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Experience recruits MSK1 to expand the dynamic range of synapses and enhance cognition

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1   **Experience recruits MSK1 to expand the dynamic range of synapses and enhance cognition**

2   Abbreviated title: MSK1 regulates the dynamic range of synapses and cognition

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41

ABSTRACT

Experience powerfully influences neuronal function and cognitive performance, but the cellular and molecular events underlying the experience-dependent enhancement of mental ability have remained elusive. In particular, the mechanisms that couple the external environment to the genomic changes underpinning this improvement are unknown. To address this we have used male mice harbouring an inactivating mutation of mitogen- and stress-activated protein kinase 1 (MSK1), a BDNF-activated enzyme downstream of the MAPK pathway. We show that MSK1 is required for the full extent of experience-induced improvement of spatial memory, for the expansion of the dynamic range of synapses, exemplified by the enhancement of hippocampal LTP and LTD, and for the regulation of the majority of genes influenced by enrichment. In addition, and unexpectedly, we show that experience is associated with an MSK1-dependent downregulation of key MAPK and plasticity-related genes, notably of EGR1/Zif268 and Arc/Arg3.1, suggesting the establishment of a novel genomic landscape adapted to experience. By coupling experience to homeostatic changes in gene expression MSK1, represents a prime mechanism through which the external environment has an enduring influence on gene expression, synaptic function and cognition.

59 **SIGNIFICANCE STATEMENT**

60 Our everyday experiences strongly influence the structure and function of the brain. Positive

61 experiences encourage the growth and development of the brain and support enhanced learning and

62 memory and resistance to mood disorders such as anxiety. While this has been known for many

63 years, how this occurs is not clear. Here we show that many of the positive aspects of experience

64 depend upon an enzyme called MSK1. Using male mice with a mutation in MSK1 we show that MSK1

65 is necessary for the majority of gene expression changes associated with experience, extending the

66 range over which the communication between neurons occurs, and for both the persistence of

67 memory and the ability to learn new task rules.

INTRODUCTION

Experience exerts a profound influence on the structure and function of the mammalian brain (Kolb and Whishaw, 1998). While this was predicted by early physiologists (von Bernhardi et al., 2017), it was the pioneering work of Donald Hebb in the 1940s that first demonstrated the enhanced cognitive abilities of rats raised in the stimulating environment of his home compared to their counterparts reared in a laboratory (Hebb, 1947, 1949). Subsequent studies have identified many cellular changes associated with the enhanced cognition that arises following exposure to larger social groups, a complex environment and exercise wheels, including neurogenesis, greater dendritic spine density and enhanced synaptic plasticity (Rosenzweig and Bennett, 1996; Sale et al., 2014; Ohline and Abraham, 2019). In experimental animals these cellular adaptations translate into enhanced cognition (Sale et al., 2014), reduced anxiety (Rogers et al., 2019), the acceleration of recovery from brain injury (de la Tremblaye et al., 2019), resistance to drugs of abuse (Stairs and Bardo, 2009) and the alleviation of signs associated with animal models of Parkinson's disease (Wassouf and Schulze-Hentrich, 2019), Huntington's disease (Mo et al., 2015) and autism spectrum disorder (Gubert and Hannan, 2019). Indeed, parallels have been described in humans, including for children with autism (Woo and Leon, 2013; Woo et al., 2015; Aronoff et al., 2016), in terms of the benefits for mental health and wellbeing, and brain structure and function, of social interaction, skills training, exercise and higher socioeconomic status (Kolb and Whishaw, 1998; Sale et al., 2014; Farah, 2018; Miguel et al., 2019; Rogers et al., 2019).

However, despite the importance of establishing how experience influences brain function, the intracellular signalling cascade and the enduring influence on the genome that underlie these cellular and cognitive adaptations to enriched environments have yet to be identified. An ideal candidate to

91 mediate such a coupling between the environment and the genome would be positioned between

92 the BDNF TrkB receptors repeatedly implicated in mediating the benefits of environmental

93 enrichment (Cowansage et al., 2010; Sale et al., 2014; Rogers et al., 2019), and the genomic changes

94 required for persistent modifications to neuronal structure, synaptic function and learning and

95 memory (Alberini and Kandel, 2014; Takeuchi et al., 2014).

