect F1cb from F1bc adults from module expression data. Significant detection of

484   F1cb from F1bc adults (P=0.0005) was observed with 88% sensitivity, 72% specificity

485   and a 20% OOB generalization error (Fig. 6A). Permuted importance values for

486   modules in the classifier revealed five significantly informative modules (P<0.05)

487   (Modules: 1, 6, 21, 24 and 40). Bar plots of the expression frequencies for the

488   significant modules show how the F1cb versus F1bc adults differentially express these

489   modules during foraging (Fig. 6B). Having identified discrete modules impacted by

490   parental effects in adults, we set out to uncover mechanisms involved.

491

492   To identify candidate genes and pathways in the brain impacted by parental

493   effects, we tested for gene expression differences between F1cb and F1bc female

494   offspring during nursing (P5), weaning (P15) and in adulthood (Fig. 6C-E). Our study

495   focuses on the dorsal raphe nucleus (DRN) region of the midbrain, which contains the

496   largest serotonergic nucleus innervating the forebrain. This region exhibits

497   developmental changes that impact feeding and activity, is linked to mental disorders,

498   and modulates diverse systems involved in foraging, including reward, feeding, stress &

499   anxiety, exploratory drive, arousal and sensorimotor systems (Bromberg-Martin et al.,

500   2010; Jean et al., 2017; Lowry et al., 2008; Monti, 2010; Nectow et al., 2017; Ranade

501   and Mainen, 2009; Rood et al., 2014). We use RNASeq datasets we previously

502   published (Huang et al., 2017) and compare DRN region gene expression in female

503   F1cb to F1bc offspring for P5 nursing pups (N=7 for each cross), P15 weaning juveniles

504   (N=7 for each cross) and 8-week old adults (N=9 for each cross). Genes subject to

505   parental effects are significantly differentially expressed between F1cb versus F1bc

506   offspring at a 5% false discovery rate (FDR) threshold using edgeR (Robinson et al.,

507   2010) (Fig. 6C-F, Table S4).

508

509   At P5, 706 genes are significantly differentially expressed between F1cb and

510   F1bc offspring, indicating parental effects (Fig. 6C,F). By P15, the number drops to only

511   40 genes, which is a 18-fold decrease compared to P5 (Fig. 6D,F). In adults, however,

512   the number rebounds to 147 differentially expressed genes - a 4-fold increase relative to

513   P15 (Fig. 6E,F). Importantly, the reduction in parental effects on gene expression at P15,

514   but subsequent increase in adulthood, is concordant with the pattern of parental effects

515   we observed at the level of foraging behavior (see Fig. 2). The results further reveal that

516   the strongest parental effects on DRN gene expression manifest at P5, during nursing.

517

518   Genes differentially expressed between P5 F1cb and F1bc mice are enriched for

519   *myelin sheath*, *plasma membrane, axon guidance* and *synapse* gene categories (Fig.

520   6G, Table S5). Changes to myelination, axon guidance and synapse function could

521   shape offspring brain function and behavior patterns. No enrichments were observed

522   from P15 differentially expressed genes. For the adult, we found enrichments for the

523   KEGG *protein digestion and absorption* pathway, which is related to enrichments for ion

524   channels (eg. *Kcnq1,* a voltage gated potassium channel) and several collagen genes

525   (eg. *Col4a3,* collagen type IV alpha 3) (Fig. 6H). Our results reveal candidate genes and

526   pathways involved in the parental effects shaping offspring foraging.

527

22

528   Among the most statistically significant candidate genes, we found several genes

529   in the PWS – Angelman Syndrome (AS) locus, including *Ube3a, Magel2* and *Ndn* (Fig.

530   6I, Table S4). PWS patients have profoundly altered feeding, activity and motivated

531   behaviors, and show developmental changes to their phenotypes (Cassidy et al., 2012).

532   Mouse models also show altered motivation, anxiety, activity and feeding (Bervini and

533   Herzog, 2013). Thus, these genes are strong candidates for shaping modules of

534   economic behavior for foraging. By focusing on one of these genes, *Magel2,* we tested

535   for age-dependent effects on foraging patterns and module expression.

536

537   **Loss of the PWS-AS Gene, *Magel2,* Shapes Adult Foraging Patterns by Modifying**

538   **the Expression of Specific Behavioral Modules**

539   *Magel2* influences circadian rhythm, feeding, anxiety, arousal and serotonin signaling in

540   mice (Kozlov et al., 2007; Mercer et al., 2009). Roles in shaping more complex,

541   ethological foraging patterns have not been examined. We bred published *Magel2*

542   mutant mice to generate *Magel2$^{+/-}$, Magel2$^{-/+}$* and *Magel2$^{+/+}$* female offspring. Since

543   *Magel2* is imprinted and expressed from the paternal allele, *Magel2$^{+/-}$* offspring are

544   mutants compared to the other genotypes. Based on our results above, *Magel2* is a

545   candidate mechanism for shaping foraging at later ages, after weaning. To test this, we

546   compared foraging patterns between *Magel2$^{+/-}$* mutants and controls at weaning (P20)

547   and again in adults (9-12 weeks of age) (Table S6).

548

549   Random forest machine learning on the data captured for foraging features did

550   not uncover a significantly distinguishing pattern in the *Magel2$^{+/-}$* mutants at P20 (Fig.

551   7A, P=0.55). However, in adults, *Magel2$^{+/-}$* mutants were identified with 86% accuracy,

23

552   which is significantly better than chance (Fig. 7A, P=9.4x10^{-7}). Therefore, loss of *Magel2*

553   more strongly impacts adult compared to P20 foraging patterns, supporting our

554   hypothesis. The informative features in the classifier detecting *Magel2$^{+/-}$* mutants from

555   controls included 10 Foraging phase features and 2 Exploration phase features (Fig.

556   7B). The top ranked feature is the total time in the arena during the Foraging phase

557   (F.TTA.0, Table S1 and S6) and radar plots of the data show that *Magel2$^{+/-}$* mutants

558   spend less time in the arena during the foraging phase and more time in the tunnel

559   entry (F.TTE.2) and tunnel zone (F.TTTZ.0), and perform more total visits to the tunnel

560   during the first time bin (F.TVT.1) (Fig. 7B). Our next goal was to determine whether this

561   distinguishing pattern of behavior involved changes to specific behavioral modules,

562   either by giving rise to new mutant modules or changing the expression of core modules

563   shared across mutants and controls.

564

565   To identify modules, DeepFeat was applied to analyze the 5,302 home base

566   excursions detected from the P20 and adult *Magel2* mutant and control mice. This initial

567   analysis identified 42 modules. However, random forest classification based on the

568   expression *frequency* of these modules was unable to distinguish adult mutants from

569   controls better than chance (P>0.05, Fisher's test of the confusion matrix). We therefore

570   concluded that loss of *Magel2* does not create new modules in mutants compared to the

571   controls, and changes to the expression frequency of modules is not a significant

572   component of the mutant phenotype. Alternatively, changes to the *contextual pattern* of

573   module expression might be involved.

574

24

575 In a revised analysis, we integrated excursion start time and phase information

576 into the phenovectors captured by DeepFeat. With these new elements added to the XY

577 movements, DeepFeat now identified 75 different modules from the 5,302 home base

578 excursions (Fig. 7-S1A, Table S6). These modules yielded statistically significant

579 detection of the adult mutant mice (Fig. 7-S1B). Nine modules were identified as

580 significantly informative in the adult mutant classifier (Importance permutation P<0.05)

581 (Fig. 7-S1C). Magel2-Module_42 is the top ranked module and involves an extended

582 visit near Pot#2 (Fig. 7C). This module is expressed relatively equally in Exploration and

583 Foraging phases by the controls. However, adult mutants exhibit increased expression

584 during the Exploration phase (Fig. 7D), consistent with a context-dependent shift.

585

586 Magel2-Module_21 is another significantly informative module for the mutant

587 phenotype, and involves prolonged lingering near the tunnel to the home base (Fig. 7E).

588 In controls, this module is preferentially expressed in the Exploration phase (Fig. 7F).

589 However, adult mutants exhibit increased expression during the Foraging phase (Fig.

590 7F). The altered contextual expression of this module is consistent with many aspects of

591 the mutant's foraging pattern, including increased time in the tunnel zone during the

592 Foraging phase (F.TTTZ) (Fig. 7B). Temporally, Magel2-Modules 42 and 21 have

593 distinct expression patterns, indicating roles at different stages of foraging behavior (Fig.

594 7G). Overall, the basis of the phenotype in adult *Magel2* mutants is revealed to involve

595 context- and age- dependent changes to the expression of nine discrete modules.

596

597 Finally, we sought to test whether the modules impacted by loss of *Magel2* are

598 related to the modules impacted by parental effects in adult mice (Fig. 6B). To compare

599   modules across different cohorts of mice, we developed a module matching strategy

600   based on ideas for comparing gene networks (Langfelder and Horvath, 2007;

601   Langfelder et al., 2011). From the phenovector data for each of the 59 reference

602   modules identified in Cast, B6, F1cb and F1bc mice, we computed the first principal

603   component for each module. This eigenvector reflects the major phenotypic

604   characteristics of a module, which we call the eigenphenotype. We then computed the

605   eigenphenotypes for the nine modules informative for the adult *Magel2* mutant

606   phenotype after removing the start time and phase information, so that the underlying

607   data matches the reference modules. The Magel2-Modules were matched to the

608   reference modules using a Pearson correlation of the eigenphenotypes (Fig. 7-S2). The

609   results show the top significant module matches, revealing how the *Magel2* modules

610   map to specific reference modules (Fig. 7H). Importantly, we found that Magel2-

611   Modules 36 and 51 match to reference modules 24 and 21 (Fig. 7H), respectively,

612   which are two of the five modules impacted by parental effects in adult F1cb versus

613   F1bc mice (Fig. 6B). Reference module 21 is a significant "risk" module (E.PTC:

614   P=0.005, F.PTC: P=0.01) and also linked to effort in the Foraging phase (F.TSD:

615   P=0.002) (see Fig. 5F). The results validate *Magel2* as a mechanism shaping the

616   expression of specific modules during later life, and establish a framework for

617   phenotypic comparisons across studies.

618

619   **DISCUSSION**

620   We set out to learn whether foraging patterns are built from discrete modules, and

621   determine whether changes to modules underlie how genetic, parental, age and context

622   dependent effects shape different behavior patterns. By coupling a rich foraging

623   behavior paradigm with custom analysis methods, we found an atlas of 59 core

624   modules of economic behavior. Each module reflects a type of behavioral sequence

625   performed during round trips from the home. Distinct modules are linked to reward, risk,

626   effort and memory responses during foraging, indicating putative functional subclasses

627   and relationships to keystone features of foraging patterns. Mice were found to express

628   different modules in different temporal sequences that depended on context. The

629   modular architecture of foraging increases in complexity over development and we

630   defined ages when specific modules emerge. Intriguingly, parental and genetic effects

631   in mice are revealed to shape foraging in a highly age-dependent manner by changing

632   the expression of core modules, rather than creating new modules. Parental effects

633   impact foraging increasingly as mice age and we identify candidate genes and

634   pathways involved. Validation of one gene, *Magel2,* confirmed increased phenotypic

635   effects in adults compared to juveniles. Further, loss of *Magel2* function, which causes

636   PWS and autism in humans, alters foraging by changing the contextual expression of

637   nine modules in adults. A subset of these modules matched to the modules sensitive to

638   parental effects, revealing a putative mechanistic link through *Magel2*. Our results help

639   elucidate the basis of normal and abnormal behaviors, and how evolution shapes new

640   adaptive patterns.

641

642   **Towards Understanding How the Genome Shapes Complex Economic Behavior**

643   **Patterns**

644   Most neuroscience studies use controlled tests aimed at specific behavioral responses,

645   rather than studying rich behavior patterns. As a consequence, the principles and

646   mechanisms involved in the construction of complex patterns are ripe for discovery. The

647   importance of deeply analyzing relatively spontaneous behavioral sequences in rodents

648   was emphasized decades ago (Kavanau, 1967). More recently, sub-second patterns of

649   body language were uncovered using machine learning in mice (Wiltschko et al., 2015),

650   revealing an important new dimension of behavior. Here, we also identified important

651   phenotypic information from short behavioral modules (1-2 seconds or less), supporting

652   the rationale for further studies in this area. Invertebrate model organisms have shown

653   how computational dissections of behavioral sequences can ultimately help resolve

654   links between specific mechanisms and behavior patterns (Robie et al., 2017;

655   Vogelstein et al., 2014), and advances are being made in mammals (Hong et al., 2015;

656   Kabra et al., 2012; Markowitz et al., 2018; Weissbrod et al., 2013). However, the study

657   of modularity in free patterns of complex ethological behaviors presents many

658   challenges in terms of how to design the assay, select features for analysis and analyze

659   the data to uncover biologically important patterns and modules. Our study makes

660   advances on these fronts for foraging and related economic behaviors.

661

662   We found that modularity in foraging emerges from behavioral sequences

663   anchored by the home base. The role of the home base in behavior is understudied,

664   with the exception of a few pioneering efforts (Eilam and Golani, 1989; Gorny et al.,

665   2002). However, the centrality of the home in the ecology of many species, including

666   humans, is undeniable. Humans spend 69% of their life in their home (Klepeis et al.,

667   2001) and homelessness is a major factor associated with poverty, mental illness and

668   addiction, yet we know little about the role of the home in structuring adult and

669   developing behavior patterns. For many species in the wild, behavioral responses are

670   structured within a defined home range (Börger et al., 2008). Neural mechanisms for

28

671 navigating and dead reckoning relative to a landmark, such as the home, have been

672 identified (Wallace et al., 2008). Thus, the modular structure of foraging that we

673 uncovered is likely to have been selected for, having a genetic basis and analogs

674 across different species. In support of this, we show that genetic differences between

675 distant strains of *Mus musculus* shape different behavior patterns by changing the

676 expression of a set of shared modules, rather than creating novel modules. In future

677 work new modules might be discovered relative to landmarks other than the home. In

678 larger environments, rodents are known to identify landmarks for navigation (Wallace et

679 al., 2008), which might serve as anchors for the expression of some modules during

680 foraging in the wild.