96

97 The nuclear kinase, mitogen- and stress-activated protein kinase 1 (MSK1), is well-placed to transduce

98 the sensory experiences associated with enrichment into the enduring cellular, molecular and

99 genomic events underpinning enhanced cognition. MSK1 is activated by BDNF and regulates gene

100 expression, notably via the phosphorylation of CREB (Arthur et al., 2004; Reyskens and Arthur, 2016),

101 and including that of the plasticity-related protein Arc/Arg3.1 (Hunter et al., 2017). In addition, MSK1

102 is expressed in hippocampal neurons (Heffron and Mandell, 2005; Sindreu et al., 2007), a major site of

103 the effects of environmental enrichment (Hirase and Shinohara, 2014). Using mice harbouring a

104 knock-in point mutation of the MSK1 gene that results in the elimination of the kinase activity of

105 MSK1 (kinase dead; MSK1 KD), but which does not affect hippocampal learning and memory or

106 synaptic plasticity under standard housing conditions (Daumas et al., 2017), we previously showed

107 that the kinase activity of MSK1 was required for homeostatic synaptic scaling in vitro, and the in vivo

108 enrichment-induced enhancement of miniature excitatory postsynaptic currents (Corrêa et al., 2012;

109 Lalo et al., 2018). However, this left unanswered the important question of the genomic, plasticity

110 and cognitive implications of these isolated observations at the synapse.

111

112 Using wild-type (WT) and MSK1 KD mice we have found that the kinase activity of MSK1 is necessary

113 for the full benefits of enrichment on cognition, in particular, in the persistence of hippocampal

114   spatial memory and cognitive flexibility. As a potential cellular correlate of this enhanced cognition,

115   we discovered that enrichment is associated with an MSK1-dependent expansion of the dynamic

116   range of synapses: both hippocampal long-term potentiation (LTP) and long-term depression (LTD)

117   are enhanced, thereby allowing synapses to code a greater amount of information. Finally, an RNA-

118   Seq analysis of the hippocampal transcriptome under standard and enriched conditions revealed a

119   predominant requirement for MSK1 in the experience-dependent regulation of gene expression.

120   Moreover, we observed an unexpected and MSK1-dependent downregulation of plasticity-associated

121   proteins and transcription factors such as Arc/Arg3.1 and EGR1. These observations suggest that

122   MSK1 couples the external environment to the genome, and through this coupling initiates both the

123   cellular and molecular events leading to synaptic and cognitive enhancement, and an experience-

124   dependent genomic homeostasis designed to maintain the stability of the enhanced brain.

125

126 **MATERIALS AND METHODS**

127 **Animals**

128 The MSK1 kinase dead (KD) mouse used in this study has been described previously (Corrêa et al.,

129 2012). Briefly, Asp194 in the endogenous MSK1 gene was mutated to Ala (D194A). This inactivates the

130 N-terminal kinase domain of MSK1. Genotyping was carried out by PCR using the primers 5'-

131 CGGCCATGTGGTGCTGACAGC-3' and 5'-GGGTCAGAGGCCTGCACTAGG-3', which gives 378 bp and 529

132 bp products for WT and targeted alleles, respectively. All the mice used in this study were on a C57-

133 Bl/6J genetic background after at least four backcrosses from the original C57-Bl/6n strain used by

134 Taconic Artemis to generate the mutant mice. Male WT C57-Bl/6J mice purchased from Charles River

135 UK were used for backcrossing with female MSK1 KD homozygous mutants. The mice used in this

136 study were maintained as homozygous and WT lines derived from founder homozygous and WT

137 breeders from an initial series of heterozygote crosses. Subsequent backcrossing occurred when the

138 founder mice had come to the end of their reproductive lifetime (typically three litters). This strategy

139 avoided genetic divergence of the two lines. While using WT and homozygous mutant littermates

140 from heterozygote crosses is experimentally desirable, our breeding strategy is appropriate when

141 homozygous mutants of both sexes are viable and fertile (Jax, 2009), allowed large numbers of

142 animals of the correct age, genotype, housing condition and sex to be bred in order that experiments

143 could be conducted in time-limited batches, minimizing variability. Our breeding strategy also avoided

144 the unnecessary breeding and culling of large numbers of heterozygote mice (50% of all litters) in

145 keeping with the drive to reduce the number of animals used in research, and with institutional and

146 funder expectations. We note that many experimental parameters were similar between WT and

147 MSK1 KD mice under standard and enriched housing conditions, and that the hippocampal expression

148 of only three genes differed between the two genotypes under standard housing conditions. This

149   suggests that the breeding strategy did not introduce confounds that could have affected our

150   observations.