681

682   Our data indicate that complex economic patterns are comprised of discrete

683 modules. Obesity, addiction, depression and anxiety disorders and others can be

684 framed in terms of biological effects on economic patterns (Camerer, 2013; Hartley and

685 Phelps, 2012; Monterosso et al., 2012; Rowland et al., 2008; Sharp et al., 2012b). We

686 found multiple modules can link to a single primary outcome, like food consumption,

687 raising the question of what functions these different modules serve and what internal

688 states drive the expression of one module over another. Further, economic decisions

689 during foraging involve tradeoffs (Stephens et al., 2007) and future studies could

690 determine which modules have antagonistic relationships with each other and shape

691 different tradeoffs. Defining the mechanisms involved in the form and expression of

692 different modules could help uncover strategies to normalize maladaptive behavior

693 patterns caused by genetic, epigenetic or environmental factors. Another goal involves

694 developing "Ontological Categories" of behavioral modules based on their functions and

695   analogs across species. Our study lays groundwork for this by developing

696   eigenphenotypes as one approach for comparing modules across studies.

697

698   **Modular Changes to Behavior During Development**

699   Much remains to be understood about how complex behavior patterns develop and

700   change with age. This is an important area since most forms of mental illness involve

701   early-life antecedents (Rutter et al., 2006) and arise at characteristic ages (Silbereis et

702   al., 2016), and economic behavior patterns are necessarily linked to physiological and

703   metabolic changes during development. Our data suggest that behavioral development

704   for foraging involves creating and changing the expression of discrete modules.

705   Currently, we do not know the functions of the modules or the mechanisms that underlie

706   their development or expression at different ages. The genomics field has described

707   developmental gene expression changes in the human, non-human primate and rodent

708   brain (Bakken et al., 2016; Kang et al., 2011; Miller et al., 2014; Silbereis et al., 2016;

709   Thompson et al., 2014), however, less is known about how specific mechanisms map

710   on to specific behavioral changes. The majority of the cellular gene expression

711   programs in the cortex of rodents and humans are highly conserved (Zeng et al., 2012),

712   as are the trajectories of most neurodevelopmental gene expression programs in the

713   developing brain (Bakken et al., 2016), and the temporal sequence of

714   neurodevelopmental processes (Workman et al., 2013). Thus, the stereotyped trajectory

715   of module development that we uncovered in mice could be rooted in conserved

716   neurodevelopmental programs. Further, the expression of specific modules may help

717   stage and study the progression of behavioral development in mouse models.

718

30

719   We found that parental effects on offspring foraging patterns unexpectedly

720   emerge most strongly at ages after weaning. From an evolutionary perspective, parental

721   effects are typically thought to be adaptive for parental fitness rather than offspring

722   fitness (Haig, 2000; J Marshall and Uller, 2007) and the functions of parental effects on

723   offspring behaviors after weaning are unclear. However, other adult behaviors have also

724   been shown to be shaped by parental effects, including maternal behavior (Francis et

725   al., 1999; Lefebvre et al., 1998; Li et al., 1999), circadian rhythm, feeding, reward,

726   learning & memory and anxiety (Curley and Champagne, 2016; Perez et al., 2016). We

727   speculate that parental effects on adult female foraging could function to influence

728   foraging patterns in daughters during offspring rearing, contributing to adaptive,

729   transgenerationally inherited economic patterns of behavior. Our study reveals

730   candidate genes and pathways for further analysis.

731

732   Finally, by analyzing *Magel2* mutant mice we uncovered a genetic mechanism

733   involved in regulating the expression of foraging modules and found that genes linked to

734   human neuropsychiatric disorders can impact the expression of specific modules at

735   specific ages. In humans, *MAGEL2* mutations cause PWS and autism (Schaaf et al.,

736   2013), which involve major phenotypic changes across development (Cassidy et al.,

737   2012; Lord et al., 2015). Further studies of *Magel2* mutants at other ages with our

738   approach may help reveal the modular basis of behavioral changes due to mutations in

739   this gene, and the mechanisms involved.

740

741   **STAR METHODS**

742   **Animals**

743   Animal experiments were performed in accordance with protocols approved by the

744   University of Utah Institutional Animal Care and Use Committee (#14-12012). C57BL/6J

745   (B6) and CastEiJ mice were obtained from Jackson Laboratory. Mice were maintained

746   on reverse 12 h light/dark cycle (lights off at 11:00) for foraging behavior experiments,

747   and given water and food ad libitum (Harlan Tecklad rodent diet 2920X; Madison, WI.).

748   All studies were performed on females. Cage bedding was Paperchip bedding

749   (Shepherd Specialty Paper) and animals were housed with sex-matched littermates (2-5

750   mice per cage).

751

752   **Mouse breeding**

753   Breeding pairs were housed together continuously for breeding. Maximum litter number

754   was capped at 6 for each breeding pair and large litters were culled to a maximum of 6

755   pups. For P15 behavior testing, offspring were returned to the parental cage after

756   testing and weaned at P21. For P20 behavior testing, mice were weaned after testing

757   was completed. For P25 behavior testing, mice had been weaned at P21.

758

759   **Mouse Behavior Experiments**

760   All B6, Cast, F1cb and F1bc mice were only tested once in the foraging assay and

761   therefore mice at all ages were naïve to the test. Adult mice for all groups were at ~ 3

762   months of age when tested. *Magel2* mice were tested twice in the foraging assay, once

763   at P20 and again as adults. For B6 females: N=20 adult, N=16 P25, N=15 P20 and

764   N=16 P15 mice; For F1cb: N=17 adult, N=15 P25, N=15 P20 and N=15 P15 mice; For

765   F1bc: 18 adult, 15 P25, 18 P20 and 15 P15; For Cast: N=15 P15 mice. For *Magel2* at

766   P20, N=22 maternal wt (*Magel2*$^{m+/p+}$), N=28 maternal het (*Magel2*$^{m-/p+}$), N=22 paternal

767   wt (*Magel2*[m+/p+']) and N=22 paternal het (*Magel2*[m+/p-]). For *Magel2* adults, N=18

768   maternal wt, N=23 maternal het, N=20 paternal wt and N=20 paternal het. Since each

769   litter yielded ~ 3 females, each group involved testing pups derived from 5-9 different

770   litters and at least five different breeding pairs. A detailed protocol for the behavior

771   analysis is provided in the Supplemental Information.

772

773   **RNA Isolation and RNA-Seq**

774   The DRN dissections include portions of the ventral periaqueductal gray and the RNA

775   isolation, RNA-Seq and imprinting studies were performed as previously described

776   (Bonthuis et al., 2015; Huang et al., 2017). In brief, RNA was extracted and DNase

777   treated using the RNAeasy Micro Kit (QIAGEN). RNA was pooled from ~6 daughters

778   from different litters to provide 3 µg of total RNA for each biological replicate. Samples

779   were prepared for RNA-seq using the TruSeq RNA sample preparation kit v2 (RS-122-

780   2001, Illumina). Single-end 59-bp sequencing of the libraries was performed using the

781   Illumina HiSeq 2500.

782

783   **Statistical Analysis of Behavior**

784   Statistical testing was performed in R and the code and sample datasets are provided in

785   the detailed protocol file in the Supplemental Information.

786

787   **Statistical Analysis of RNASeq Data**

788   Statistical testing of gene expression differences in the RNASeq data was performed

789   using the edgeR package (Robinson et al., 2010).

790

791   **SUPPLEMENTAL INFORMATION**

792   Supplemental Information includes 2 supplementary movies, 8 supplementary figures

793   and 6 tables, supplemental data and a detailed protocol for the foraging assay with code

794   and 3D printing files.

795

796   **AUTHOR CONTRIBUTIONS**

797   Conceptualization: C.N.S.H and C.G., Methodology: C.N.S.H., E.W., P.T.F. and C.G.;

798   Investigation: C.N.S.H and A.N.R; Software: C.G., C.N.S.H., E.W.; Formal Analysis:

799   C.N.S.H, E.W., P.T.F., E.F., C.G.; Visualization: C.N.S.H., E.W. and C.G.; Writing:

800   C.N.S.H., E.F. and C.G.; Supervision: P.T.F. and C.G.; Funding: C.G.

801

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811

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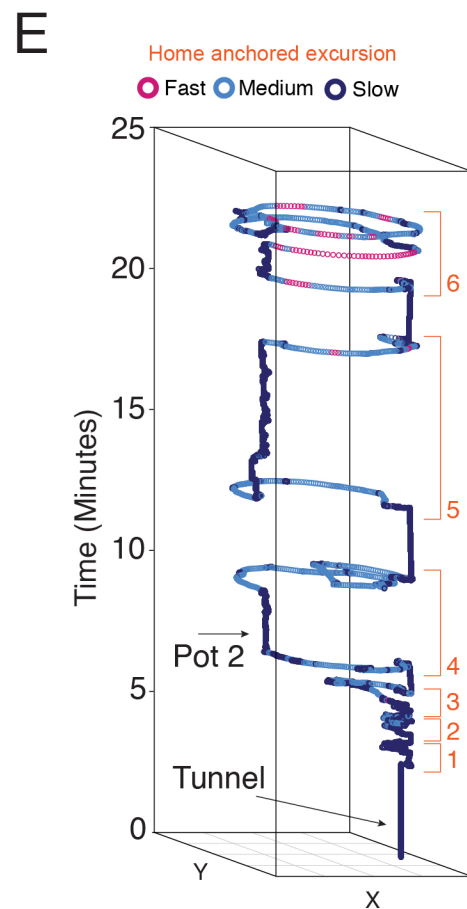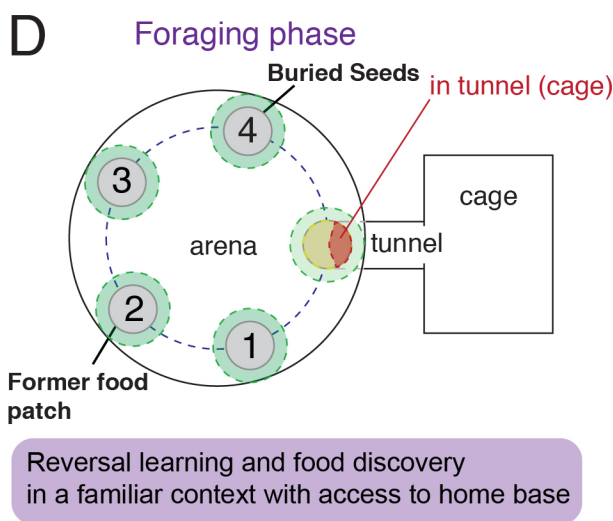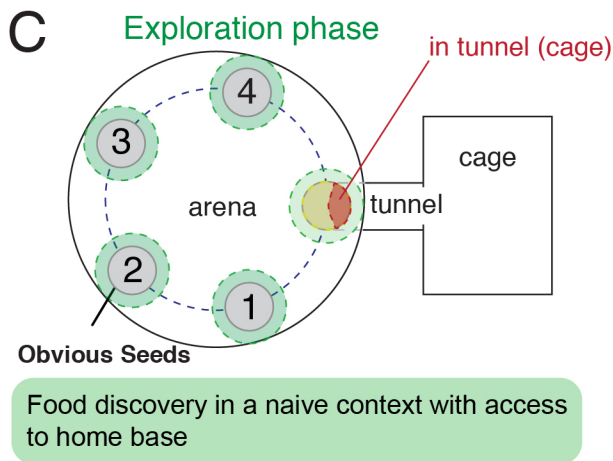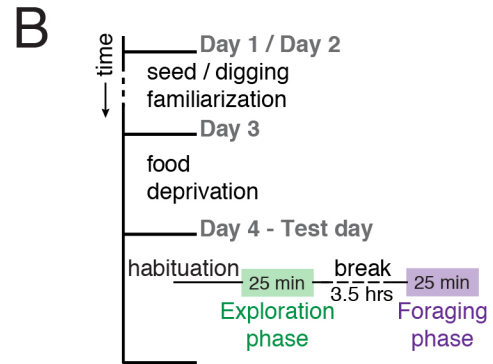864   Galef, B.G., Jr, 1981. The ecology of weaning. Parental Care in Mammals
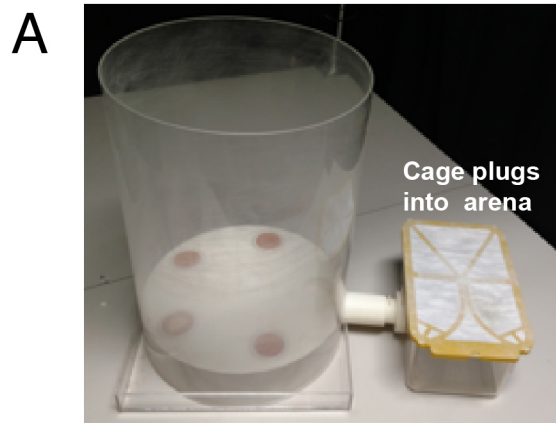
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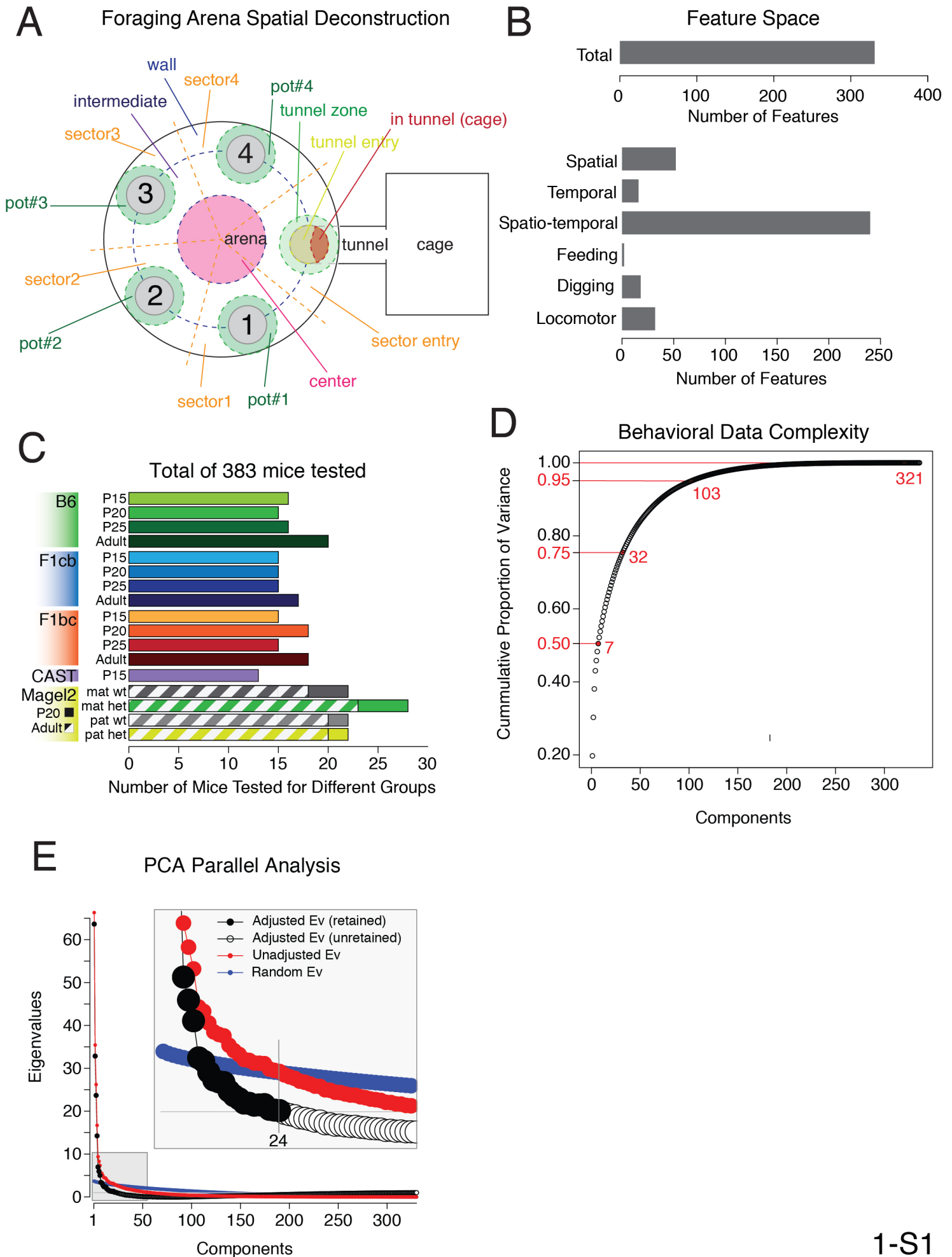1008 Schaaf, C.P., Gonzalez-Garay, M.L., Xia, F., Potocki, L., Gripp, K.W., Zhang, B., Peters,

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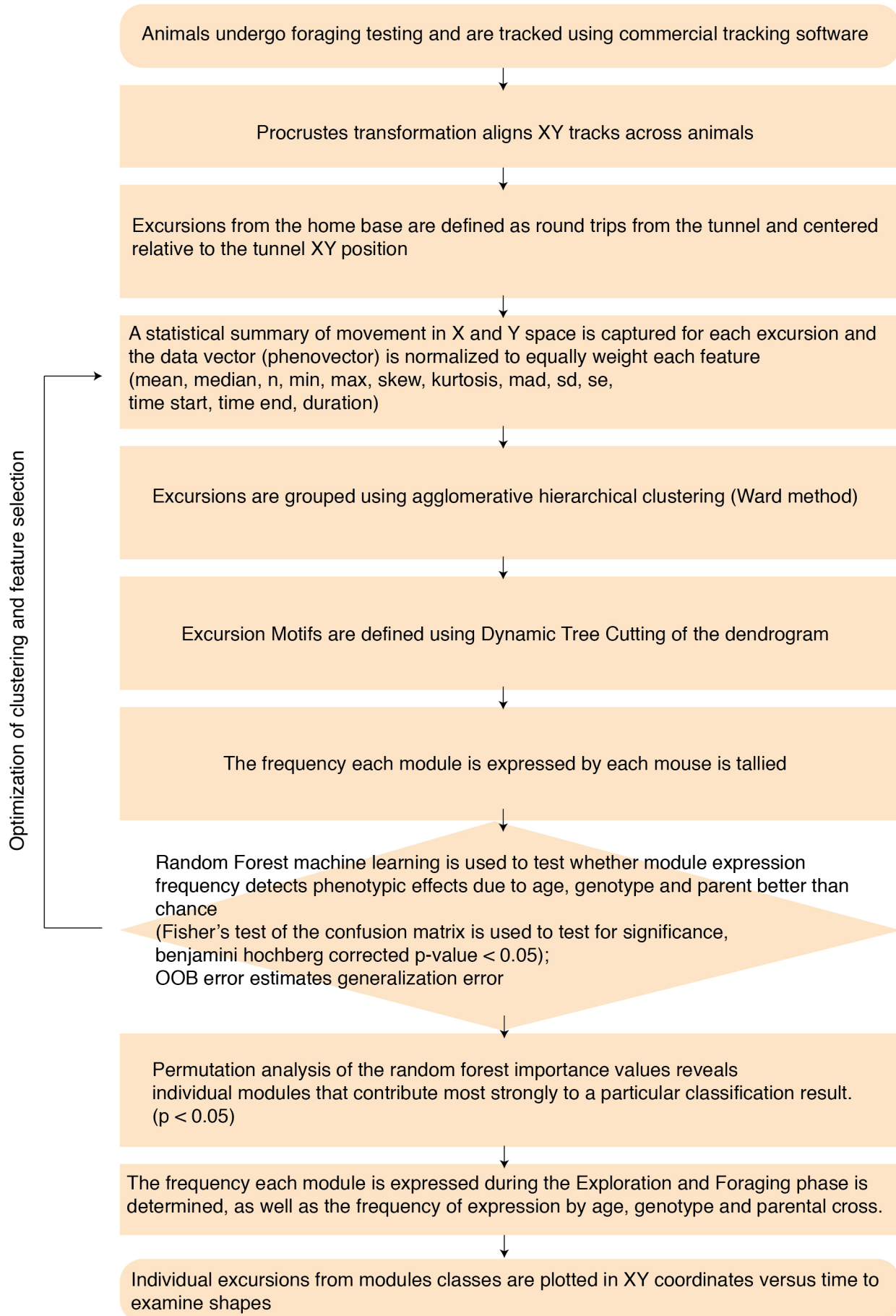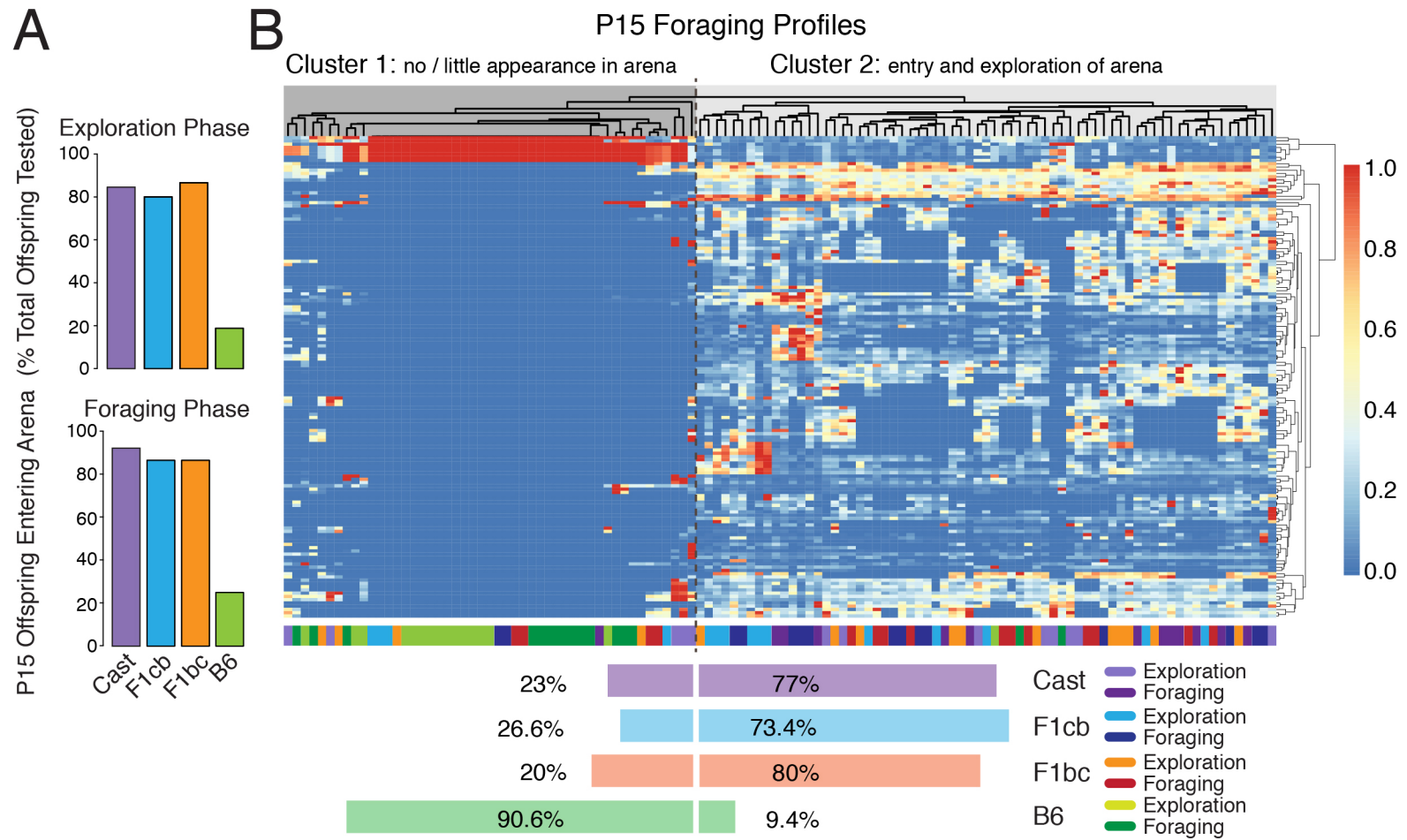1072   126–131. doi:10.1016/j.mito.2014.07.006
1073

A Cage plugs into arena

B
time
Day 1 / Day 2
seed / digging familiarization
Day 3
food deprivation
Day 4 - Test day
habituation | 25 min | break 3.5 hrs | 25 min
Exploration phase | Foraging phase

C Exploration phase
in tunnel (cage)
4
3
arena
cage
tunnel
2
1
Obvious Seeds
Food discovery in a naive context with access to home base

D Foraging phase
Buried Seeds — in tunnel (cage)
4
3
arena
cage
tunnel
2
1
Former food patch
Reversal learning and food discovery in a familiar context with access to home base

E
Home anchored excursion
Fast Medium Slow
25
20
6
Time (Minutes)
15
5
10
4
3
5
Pot 2
2
1
Tunnel
0
Y
X

DeepFeat Pipeline (see Fig. S1)

Optimize clustering & tree cutting based on RF accuracy (repeat)

1. Agglomerative hierarchical clustering groups excursions into motif classes
2. Dynamic tree cutting identifies motif classes
3. Quantification of motif class expression frequency for each animal
4. Random forest machine learning classification of animals by motif expression
5. Permutation of importance values identifies major motif classes distinguishing animals

1

A — Foraging Arena Spatial Deconstruction

B — Feature Space

C — Total of 383 mice tested

D — Behavioral Data Complexity

E — PCA Parallel Analysis

1-S1

DeepFeat WorkFlow

Animals undergo foraging testing and are tracked using commercial tracking software

Procrustes transformation aligns XY tracks across animals

Excursions from the home base are defined as round trips from the tunnel and centered relative to the tunnel XY position

A statistical summary of movement in X and Y space is captured for each excursion and the data vector (phenovector) is normalized to equally weight each feature
(mean, median, n, min, max, skew, kurtosis, mad, sd, se,
time start, time end, duration)

Excursions are grouped using agglomerative hierarchical clustering (Ward method)

Excursion Motifs are defined using Dynamic Tree Cutting of the dendrogram

The frequency each module is expressed by each mouse is tallied

Random Forest machine learning is used to test whether module expression frequency detects phenotypic effects due to age, genotype and parent better than chance
(Fisher's test of the confusion matrix is used to test for significance,
benjamini hochberg corrected p-value < 0.05);
OOB error estimates generalization error

Permutation analysis of the random forest importance values reveals
individual modules that contribute most strongly to a particular classification result.
(p < 0.05)

The frequency each module is expressed during the Exploration and Foraging phase is determined, as well as the frequency of expression by age, genotype and parental cross.