151

152   Mice were maintained under a 12/12 light dark cycle (lights on at 7.00 am) in a facility kept at 20-24

153   °C and were given ad libitum access to standard mouse chow and water. All animal procedures

154   conformed with local, national and EU guidelines concerning the welfare of experimental animals.

155   Behavioral studies were performed under the auspices of Home Office licence PPL 70/7821 granted to

156   BGF. Male mice were used in this study to facilitate comparison with previous studies on MSK1 KD

157   mice (Correa et al., 2012; Daumas et al., 2017). The mice have been deposited with the

158   INFRAFRONTIER/EMMA repository at MRC Harwell, UK (https://www.har.mrc.ac.uk).

159

160   **Environmental enrichment**

161   Environmental enrichment was provided via the rearing of WT and MSK1 KD mice in large individually

162   ventilated rat cages (Tecniplast 1500U; 480 x 375 x 210 mm; 1500 cm^2 floor area) containing bedding

163   material, a cardboard tube, one running wheel and several plastic toys (tunnels, platforms, see-saws)

164   and a metal ladder. To provide novelty, toys were moved around twice per week and new toys

165   introduced once per week. Two to three pregnant dams (E14-15, based on vaginal plugs) were

166   randomly selected and placed in enriched cages to provide additional mothering (D'Amato et al.,

167   2010) and larger groups for social interactions. Dams typically gave birth within 1 – 2 days of each

168   other. At weaning (P23-24), all females were removed and the males (typically 8) remained in the

169   enriched environment for the remainder of the experimental period (to ~5 months of age). Age-

170   matched male mice were born and maintained in standard housing (Tecniplast 1284L; 365 x 207 x 140

171   mm; 530 cm^2 floor area; two to four mice with bedding material and a cardboard tube) and served as

9

172   controls for the environmental enrichment groups. Cage cleaning was done on Mondays for all

173   standard and enriched cages. Toys in enriched cages were changed on Tuesdays and were moved

174   around the enriched cages on Mondays and Thursdays. To keep disruption of the home environment

175   to a minimum, sawdust and bedding were never changed at the same time as toys. To minimise

176   disruptions to established hierarchies, during cage cleaning and behavioural testing all mice (standard

177   and enriched) were removed to a different cage (standard cage size with one toy from the enriched

178   cage for enriched mice) and then were returned together. This was effective in reducing within-cage

179   aggression between the males.

180

181   **Analysis of dendritic spine density**

182   Male WT and MSK1 KD mice were killed at 17-18 weeks of age, two weeks after the end of

183   behavioural testing, by cervical dislocation in accordance with the UK Animals (Scientific Procedures)

184   Act 1986 and with local Animal Welfare and Ethical Review Board approval. The brains were removed

185   and processed with the FD Rapid Golgi Stain kit (FD NeuroTechnologies, Inc.) in accordance with the

186   manufacturer's protocol. Impregnated brains (4 per group) were sectioned with a vibratome (coronal

187   sections; 200 μm thick) stained and mounted. Dendritic spines on the secondary branches of apical

188   dendrites of hippocampal CA1 neurons were counted. Spine count was conducted blind to genotype

189   and housing condition. ImageJ software was used to measure dendritic length and the numbers of

190   spines on each dendritic segment. Images for spine density analysis were captured with a 40x

191   objective on a Zeiss Imager 2 AXIO microscope.