Individual excursions from modules classes are plotted in XY coordinates versus time to examine shapes

Optimization of clustering and feature selection

1-S2

2-S1

2-S2

A Module Detection

(191 Mice; 6585 Excursions
Genotypes: B6, Cast, F1cb, F1bc;
Ages: P15, P20, P25, Adult)

Ward, DeepSplit = False

59 clusters

B log(Expression Frequency)

Chi-Square P value: ***P = 2.3x10^-85   ***P = 7.0x10^-64

C Module 37
Adult F1cb #1   Adult F1cb #2

Module 6
Adult F1bc #1   Adult F1bc #2

Module 1
Adult F1bc #1   Adult F1bc #2

D Module 34
P20 F1bc #1   P20 F1bc #2

E Not Expressed / Expressed

% of Total Modules Expressed at Each Age

3

A

	N	Sensitivity (%)	Specificity (%)	OOB error	P. Adjust
B6 Adult	20	35	100	6.8	*** 2×10^{-7}
B6 P25	16	17	100	6.8	** 2×10^{-3}
B6 P20	15	33	99	5.8	*** 4×10^{-5}
F1 Adult	17+18	51	99	10	*** 6×10^{-13}
F1 P25	15+15	10	100	14.1	** 7×10^{-3}
F1 P20	15+18	6	100	16.2	* 3×10^{-2}
F1 P15	15+15	66	99	6.3	*** 4×10^{-16}
CAST P15	15	31	100	4.7	*** 5×10^{-5}

B

Adult F1 / Adult B6 Venn diagram: 9 / 0 / 5

Permuted Importance ($p<0.05$)

Adult F1 Motifs	Adult B6 Motifs
3	1
4	7
6	9
10	38
11	52
14	
21	
27	
36	

C

Motif 10

D

Motif 10

E

Motif 7

F

Motif 7

4

4-S1

A

■ 30 second time bins

Time at Pot #2 (seconds)

P20 / P25 / Adult

Foraging phase

Foraging phase Food Patch

Arena

Tunnel

Previous Food Patch (Exploration Phase)
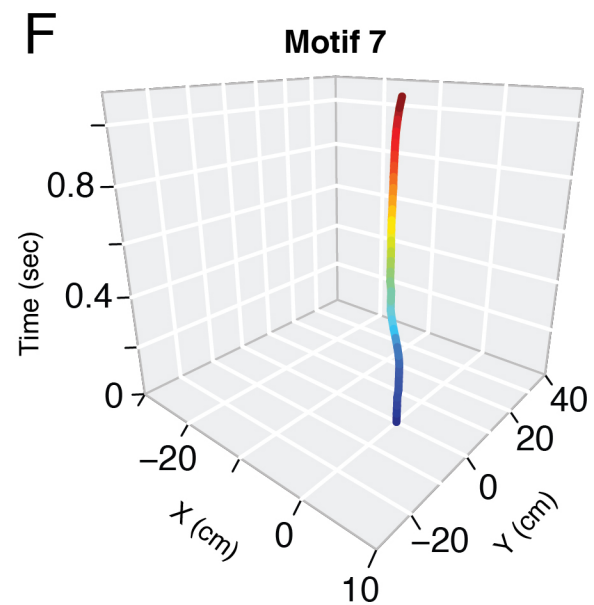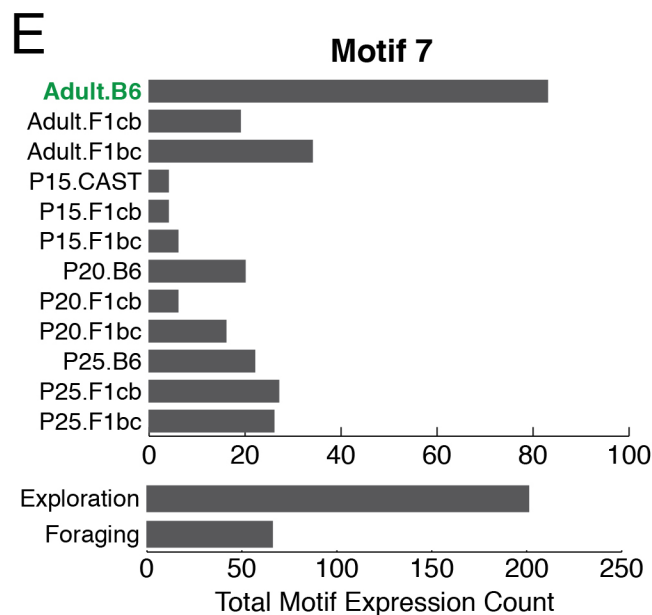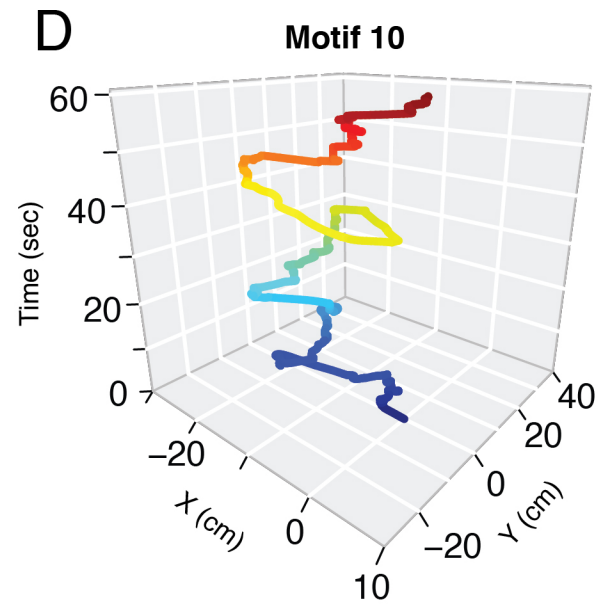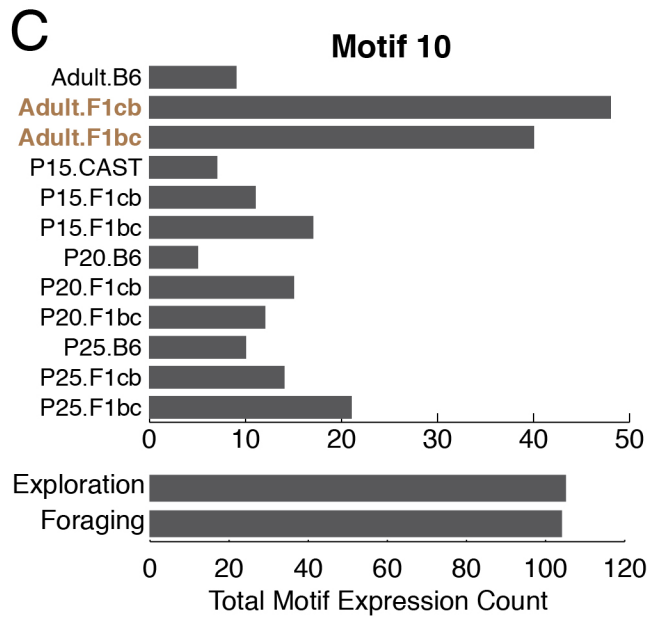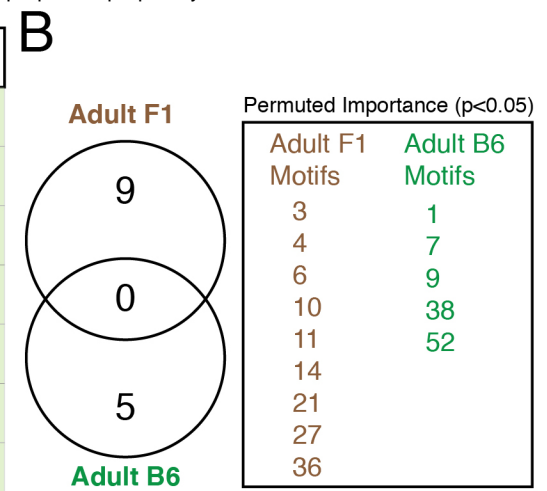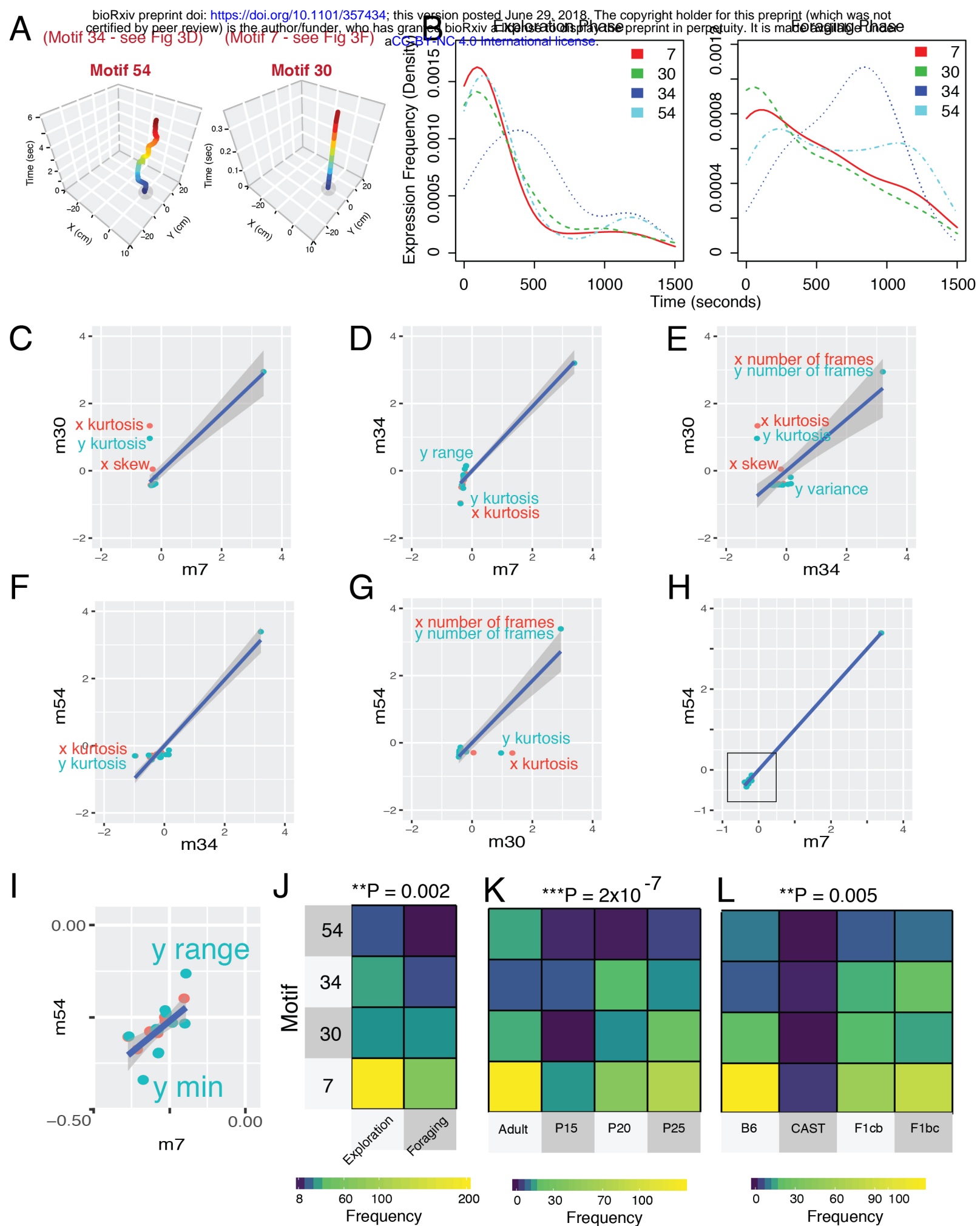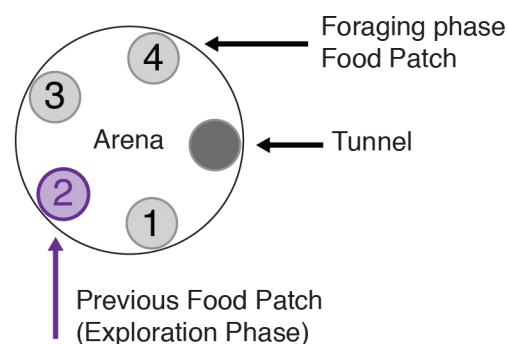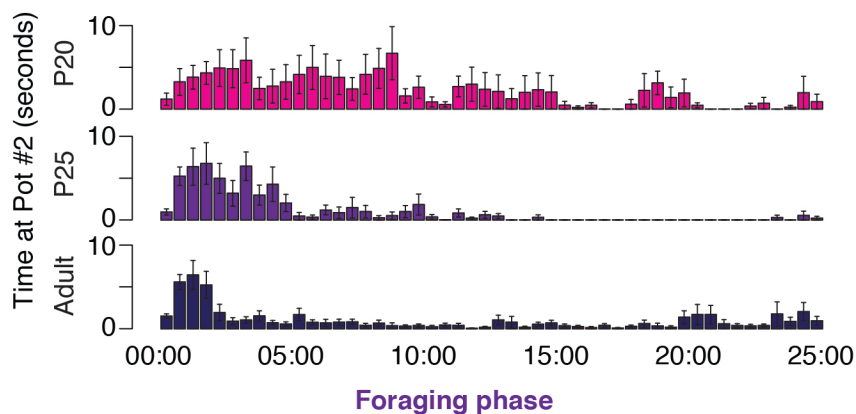
B

Modules correlated to sand dug from Pot# 2 (Spearman rho)
(across all ages, genotypes and crosses)

Sand Dug from Pot# 2

2**
31**
45**
6**
18**
14**
0**

Module 31

Mean Expression Frequency

Mean Sand Dug from Pot#2 (Foraging phase)

rho = +0.8

E
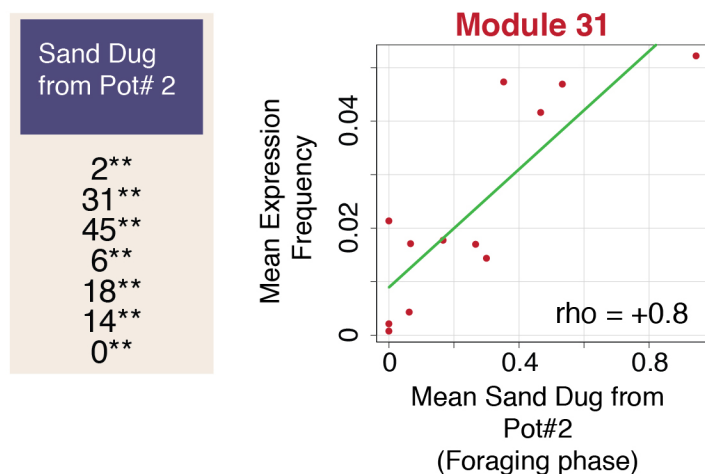
Expression differences for different memory response modules

Module Expression Frequency Density

31
14
18

Foraging Phase

Time (seconds)

C Module 31

Time (sec)

Pot #2

X (cm) / Y (cm)

D Module 14

Time (sec)

X (cm) / Y (cm)

F

REWARD
Food Consumed — E / F

RISK
Percent Time in Exposed Center Zone — E / F

EFFORT
Total Sand Excavated — E / F

Total Distance Traveled — E / F

Number of Significantly Correlated Modules

5

A

Exploration (min 0-25)

Pot Time / Arena Time

P20 P25 Adult

1 ****
2 ****
3 ****

Foraging (min 0-2)

Pot Time / Arena Time

P20 P25 Adult

1 ****
2 ***
3 ns

B

High Reward Module

Module 27 (high time at Foraging phase food patch)

Pot #4

C

High Risk Module

Module 2 (high percent time in center zone)

D

High Effort Module

Module 19 (high total distance)

(see Module 31 for high sand excavated)

A Parental Effects on Module Expression

Adult
Sen: 88%
Spc: 72%
OOB: 20%
***P = 0.0004

C DRN Genes Sensitive to Parental Effects (FDR 5%)

G P5

H Adult

I PWS-AS Locus

7

A

P20 and Adult Offspring

Magel2 $^{m+/p-,\ m-/p+,\ m+/p+',\ m+'/p+}$

75 Modules

B

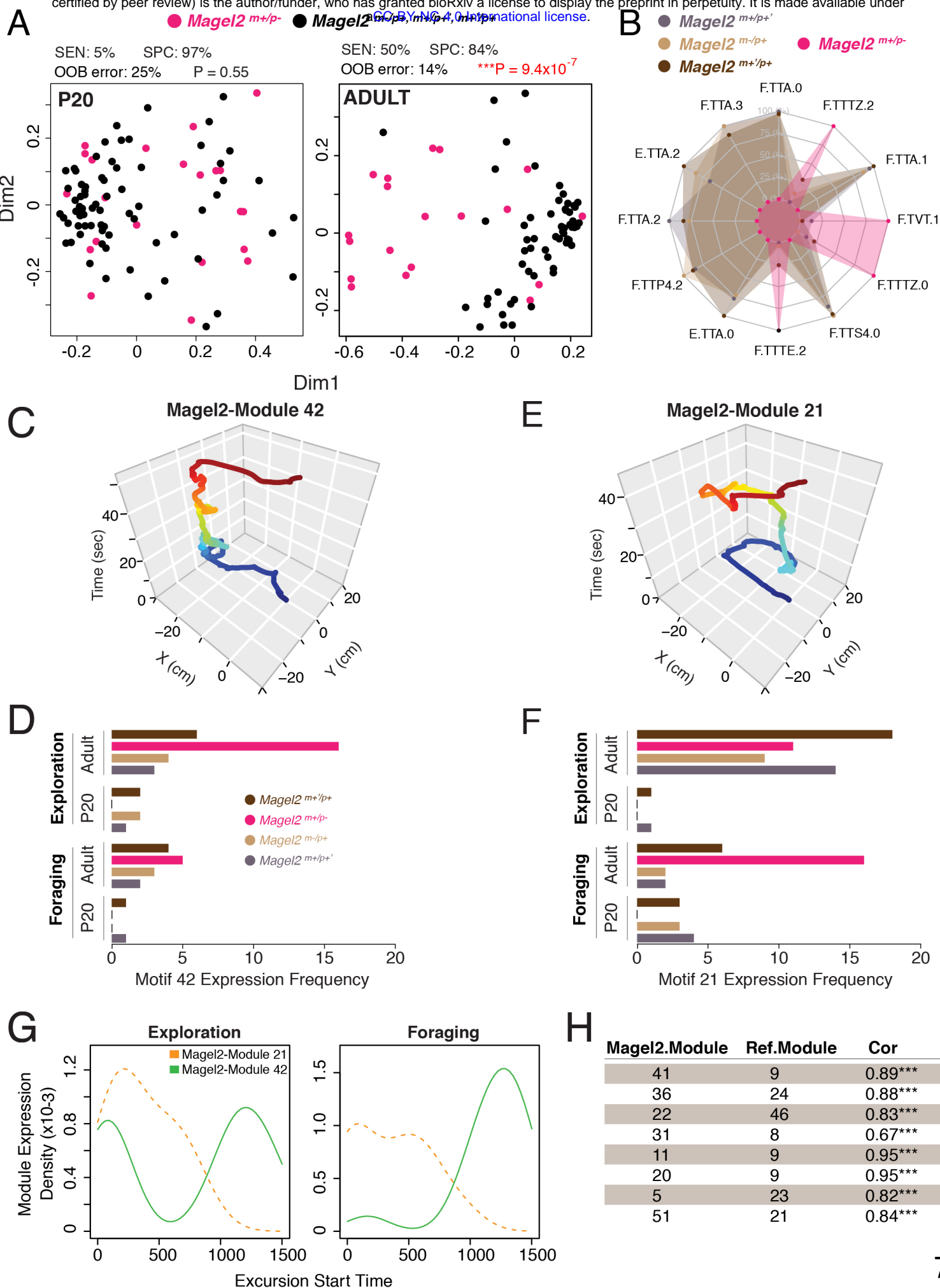|	N	Sensitivity (%)	Specificity (%)	OOB error	P. Value
Adult Magel2 +/-	20	10%	100%	10.4%	* 0.01

C
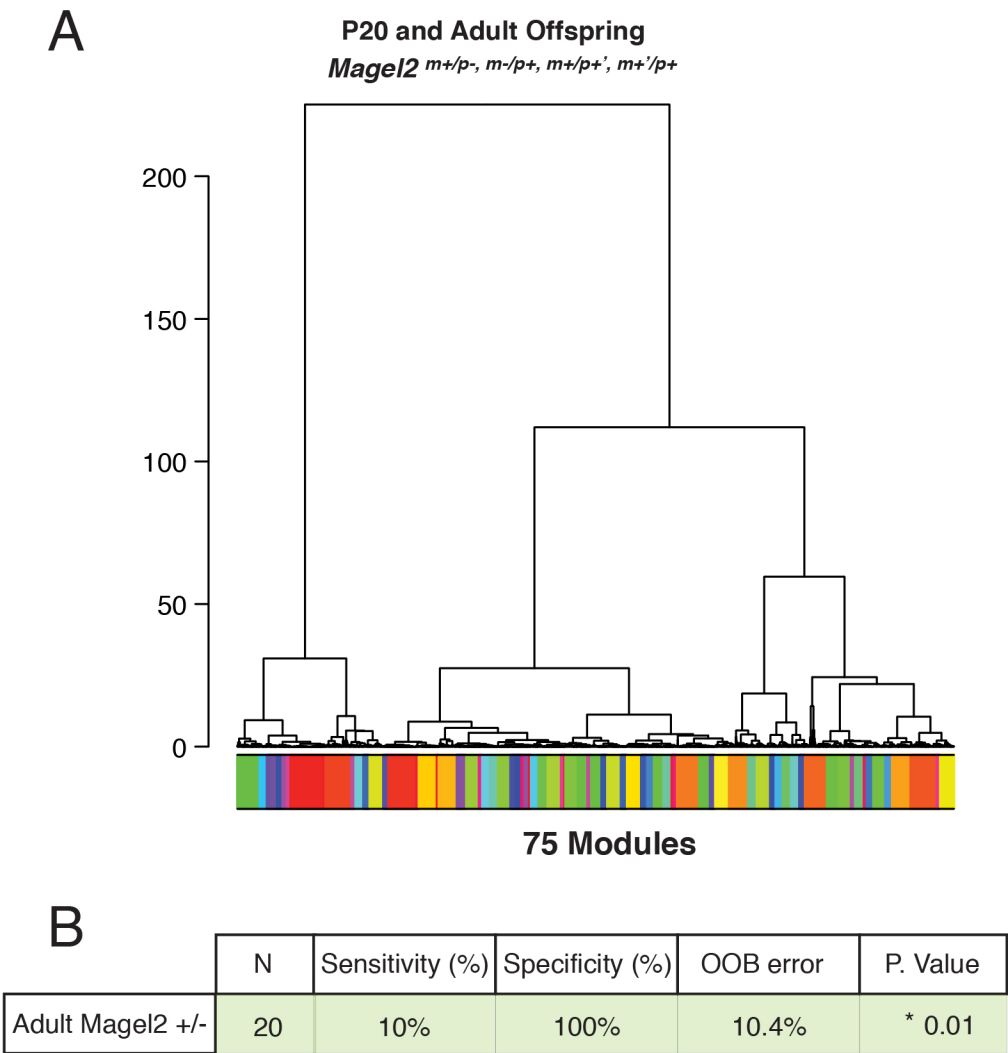
Significantly informative Magel2-Modules:

Magel2-Module 42***
Magel2-Module 37**
Magel2-Module 23*
Magel2-Module 32*
Magel2-Module 12*
Magel2-Module 21*
Magel2-Module 6*
Magel2-Module 52*

7-S1

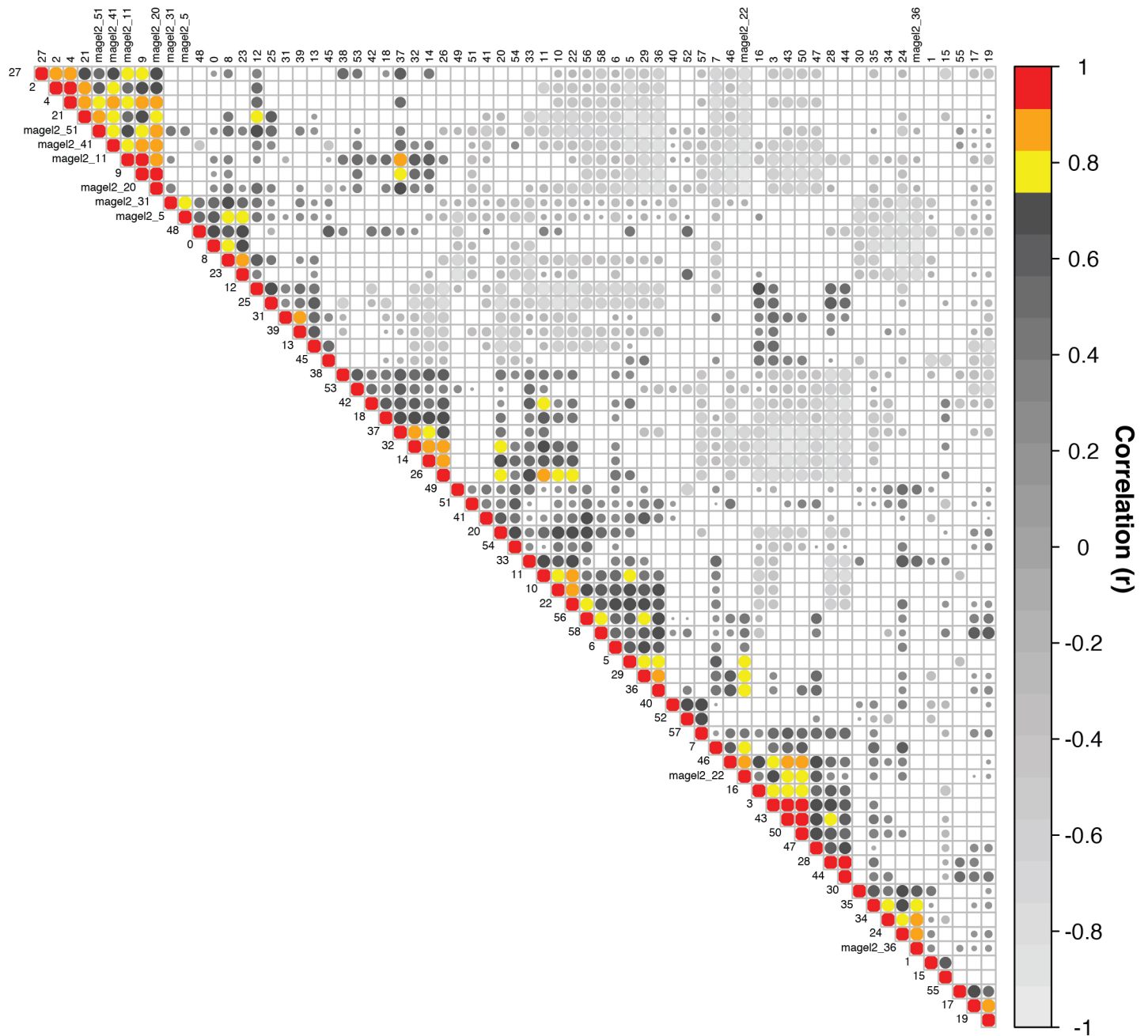**Magel2 Mutant Module to Reference Module Matches**

7-S2

FIGURE LEGENDS

Figure 1. A paradigm to study rich foraging patterns in mice.

(A) Image shows the foraging arena. The 3D printed arena platform holds pots containing sand and one food patch with seeds. The home base cage plugs into the arena through a 3D printed tunnel on the side. Access to the arena is controlled by a door in the tunnel and the experimenter.

(B) A summary of the assay workflow.

(C and D) Overviews of the pots, tunnel zone and seed location during the naïve Exploration phase (C) and familiar Foraging phase (D). The mice learn the food patch is Pot#2 in the Exploration phase. In the Foraging phase, the food is moved and hidden in Pot#4.

(E) An example of XY tracking of body position over time during the foraging assay (Exploration phase shown). Excursions are defined from round trips from home base cage (orange numbered track sections). Foraging behavior modules are defined from these excursions using DeepFeat (see text, Fig. S1 and Supplemental Information).

Figure 1-S1. Identification of a high dimensional feature space for the analysis of complex foraging patterns.

(A) Schematic overview of the different zones of the arena for systematically deconstructing spatiotemporal features of foraging.

(B) The bar chart shows the total feature space (338) and a break down of numbers of different types of features defined according to different types of measures (see Table S? and Supplementary Information).

(C) The bar chart summarizes the number of female mice profiled for each age and genotype in our study (C).

(D-E) A PCA analysis of the feature data for all 383 mice reveals high dimensionality for the data. The dot plot in (D) shows the cumulative proportion of the variance that is explained by different numbers

1

of principal components. The plot in (E) shows the results of a parallel analysis, in which 24 principal components are retained to explain the variance feature data. Ev, eigenvalue.

Figure 1-S2. Summary of the workflow for DeepFeat analysis of behavioral modules.

The graphic shows the different steps involved in DeepFeat. Custom scripts were generated to perform each step of the analysis.

Figure 2. Random forest analysis of foraging patterns reveals parental effects on economic patterns emerge after weaning, while genetic effects are robust at all ages

(**A**) Schematic summary of mouse genetics strategy to identify parental and/or genetic effects at P15, P20, P25 and adulthood. Different mice are tested at each age. Parental effects potentially encompass genetic parental effects and environmental parental effects (see text).