192

193   **Hippocampal slice preparation and extracellular recordings**

194   Male WT and MSK1 KD mice (3-5 months old) were killed by cervical dislocation in accordance with

195   the UK Animals (Scientific Procedures) Act 1986 and with local Animal Welfare and Ethical Review

196   Board approval. Hippocampal slices (400 μm) were cut in ice cold aCSF using either a Stoelting tissue

197   chopper or a Microm HM650V tissue slicer. Upon cutting, slices were transferred to a recording

198   chamber and placed upon a mesh support at the interface of an oxygen-rich atmosphere and

199   underlying aCSF where they remained for the duration of the experiment, which typically started

200   some 2 hours after slice cutting. The temperature of the aCSF was set at 31°C and the flow rate was

201   1.5 ml/min. The aCSF used for the preparation, maintenance and recording of slices contained in mM:

202   124.0 NaCl, 4.4 KCl, 1.0 Na_2HPO_4, 25.0 $NaHCO_3$, 2.0 $CaCl_2$, 2.0 $MgCl_2$, 10.0 D-glucose. aCSF was

203   bubbled with 95 % O_2/5 % CO_2; pH 7.4. All salts used in the aCSF were obtained from either Fisher

204   Scientific or Sigma-Aldrich.

205

206   To make extracellular recordings of field excitatory postsynaptic potentials (fEPSPs), an aCSF-filled

207   glass microelectrode was placed in stratum radiatum of area CA1 and two concentric bipolar

208   stimulating electrodes (CBBRC75, FHC) were placed either side of the recording electrode. This

209   allowed alternating recordings to be made from two independent but convergent afferent Schaffer

210   collateral/commissural fibre pathways. Each pathway was stimulated every 90 s with a monophasic

211   pulse of 0.1 ms duration. Pathway-independence was assessed via a crossed paired-pulse facilitation

212   protocol (at 50 ms interpulse interval). Independence was accepted when facilitation of the second

213   pulse was ~10 % or less. To assess basal synaptic transmission, stimulus input/fEPSP slope output

214   curves were constructed over the range of 20 - 300 μA. A minimum of four fEPSPs were averaged to

215   yield a fEPSP slope measurement at each stimulus intensity. At the highest stimulus intensity (300

216   μA), and where visible, the presynaptic fibre volley was measured as an indicator of the recruitment

11

217   of afferent axons. Paired-pulse facilitation (PPF), a commonly used index of the probability of

218   neurotransmitter release, was assessed over an inter-stimulus interval of 50 – 350 ms, with the

219   average of at least 2 fEPSPs yielding the slope measurement at each paired-pulse interval. In all

220   experiments both pathways in each slice were tested for input-output and PPF profiles and all were

221   taken into consideration in subsequent analyses.

222

223   For the LTP and LTD experiments a stable baseline of at least 30 mins was achieved before theta-burst

224   (TBS) or low-frequency stimulation (LFS) was delivered to one pathway. TBS consisted of bursts of 4

225   stimuli at 100 Hz with 10 such bursts comprising a train. Each burst within a train was separated by

226   200 ms. Trains were repeated 3 times with an inter-train interval of 20 seconds. LFS consisted of 900

227   pulses at 1 Hz. The second pathway was not subject to TBS or LFS and served as a control for the

228   stability of the recordings. Experiments were excluded from analysis if the control pathway

229   deteriorated by more than 10 % within the 3 hours post-TBS, and 1 hour post-LFS, monitoring period.

230

231   Given the deficit in basal synaptic transmission observed in MSK1 KD mice, care was taken to match

232   the baseline strength of synaptic transmission, which involved adjusting the stimulus intensity to yield

233   fEPSPs of ~3 mV across all groups. A one-way ANOVA showed no difference in baseline fEPSP

234   amplitudes across the four experimental groups for either the LTP ($F_{(3,28)}$ = 0.676; p = 0.574) or LTD

235   ($F_{(3,22)}$ = 0.975; p = 0.422) experiments. Electrophysiological recording parameters and the analysis

236   of fEPSPs were under the control of WinLTP program (Anderson and Collingridge, 2007). LTD

237   experiments, and the majority of LTP experiments, were performed in experimentally naïve mice of 3

238   – 4 months of age. Experiments were interleaved and performed blind to the identity and housing

239 condition of the mice, which was revealed only after the experiments had been analysed and

240 genotype confirmed with post-hoc genotyping as required.