(**B**) Multidimensional scaling plots of the random forest classifier proximity values for each mouse reveals parental and/or genetic effects in P15 (early weaning), P20 (weaning), P25 (post-weaning) and adult females. The sensitivity (Sen), specificity (Spc), out-of-bag error (OOB) and two-tailed Fisher test P-value for the confusion matrix of the classification results are shown above each plot.

(**C**) Venn diagram shows that the significantly informative features in the classifiers detecting parental effects in P25 and adult F1cb vs F1bc mice differ (PIMP P<0.01).

(**D and E**) Radar plots show the data for significantly informative features for the classifiers detecting parental effects in P25 (D) and adult (E) offspring. The results show the relative values for the features that most strongly distinguish F1cb and F1bc offspring foraging patterns at each age (see main text). Feature data points are the mean values from F1cb (blue) and F1bc (orange) mice, presented as percentages of the relative maximum (100%) and minimum (0%) values for each feature. Feature codes are: [Phase.Feature.TimeBin]. F, Foraging phase; E, Exploration phase. See Table S1 for feature definitions.

Figure 2-S1. Foraging is immature in P15 mice, but early stage patterns are detectable from many features for P15 Cast, F1cb and F1bc offspring.

(A) Bar plots show the percentage of P15 juveniles that appear in the foraging arena in the Exploration phase and Foraging phase (N=15-20 per cross). Few B6 offspring (20%) enter the arena, but most Cast, F1cb and F1bc (~80%) offspring enter.

(B) Heat map and clustering results for the profiles from all Exploration and Foraging trials for B6 (green), Cast (purple), F1cb (blue) and F1bc (red) P15 animals. Columns are single trials. Rows are behavioral measures. Values are normalized to maximum value in each row. Colored bars underneath the heatmap indicate the cross (B6, Cast, F1cb, F1bc) and phase for each trial (column). Horizontal bars indicate the percentage of trials from each cross that cluster into Cluster 1 (no/little appearance) or Cluster 2 (data captured from foraging trials). The results in Cluster 2 show that data for many features was successfully obtained for P15 Cast, F1cb and F1bc offspring.

Figure 2-S2. Genetic effects in B6 versus F1 hybrid mice shape foraging patterns in an age-dependent manner.

(A) A venn diagram of the features with significant importance values in the random forest classifiers detecting B6 from F1 hybrid offspring at P20, P25 and adult. No feature is significant across all ages and most features are age-specific.

(B-D) The radar plots depict the data for a subset of the top ranked features (PIMP P<0.01) in the classifier detecting B6 offspring from F1cb and F1bc offspring at P20 (B), P25 (C) and for adults (D). The data points for each behavioral feature are the mean values from B6 (green), F1cb (blue) and F1bc (orange) mice, presented as percentages of the relative maximum (100%) and minimum (0%) values for each feature. Feature codes are: [Phase.Feature.TimeBin]. F, Foraging phase; E, Exploration phase. See Table S1 for feature definitions.

3

Figure 3. Identification of 59 behavioral modules underlying mouse foraging patterns across different ages reveals contextual and developmental changes to module expression.

(A) Dendrogram of the clustering results to define modules from home base excursions. DeepFeat identified 59 modules from over 6500 home base excursions performed by 191 mice in our cohort of B6, Cast, F1cb and F1bc offspring from four ages. Distinct clusters (modules) are indicated by color.

(B) The heatmap shows the relative expression frequency of different modules by phase (Exploration versus Foraging) and age. The module expression counts are centered by row. Clustering on the rows identified 11 groups based on expression frequency similarities. P values for a Chi-Square test of independence are shown for Phase and Age on the top of the chart. The results show that module expression patterns are significantly dependent on phase and age.

(C and D) Examples of representative modules from two different adult F1bc mice for Modules 37, 6 and 1 (C), and for two different P20 F1bc mice for Module 34 (D). The data show the XY tracking pattern of the mice over time during one round trip excursion from the tunnel to the home base cage (0,0). Behavioral sequences are colored according to time (normalized to total time).

(E) Bar plot shows the percentage of the 59 modules expressed (purple) by mice according to age. Modules not expressed (grey) have a frequency ≤ 1 across all mice tested for that age.

Figure 4. Changes to the expression of specific behavioral modules underlie genetic effects on foraging patterns.

(A) The table shows the results of the random forest classification of animals by genotype (B6, F1 hybrid and Cast) for each age (P15, P20, P25 and adult). A two-tailed Fisher's test on the resulting confusion matrix showed statistically significant detection of each group (Benjamini-Hochberg adjusted p-values shown). OOB, out-of-bag error. N shows number of animals tested (F1 hybrid: F1cb + F1bc).

(B) Permuted importance analysis for the classifiers trained to detect adult F1 hybrid (F1cb and F1bc) and B6 mice identified significantly informative modules (P<0.05). The Venn diagram and box inset

4

show the modules informative for adult B6 versus F1 hybrid mice phenotypes, and they are different (refer to Figure 2B for context and age expression details).

(C and D) Module 10 is a significantly informative module discriminating F1 hybrid adult behavior. Counts of the module's expression frequency show it is expressed by all strains and ages, but is relatively highly expressed by F1cb and F1bc adults (C), and equally in the Exploration and Foraging phases. A representative trace of the module is shown from an F1bc adult, indicating a long, looping trajectory with multiple pauses in different locations on the side of the arena harboring Pots 1 and 2 (D).

(E and F) Module 7 is a significantly informative module discriminating B6 adults. It is expressed highly by B6 adults, and more frequently in the Exploration compared to the Foraging phase (E). A representative adult B6 trace reveals that Module 7 is a brief, sub-second emergence from the tunnel to the threshold of the arena and back (F).

Figure 4-S1. Modules involving brief behavioral sequences capture important phenotypic information.

(A) Four short modules were analyzed in depth. Representative traces are shown for two of the short modules, including Module 54 and Module 30. Traces for Module 34 and Module 7 are presented in other main figures. The short module subtypes show differences in temporal length and/or XY movement architecture.

(B) Density plots of the expression pattern (excursion start times) for all excursions classified as Modules 7, 30, 34 or 54. The temporal expression pattern is shown across the entire 25 minute Exploration and Foraging phases. The data shows that Module 34 exhibits a distinct temporal expression profile in the Foraging phase compared to the other short modules.

(C-I) Plots compare descriptive statistics calculated from the XY movement patterns for excursions falling under the same module class. Data for each module (m) is plotted in a comparison with a different short module (7, 30, 34, 54). The results show specific differences and outlying measures

5

relative to a regression line indicating the pattern for a linear relationship. The data in (I) is a zoom of the data in the black box in (H). Prominent outlying descriptive statistics are labeled in each contrast.

(J-L) The heatmaps show the frequency each short module is expressed according to phase (J), age (K) and genotype (L). Different modules show different expression patterns. A Chi-Square test of independence determined that the short module expression frequencies are significantly dependent on the biologically meaningful factors of phase, age and genotype (P values shown on top).

Figure 5. Different modules are linked memory, reward, risk and effort responses during foraging.

(A) P20, P25 and adult mice spend increased time investigating the former food pot (Pot#2) during the Foraging phase, showing perseverative effects of the learned food location. The bar chart shows time at Pot#2 over the course of the 25 minute Foraging phase trial for B6 mice in 30 second time bins. The error bars are SEM. The schematic indicates the layout of the arena and location of Pot#2.

(B) Top putative memory and perseveration response modules are revealed from significant correlations to the amount of sand dug from Pot#2 in the Foraging phase are shown (P<0.01, Spearman Rank correlation). An example of the data is shown for Module 31. The average expression frequency for the module across the 12 different genotype, cross and age groups is correlated to the average sand dug from Pot#2 in the Foraging phase.

(C and D) Representative traces for the memory and perseveration Modules 31 and 14. Both modules are significantly associated with the amount of sand dug from Pot#2, but have distinctive temporal and movement patterns. Module 31 is longer and involves a looping visit to Pot#2, while Module 14 is a brief, multi-second emergence from the tunnel and back.

(E) The density plots show the expression start times for all Foraging phase excursions classified as the memory and perseveration Modules 31, 6, 14 or 18. The data reveals differences in the temporal expression patterns of the modules across the entire 25 minute Foraging phase trial (see text).

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(F) The bar chart shows the number of modules significantly correlated to reward (blue, total food consumed), risk (purple, percent time in center zone) and effort (dark blue, total sand dug and total distance traveled) keystone features in the Exploration (E) and Foraging (F) phases. Spearman rank correlation P<0.05.

Figure 5-S1. Top modules linked to reward, risk and effort responses during foraging.

(A) Bar charts show the time spent at each pot (1, 2, 3 and 4) during the entire Exploration phase and the first two minutes of the Foraging phase. The time at each pot is normalized to the total time an animal spends in the foraging arena. The data show that P20, P25 and adult mice find the food in Pot#2 in the Exploration phase and retain a perseverative memory of that food's location in the Foraging phase. Significance relationships are shown in the legend. One way ANOVA and Tukey posttest. N=20-25 B6 mice. Error bars show SEM. The data show memory and perseveration responses, providing the basis for the identification of putative memory and perseveration modules.

(B-D) Representative traces of the top modules linked to total food consumption (B), percent time in the center zone (C) and total distance traveled (D). The inset rotation of Module 27 shows overlap with the Foraging phase food patch in Pot #4 (B). Module 2 is characterized by extensive time in the center zone of the arena (see Fig. 5-S1 for location of center zone). Module 19 involves long, wide looping movements.

Figure 6. Discrete behavioral modules, genes and pathways are impacted by parental effects in offspring in an age-dependent manner.

(A) Multi-dimensional scaling plot of random forest classification results for adult F1cb versus F1bc mice based on module expression data. The results show significant discrimination by the trained classifier.

(B) The bar plots show the adult F1cb and F1bc expression frequency data for significantly informative modules in the trained classifier. Permuted Importance Analysis (P<0.05).

(C-E) MA-plots of RNASeq analysis of differential gene expression in the DRN region of F1cb versus F1bc offspring at P5 (C, N=7), P15 (D, N=7) and in adults (E, N=8). Significant genes are shown in red (5% FDR). The data show a relative reduction of parental effects in P15 juveniles, consistent with our analysis of behavior patterns (Fig. 2B).

(F) Venn diagram of P5, P15 and adult differentially expressed (DE) genes due to parental effects.

(G and H) Bar plots show the results of DAVID gene ontology and pathway enrichment analysis for genes with significant parental effects in the P5 (G) and adult (H) DRN. Only statistically significant enrichment terms are shown (5% FDR threshold indicated by red line). The x-axis shows the adjusted p-value for the ontology term enrichment.

(I) Bar chart of the adjusted p-values for DRN differential expression between F1cb and F1bc offspring for imprinted genes in PWS-AS locus. *Ube3a, Magel2* and *Ndn* show significant expression level differences at P5. *Ndn* is also significant in adults and *Ube3a* is significant at P15. Red line indicates the threshold for a 5% FDR (see Table S?).

Figure 7. The PWS gene, *Magel2,* most strongly shapes adult behavioral patterns associated with the home.

(**A**) Multidimensional scaling plots of the random forest classifier proximity values to detect *Magel2* mutants (*Magel2$^{m+/p-}$*) from controls (*Magel2$^{m+/p+'}$*, *Magel2$^{m-/p+}$* and *Magel2$^{m+'/p+}$*) at P20 and in adulthood. The sensitivity (SEN), specificity (SPC), out-of-bag error (OOB) and the two-tailed fisher test P-value for the confusion matrix of the classification results are shown above each plot.

(B) Radar plot shows the relative values of data for the significantly informative features for the classifier detecting *Magel2$^{m+/p-}$* adults (yellow) from *Magel2$^{m+/p+'}$* (brown), *Magel2$^{m-/p+}$* (green) and *Magel2$^{m+'/p+}$* (pink) controls (P<0.01, PIMP). The results show the distinguishing pattern in *Magel2$^{m+/p-}$* adults (see main text and Table S1 for feature descriptions).

(C-F) A representative trace of Magel2-Module 42 (C) and Magel2-Module 21 (D), significantly informative modules in the adult *Magel2$^{m+/p-}$* classifier. The bar plots show the expression frequency

8

of the modules for all genotypes and ages (D and E). The results show phase (context) dependent changes to module expression in the mutants.

(G) Density plots show distinct temporal expression patterns (excursion start times) across the 25 minute Exploration and Foraging phase trials for excursions classified as Module 42 versus Module 21, two module types significantly linked to phenotypic effects in *Magel2$^{m+/p-}$* adult mutants.

(H) The table shows the top reference module matches for each of the nine Magel2-Modules that are significantly informative for the *Magel2$^{m+/p-}$* adult mutant phenotype. Magel2-Module and reference modules IDs are shown. Cor, is the Pearson correlation for the match of the eigenphenotype. ***Holmes adjusted p-value < 10^{-10}.

Figure 7-S1. Identification of behavioral modules expressed by P20 and adult *Magel2* mutant and control mice.

(A) Dendrogram of the clustering results to define modules from home base excursions. DeepFeat identified 75 modules from 5302 home base excursions performed by 60 mice in our cohort of P20 and adult *Magel2* mutants and controls. Distinct clusters (modules) are indicated by color.

(B) The table shows the results of the random forest classification of adult *Magel2$^{+/-}$* mutants based on module expression frequency data. A two-tailed Fisher's test on the resulting confusion matrix showed statistically significant detection of adult *Magel2* mutants (p-value shown). OOB, out-of-bag error.

(C) The table shows the identity of the nine significantly informative Magel2-Modules for the adult *Magel2* mutant phenotype found from permuted importance analysis. ***P<0.0001, **P<0.01, *P<0.05

Figure 7-S2. Eigenphenotype correlations reveal matches between Magel2-Modules and Reference Modules derived from Cast, B6, F1cb and F1bc mice.

The plot shows the upper triangle of the correlation matrix matching nine Magel2-Module query modules to all 59 core reference modules. Top hits are colored according to the legend and the best

reference module matches for each Magel2-Module are shown in Fig. 7H. Only statistically significant matches are shown in the plot after adjusting for multiple testing. Holmes adjusted p-value < 0.05

SUPPLEMENTAL MOVIE LEGENDS

Movie S1: Exploration trial

Movie showing short sequences of a representative adult Exploration phase trial. The adult B6 female appears in the arena, retreats to the home cage and returns several times to the arena. The mouse explores various features and regions of the arena environment and finally finds the food in Pot #2 and starts to eat. This phase examines how mice behave in a novel foraging environment and discover and consume food. We deconstruct the behavior patterns in this phase into 152 different measures.

Movie S2: Foraging trial

Movie showing short sequences of a representative adult Foraging phase trial. The adult B6 female appears in the arena and visits the previous food pot (Pot #2), digs and searches for food in Pot #2. The mouse eventually gives up searching Pot #2, searches and finds the hidden food in the new location (Pot #4). This phase examines how mice behave in a more familiar foraging environment, adapt to changes in the environment and discover the novel food source. We deconstruct the behavior patterns in this phase into 152 different measures.

SUPPLEMENTAL TABLES

Supplemental Table 1. Summary of all behavioral and locomotor features measured. For Figures 1-7.

This table details the features measured in the foraging assay. Each feature is measured in the Exploration phase and the Foraging phase and several measures are analyzed at different time bins (1, 2, 3, 4 and 5) and/or across the entire phase (0). Each measure is assigned a unique ID with the following structure [Phase.Feature.TimeBin].

Tab#1 Behavior Features: phase, Exploration or Foraging phase; measure, description of each behavioral feature; code, unique ID for each behavioral measure; unit; non-normalized data, x indicates if a measure is included in the non-normalized data set; normalized data, x indicates if a measure is included in the normalized data set; normalization, values indicate how each measure was normalized for the normalized data set.

Tab#2 Locomotor Features: The locomotor features captured in the assay are indicated with descriptive IDs.

Supplemental Table 2. Mouse phenome project detected phenotypic differences between Cast and B6 female mice that could contribute to putative parental effects on offspring phenotypes. For Figure 2A. The table shows phenotypic differences between Cast and B6 female mice uncovered by the Mouse Phenome Project. Significant differences in female reproductive behaviors were identified, as shown. These phenotypic differences are diverse and support the rationale for investigating parental effects on offspring in F1cb and F1bc mice.

Supplemental Table 3. Raw data for Cast, B6, F1cb and F1bc foraging features and motifs. For Figures 2-5. The raw data for features as defined in Table S1 and for motif expression counts identified by DeepFeat.

Supplemental Table 4. Significant differentially expressed genes identified by RNASeq analysis of F1cb versus F1bc female mice at P5, P15 and adulthood for the DRN region. For Figure 6. The results show the output from edgeR for the analysis of differential gene expression due to parental effects at different ages. Separate tabs indicate the data for P5, P15 and adult female mice. CPM, counts per million reads; FDR, false discovery rate.

Supplemental Table 5. DAVID gene ontology and pathway enrichment results for genes differentially expressed in the DRN between F1cb and F1bc mice at P5 and Adult. For Figure 6. The ontology enrichment analysis was performed on differentially expressed genes defined at a 10% FDR threshold relative to a background of all genes expressed in the DRN. Separate tabs are provided for the P5 and adult DRN results.

Supplemental Table 6. Raw data for Magel2 mouse foraging features and motifs. For Figure 7. The raw data for features as defined in Table S1 and for motif expression counts identified by DeepFeat.

1   **SUPPLEMENTAL INFORMATION**

2   **Rationale for the Foraging Assay Design and Feature Selections**

3   _Foraging Assay Design_

4   Our primary goal in the design of the assay for the foraging screen is to create a broad,

5   ethologically-relevant screening platform in which diverse phenotypic patterns can

6   potentially manifest due to effects from a wide variety of different neural systems. In our

7   design, we attempt to incorporate important elements from behavioral ecology and lab-

8   based approaches to the analysis of foraging. To investigate rodent foraging in the wild,

9   behavioral ecologists frequently monitor trays of sand that contain seeds (Brown,

10   1988b). These food patches are placed strategically in different regions of the

11   environment to test different hypotheses about rodent foraging patterns and to measure

12   giving up densities. The giving up density is the point at which rodents abandon a food

13   patch because the density of seeds in the sand is too low, and therefore the animal has

14   made a decision about the costs of continued foraging in that patch versus the rewards

15   (Brown, 1988a; Brown and Kotler, 1994a; 1994b). We incorporate this idea into our

16   assay by designing the food patches in the foraging arena as pots of sand with or

17   without seeds. Thus, mice are challenged to extract seeds from the sand and

18   apparently make decisions regarding the costs versus the rewards (see below). Our

19   design also incorporates features from several standard lab tests (Wahlsten, 2010),

20   including the open field test (thigmotaxis/activity), marble burying/digging

21   (repetitive/perseverative behaviors), Barnes maze (spatial navigation/memory), Morris

22   water maze (spatial memory/reversal learning), the buried food test (sensory) and basic

23   food consumption analysis (hunger/satiety), as we describe further below. From

24 relatively free and spontaneous foraging, these different components of the design

25 create an opportunity for diverse behavioral patterns to manifest in mice, as we show in

26 our study.

27

28   We wish to minimize the handling of the mice and allow them free access to the

29 home base cage during the foraging trials. Therefore, we designed the home base

30 cages with a tunnel and door (see Protocol – Arena Construction). The foraging arena is

31 also designed with a tunnel, which leads into the arena, and during a trial, the home

32 cage tunnel plugs into the arena tunnel, the door is opened and the mouse can now

33 freely leave the cage to access the foraging arena through the tunnel, and vice versa.

34 An important point to note is that in a typical lab-based behavior test, the mouse is

35 captured, lifted and placed into a behavioral testing apparatus. However, in the wild,

36 animals freely and spontaneously move between their home base and the outside

37 environment and the home base is an important safe shelter that can shape exploratory

38 patterns (Eilam and Golani, 1989).

39

40   We hypothesize that genetic mechanisms in mice shape many different foraging

41 behavior patterns relative to the home base and therefore this is an important element

42 of our design. Some known genetically driven behaviors associated with the home

43 include territorial behaviors, burrowing and nest construction (Sauce et al., 2012; Weber

44 et al., 2013), but other genetically-driven behavioral patterns likely exist. We attempt to

45 capture elements of home base related behaviors during foraging in our screen by

46 deconstructing spatiotemporal features related to the home base. For example, each

47 round trip from the home base into the arena and back is considered a single excursion

48 (Drai et al., 2000) and many discrete features of these excursions from the home base

49 are analyzed in our assay. We capture data for the length of excursions from the home

50 base at five different time windows during the assay, time spent in close proximity to the

51 home base, the number/frequency of excursions, the length/frequency of fast velocity,

52 medium velocity and slow velocity bouts of movement in each excursion from the home

53 base, the duration of time away from the home base, excursion patterns from the home

54 base during the naïve Exploration phase versus the more familiar and goal-directed

55 Foraging phase, preferences for exploring potential food patches closer to the home

56 base and the latency to enter the arena from the home base. These are some examples

57 of patterns and features that could be shaped by genetic mechanisms that influence

58 home base related behaviors.

59

60   Optimal foraging theory makes predictions about the strategies animals should

61 use during foraging to maximize rewards and reduce costs while seeking out and

62 exploiting food patches (Charnov, 1976; Pulliam and Charnov, 1977). Seeking out new

63 food patches is risky, since it is unclear whether new resources will be uncovered during

64 a search and the effort can result in energy expenditure and predation costs. Thus,

65 familiar foraging environments where known resources exist are valuable, and an

66 animal faces the ongoing challenge of deciding when to give up on a known food source

67 and take the risk of seeking out a new resource. Many neural systems are engaged in

3

68   these aspects of foraging, including neural systems for exploring, navigating and

69   detecting resources, learning the location of resources in the environment, remembering

70   the location of resources and giving up on food patches that are exhausted (Stephens

71   et al., 2007). Our assay is designed to capture some patterns related to these important

72   aspects of foraging, as follows.

73

74   First, mice search pots of sand in order to discover and obtain seeds for food.

75   This aspect of the design is ethologically relevant and innate for mice, but it also

76   demands effort, since the animals dig through the sand to explore, discover, extract and

77   consume seeds. Secondly, by incorporating a naïve Exploration phase with an obvious

78   food patch and then a relatively more goal-directed and familiar Foraging phase, in

79   which the food patch is switched and hidden, we expect that the assay can reveal a

80   variety of different phenotypic effects and patterns. For example, we expect to uncover

81   patterns related to: (1) the discovery of a food patch (Pot#2) in a new environment

82   (Exploration phase), (2) the subsequent return to, investigation of and then

83   abandonment of a former food source (Pot#2) in a now familiar environment (Foraging

84   phase), and (3) the discovery and exploitation of a new hidden food patch (Pot#4) in a

85   familiar environment (Foraging phase). In the current design, mice are provided with a 4

86   hour break between the Exploration and Foraging phases, which challenges the

87   formation and maintenance of memories of the food location beyond short-term working

88   memory, but allows for several animals (~6-8 animals) to be tested within a single day,

89   which scales to ~48 animals per week for screening (see Protocol). Each testing phase

90   is 25 minutes long, which is designed to allow for some adaptation and the expression

91   of different early, mid and late stage behavior patterns, which we show occurs in the

92   main text.

93

94   In addition to using the safety of the home base, animals have innate behaviors

95   to find food and avoid predation during foraging that influence how they navigate and

96   behave in exposed spaces versus less exposed spaces in the environment. One

97   example of this dimension of behavior is thigmotaxis, which involves a natural

98   preference for walls in an otherwise exposed area and occurs in mice (Prut and

99   Belzung, 2003) and humans (Walz et al., 2016). Our arena is designed with an exposed

100   center region and wall zones to capture behavioral features related to this dimension of

101   foraging. Further, the pots are positioned so that animals have the option of

102   approaching, exploring, digging and feeding at the pots while their body is positioned

103   toward the wall or out toward the exposed center. To capture data for features

104   potentially shaped by thigmotaxis, we break the arena into concentric circles (Fig. S1A)

105   that capture visits and the duration of time in (1) the most exposed center region, (2) in

106   an intermediate zone where the animal can interact with the pots with its body

107   positioned toward the center, and (3) a wall zone where the animal positions its body

108   close to the wall. By analyzing behavioral patterns in these different zones, we

109   potentially learn about some strategies the animal is using to reduce risks of exposure

110   during the Exploration and Foraging phases of the assay.

111

112   Finally, an important point regarding the design is that rodents explore and

113   forage instinctively, and do not require food deprivation to motivate these behaviors

114   (Collier et al., 1972; Kavanau, 1967; Moran, 1975; Pierre et al., 2001); however an

115   increased motivational state enhances learning (Makowiecki et al., 2012), which is a

116   component of our assay. Therefore, we food deprived the mice prior to the assay such

117   that the animals experience the same degree of body weight loss (~10% of starting

118   body weight), setting a common baseline for comparison across ages (see

119   Supplemental Experimental Procedures). A second point to note is that young animals

120   (P15 and P20) are maternally deprived since they are removed from the mother during

121   the assay, which could shape their behavior patterns. However, maternal attachment

122   changes and the anxiety that arises due to maternal separation declines postnatally and

123   is a natural part of the development of independent foraging behavior (Galef, 1981).

124

125   *Heuristic Feature Selection for Pattern Detection from Machine Learning*

126   The approach we took to selecting features for analysis in our assay is intended to

127   achieve three main goals. First, we wish to uncover novel behavior patterns that would

128   not have been predicted *a priori.* Second, we wish to be able to interpret and have at

129   least some understanding of the observed patterns, the features involved, and their

130   potential relevance to the principle objective of foraging – the discovery and

131   consumption of food through strategies that reduce effort and predation costs. Some

132   machine-vision based approaches can potentially uncover complex patterns that are

133   challenging to interpret, and we sought to reduce this in our initial screen, though it

134   remains an area for future development. Finally, we wish to be able to study and

135   compare behavior patterns across ages, and therefore between mice with different

136   sizes and shapes. For this application, the features and detected patterns need to be

137   comparable and generalizable across ages, which can also be challenging for some

138   machine-vision based strategies (Kabra et al., 2012; Wiltschko et al., 2015).

139

140   To meet these objectives, we first systematically deconstruct behavior patterns in

141   the assay into progressively more restricted spatiotemporal features (Fig. S1A). Spatial

142   features are deconstructed first by breaking the arena into pie shaped sectors that

143   encompass each major element of the arena (the pots and tunnel entry zone). Next, we

144   define regions immediately surrounding each major element in the arena (pot zones and

145   the tunnel zone). The tunnel zone is further subdivided into an entry zone, where the

146   mouse may peak into the arena from the tunnel and a tunnel zone, where it is in the

147   arena, but near the tunnel. Finally, we break the arena into domains according to two

148   concentric circles, one small circle encompassing the exposed center region and a

149   second larger circle that divides the pots and tunnel zone in half, with one half proximal

150   to the wall and the other half facing the center. This approach systematically divides the

151   arena into a large number of increasingly restricted zones with interpretable significance.

152

153   Temporal patterns are derived by collecting data for visits and time spent at

154   different zones in the arena in five 5-minute time bins for each phase and in aggregate

155   over the entire phase. In our main study, we found that 5-minute time bins perform well

156   for phenotype detection and are relatively easily interpreted as early, mid and late stage

157   phenotypic effects. Nonetheless, it is possible that new phenotypic information could

158   potentially be uncovered by further atomizing behaviors with finer temporal resolution.

159   Additional, temporal dimensions of behavior patterns are revealed from latency

160   measures, as well. As an illustration of how these different temporal dimensions might

161   find different patterns, a mouse may obtain a lot of food from Pot #2 during the

162   Exploration phase by simply stopping there and eating, which could be reflected in a

163   long duration of time at Pot #2. Alternatively, the mouse could obtain a lot of food by

164   darting in and out quickly from the food pot many times and performing many short

165   visits. For this sort of potential effect, examining both visits and durations at different

166   areas in the arena at different times during the assay can potentially reveal important

167   patterns. Similarly, mice may initially spend a lot of time near the tunnel to the home

168   base during the Exploration phase, but gradually become comfortable exploring pots

169   further away from the home base cage (Pots #2 and #3) at later stages, performing

170   longer and longer excursions into the arena. They may sprint through an exposed

171   region, like the center, to reach an objective at early stages, like a pot of interest, or they

172   may slowly creep around the arena, cautiously and systematically exploring. Overall, we

173   don't know which features or patterns might be shaped by particular genomic elements

174   or what patterns might emerge at different times. Therefore, we systematically capture

175   visit, duration and latency data for all of the different regions defined in the arena.

176

177   Locomotor features and patterns are major component of our analysis of foraging

178   phenotypes with our screen. Locomotor patterns can potentially reflect diverse

179   phenotypic effects that could be related to anxiety & fear levels, activity, motivational

180   state, motor patterns, anatomical phenotypes and many other effects (Drai et al., 2000;

181   2001; Fonio et al., 2009; Kafkafi et al., 2003). Indeed, human studies have found

182   important and unexpected relationships between motor patterns and various psychiatric

183   symptoms (Peall et al., 2017). In the current version of our screen, we analyze the

184   length, frequency and duration of slow, medium and fast bouts of velocity at different

185   stages of the testing periods. We also classify Exploration and Foraging phase

186   excursion patterns into major subclasses based on the elements of the arena that are

187   visited, the distance traveled, the duration of the excursion, and the frequency/duration

188   of the different velocity bouts. By deeply analyzing these different features of locomotion

189   we expect to be able to uncover diverse phenotypic effects that manifest in this

190   dimension of foraging behavior.

191

192   Finally, food consumption and digging behaviors are important features of

193   foraging that can be shaped by a variety of phenotypic effects. For example, while

194   hunger can increase food consumption, stress & fear can potentially reduce the amount

195   of food consumed (Brown and Kotler, 2007). Additionally, digging is a normal part of

196   mouse exploratory and foraging, but could indicate abnormal activity, repetitive or

197   perseverative behaviors in our assay. By analyzing visits, time spent and the mount of

198   digging in Pots #1 and #3, which don't contain food, we may learn about the animal's

199   strategies for exploring other potential patches even when an abundant and obvious

200   resource has been uncovered (eg. Pot#2 in the Exploration phase). Thus, measuring

201   the total sand dug from each pot and the total food consumed during each phase

202   provides us with important insights into the behavioral patterns expressed and their

203   impact on the central goal of the behavior – finding and consuming food.

204

205   Our discussion above explains the rationale for the assay design and the

206   features we detect. While there are many dimensions of natural foraging that we cannot

207   capture, our screen nonetheless examines a variety of different behavioral and

208   locomotor features to detect diverse patterns that we would not have predicted *a priori*.

209   In total, we obtain 336 features from the assay and each is assigned a unique ID (Table

210   S1). We do not claim that the features we defined are the only important features that

211   could be extracted from our assay and we expect that future studies will continue to

212   resolve new and important features of foraging in our screen. However, in the main text,

213   we provide evidence that our approach robustly detects a variety of different phenotypic

214   effects at different ages. For some phenotypes, some features may be redundant, but

215   they are not necessarily redundant and might reveal important phenotypic information in

216   other mouse models not tested in the current study. Importantly, random forest

217   ensemble learning, which we use to detect distinguishing behavioral patterns, is not

218   confounded by correlated features or high dimensional data, and therefore the addition

219   of more spatiotemporal and locomotor features simply expands the landscape for

220   pattern discovery.

221

222 **Known Phenotypic Differences between Cast and B6 Mice Could Potentially**

223 **Contribute to Parental Effects on Offspring Phenotypes**

224 By analyzing data in the Mouse Phenome Database (Grubb et al., 2013), we found that

225 Cast females have a significantly lower Parenting Index (ratio of weaned to born pups)

226 compared to B6 females, indicating differences in maternal behavior/physiology that

227 impact offspring postnatal survival (Table S2). In addition, Cast and B6 females differ

228 significantly in terms of litter size, fecundity, body size, hormone levels, metabolic traits,

229 activity, body composition, immune cells and cardiovascular traits (Table S2).

230 Differences in these traits can influence offspring development (Asvold et al., 2011;

231 Boersma et al., 2014; Lim et al., 2014; Moog et al., 2015; Schlotz and Phillips, 2009;

232 Westberg et al., 2016). Beyond behavioral/physiological differences, cis-regulatory

233 differences between Cast and B6 mice cause differences in the expression levels of

234 some imprinted genes in F1cb versus F1bc offspring (Bonthuis et al., 2015), and

235 mitochondrial genes are Cast-derived in F1cb mice, but B6 derived in F1bc mice. Thus,

236 F1cb and F1bc offspring have various potential differences in environmental and

237 genetic parental effects that could shape offspring behavioral phenotypes in our screen.

238

239 **SUPPLEMENTAL EXPERIMENTAL PROCEDURES**

240 The initial prototype foraging assay design and protocol, as performed in this study, is

241 provided below. In a separate supplemental Protocol file, we provide a detailed protocol

242 for an updated arena design, assay methodology and computer code for the data

243 analysis.

11

244

245   Foraging Assay Protocol:

246   In preparation for the foraging assay, mice were first habituated with sand (Jurassic play

247   sand, Jurassic Sand) and seeds (Whole millet, Living Whole Foods) for two days in their

248   home cage. On day one, seeds are spread on top of sand in the bottom of a petri dish

249   and the dish is placed on the bedding in the home cage for the mice and pups to

250   explore. On day two, seeds are covered with sand in the bottom of the petri dish in the

251   home cage for the mice and pups to dig in and explore. To motivate animals to feed,

252   mice were food deprived prior to testing to achieve 8-10% weight loss at the time of

253   testing. For P15, P20 and P25 mice, weight gain normally occurring during the period of

254   food deprivation was taken into account to achieve the intended weight loss. We

255   selected this weight loss target after several pilot studies with the goal of achieving

256   some consistency in the motivational states of the animals at different ages and not

257   compromising health or activity. To achieve the intended weight loss and motivational

258   state, adult mice were food deprived for 24 hours, P25 mice were food deprived for 17

259   hours, P20 mice were food deprived for 17 hours and housed with the mother, P15 mice

260   were food deprived for 4 hours prior to testing in the absence of the mother. For food

261   deprivation, mice are moved into a fresh cage with some soiled bedding to ensure

262   familiar smell, but no leftover food in the cage. Water is available ad libitum at all times

263   except when mice are in testing cage (2 x 1 hour).

264   Mice are housed in a room with an 11:00 – 23:00 dark cycle, so that testing is

265   performed during the dark cycle. For testing, mice are moved into the behavior room

266   prior to the start of testing for at least 1 hour for habituation to the new room. All testing

12

267   is performed in the dark and video recording is done using infrared illumination and all

268   manual procedures are done in the dark using a headlamp with red light. The mouse to

269   be tested, and their home cage soiled bedding, are moved to the testing-cage attached

270   to the arena 30 minutes prior to testing and allowed to habituate. At the start of testing,

271   the testing-cage is attached to the arena via the tunnel, the mouse now has access to

272   the arena and video recording starts for the Exploration phase. Mouse behavior is

273   recorded continuously during the 30 min Exploration phase trial under infrared lights.

274   Noldus Ethovision software v8 and v9 were used for video tracking. After completing the

275   Exploration phase, the mouse is transferred to a holding cage with water but no food

276   until the Foraging phase four hours later. For the Foraging phase, mice are again

277   placed in the testing cage and habituated for 30 min. The testing cage is then gently

278   attached to the tunnel and access to the arena is possible and the Foraging trial begins.

279   Video recording of the Foraging phase is performed for 30 minutes. After testing, mice

280   are placed in a new cage with food and are returned to the mouse colony room.

281   Importantly, between each Exploration and Foraging phase trial, the entire arena,

282   including walls, platform, tunnel and steel pots, are wiped clean with 70% ethanol.

283

284   Preparation of Sand and Seed Pots for the Foraging Assay:

285   Three stainless steel pots (Resco, diameter 5.5cm, depth 4cm) were filled with 95g of

286   sand. For the Exploration phase, 1 pot is filled with 80g of sand covered with 2.5g of

287   seeds. On top of seeds, a layer of 12g of sand is added to cover seeds. This sand is

288   then covered with 0.5g of seeds. This pot is placed in position 2 in the arena. For the

289   Foraging phase, 1 pot is filled with 80g of sand, 3g of seeds on top of sand and

13

290   additional 12g of sand to cover all seeds. This pot is placed in position 4 in the arena.

291   All pots are weighed before and after the trial to measure the sand displaced from each

292   pot. Remaining seeds and hulls left in the pot and on the platform are measured after

293   each Exploration or Foraging trial to determine the amount of seeds consumed by the

294   mouse during the trial. Used sand is collected after every trial and set aside. At the

295   completion of all testing, the used sand is autoclaved before reuse in future trials.

296

297   <u>Foraging Arena Construction:</u>

298   The foraging arena, tunnel and testing cage were custom built with acrylic plastic

299   (Delvies Plastics, Salt Lake City, UT, USA). The 14 cm long tunnel enters the platform

300   from underneath through one of the five 5.5 cm diameter holes in the arena platform.

301   The platform is 8 cm above stage level and is made from 0.5 cm thick white Plexiglas.

302   The arena is made from a transparent Plexiglas tube and the 0.4 cm thick walls raise 42

303   cm above the platform. The arena has a diameter of 35 cm. The walls of the arena were

304   roughened with sand paper to limit glaring and recording artifacts.

305

306   <u>Arena Tracking Zones:</u>

307   The arena is organized into zones that are used to breakdown the behavior and

308   foraging strategies used by each animal in the assay as detailed in Figure S1. Here, we

309   provide additional details regarding the definition of these zones. The arena is divided

310   into five sectors and the boundary of each sector is the mid point between two pots (see

311   Figure S1). The arena is further divided into three concentric circles, including the

312   middle center zone, the intermediate zone and the outer wall zone. The outer radius of

313   the *Intermediate* zone intersects with the center of the pots and tunnel entry. The radius

314   of the *Center* zone is half the radius of the *Intermediate* zone. A zone is also created

315   around each pot in the arena. *Pot* zones have a radius of 1.7x the radius of the pot itself.

316   Finally, to learn about the behavior of the animal related to entries to and exits from the

317   arena, we define zones around the tunnel entry. The *Tunnel Entry* zone aligns with the

318   entry hole of the actual tunnel. The *In Tunnel* zone is covering the most peripheral area

319   of the *Tunnel Entry* and tracks the mouse just before leaving the arena completely.

320   Whenever the mouse is in the cage, the tracking system is recording the mouse as

321   being in the *In Tunnel* zone. The *Tunnel Zone* area has the same radius as the *Pot*

322   zones.

323

324   <u>Automated Tracking:</u>

325   At the start of the trial, the tracking begins with a 10 second delay to allow time for the

326   connection of the testing-cage to the arena. The mouse is first tracked when it appears

327   in the *In Tunnel zone* (Figure 1) and position and movement is continuously recorded

328   after this time point until the end of the 30 min Exploration or Foraging Phase. The XY

329   position of the center of the mouse is video tracked with at a rate of 30 frames per

330   second. For the data analysis, tracking data from 0-25 minutes are used. Time spent in

331   each zone, latency to visit a zone and number of visits to each zone, as well as the

332   distance traveled, are calculated using the Ethovision software. The data is exported as

333   results for the total 25-minute trial duration, as well as in 5 minute time bins. Sand

334   displacement and food consummation measures are collected and calculated manually.

335

336   <u>Behavioral Measures:</u>

337   All 152 measures captured during Exploration or Foraging phases of the assay are

338   presented in Table S1. Each measure is assigned a custom ID, as indicated in the table.

339   To calculate the time spent in the *Tunnel Entry* zone, time in the *In Tunnel zone* is

340   subtracted from the raw time spent in the *Tunnel Entry zone*. To calculate the time

341   spent in *Tunnel Zone*, the raw time in the Tunnel Entry zone is subtracted from the raw

342   time spent in *Tunnel zone*. To calculate the total time spent in the arena, the time spent

343   in the *In Tunnel* zone is subtracted the raw time in the arena. For calculation of these

344   measures for specific time bins, the corresponding measures for each time 5 min time

345   bin output by Noldus Ethovision are used instead of the total time measures. For the

346   time spent in all other zones in the arena, the Noldus Ethovision output was used

347   directly for both total time and time binned results. The latency to appear on the platform

348   is scored manually, by selecting the last video frame when the center of the mouse is

349   tracked in the *Tunnel Entry* zone before climbing onto the arena and standing with all

350   four paws on the platform.

351

352   <u>Behavioral Measure Data Normalization:</u>

353   As indicated in Table S1, all 'time spent in zone', sand displaced, food consumed and

354   zone visit measures were normalized to the total time spent in the arena (TTA) by

355   dividing each value by TTA (x/TTA). For time bin values, the TTA for the corresponding

356   time bin is used for normalization (i.e. x-1/TTA-1). All latencies to visit pots were

357   normalized to the latency to enter the platform (LEP) by subtracting the LEP (x-LEP).

358   Latency to the center zone after arena entry (LCAE) is already normalized to LEP and

16

359 does not need any further normalization. All percentage measures do not need any

360 normalization. Time on the platform itself and time in the tunnel (cage) are not included

361 in the normalized dataset because they are closely related and redundant to TTA.

362

363 <u>Locomotor Measures:</u>

364 The raw data files generated by Noldus Ethovision listing all XY coordinated and all

365 zones as well as distance traveled and velocity for each frame were used to extract data

366 describing excursions and locomotor patterns using custom code, including the duration

367 and number of bouts at different velocities. An excursion is defined as beginning when

368 the mouse leaves the tunnel (In Tunnel zone) and ending when the mouse returns to

369 the tunnel (one round trip). The minimum number of frames for an excursion sequence

370 is 10. Continuous velocity values in the data were categorized into three velocity

371 classes, *slow*: velocity <= 5 equals; *medium*: velocity >5 <= 15; *fast:* velocity >15. The

372 length of the bout is calculated using the number of frames in the sequence and all

373 sequences of the same velocity class longer than 3 frames are counted as a single

374 velocity bout.

375

376 <u>DeepFeat and Data Analysis:</u> **See Detailed Protocol Folder**

377

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