241

242 **Western Blotting**

243 Experimentally naïve mice (3-4 months of age) were killed by cervical dislocation as described above.

244 The brain was removed and individual hemispheres were snap-frozen in liquid nitrogen and stored at

245 -80°C. When required, samples were defrosted, the hippocampus dissected free and lysed in lysis

246 buffer containing: 50 mM Tris-HCl, pH 7.5, 1% Triton X-100, 0.1% SDS, 1 mM Na_3VO_4, 50 mM NaF, 5

247 mM $Na_4P_2O_7$, 0.27 M sucrose, 0.02% NaN_3, and protease inhibitor mixture tablets (Roche). The tissue

248 underwent mechanical disruption using a Dounce homogeniser. Samples were then stored on ice

249 before rotation at 4°C for 30 minutes, followed by centrifugation for 20 minutes and 12,000 g at 4°C.

250 The protein concentration of each sample was calculated using a standard BCA curve. Samples were

251 aliquoted, mixed with loading buffer, and stored at -20°C until required for western blotting.

252

253 After defrosting, samples were brought to 80°C for five minutes, spun briefly and the proteins were

254 separated using SDS-PAGE electrophoresis in an 8% gel. After separation, proteins were transferred

255 onto nitrocellulose blotting membrane (GE Healthcare) in a semi-wet system for 2.5 hours at 200 µA.

256 The membrane was blocked in 10% Marvel milk powder and 0.5% TWEEN for one hour. Membranes

257 were incubated in GAPDH primary antibody (Table 1) in 1% milk powder 0.05% PBS TWEEN (PBS-T)

258 solution for two hours at room temperature, following which they were washed for 10 minutes four

259 times in 0.1% PBS-T. Samples were incubated overnight at 4°C with a second primary antibody (GluA1,

260 GluA2, EGR1, or Arc/Arg3.1; Table 1) then washed four times for 10 minutes in PBS-T. Membranes

261 were incubated for 1-2 hours in HRP-conjugated anti-rabbit antibody (1:10,000 dilution; ThermoFisher

262 #31460). After four 10-minute washes with PBS-T, membranes were incubated for two minutes in

263 Clarity Western ECL Substrate (BioRad) and imaged using the Image Quant LAS 4000 CCD

264 biomolecular imager. Image Studio Lite vs 5.2.5 was used to analyse the signal of the bands and the

265 protein of interest was normalised to GAPDH. Control blots confirmed that the GAPDH antibody gave

266 no detectable bands at the predicted molecular weights of GluA1, GluA2, EGR1 or Arc/Arg3.1.

267

268 Table 1 Antibodies used in the study

Primary antibody	Supplier (Cat #)	Concentration of primary Ab
EGR1	CST (4153S)	1:500
Arc/Arg3.1	Abcam (AB183183)	1:500
GluA1	Abcam (AB31232)	1:1000
GluA2	Abcam (AB 1768)	1:2000
GAPDH	CST (2118S)	1:160,000 (for Egr1 or Arc/Arg3.1 blots) 1:40,000 (for GluA1 or GluA2 blots)

269

270 **Behavioural Procedures**

271 Mice used were male aged 2.5 to 3.5 months and were scored for weight and against a battery of

272 tests for neurological signs (Wolf et al., 1996) before any behavioural experiment began. No

273 neurological signs were observed across any of the groups (data not shown). Different tests were

274 conducted at weekly intervals to avoid one test influencing another: the week commencing (w/c)

275 postnatal day (P) 70 open field and novel object; w/c P77 elevated plus maze; w/c P84 spontaneous

276 alternation; w/c P91- w/c 104 watermaze, a 2 week protocol.

277

278 *Open field and novel object*

279   These tests were run as two consecutive stages of the same experiment. Four open field boxes (Ugo

280   Basile; 44 x 44 x 44 cm) were placed inside the empty water maze arena to form a square. Four mice

281   were tested simultaneously. Each mouse was singly released in each box and tracked. Exposure to the

282   open field lasted for 1 hour after which, for the novel object stage of testing, a 50 ml plastic vial

283   (Falcon) was secured upside-down to the centre of the arena and the mouse was tracked for an

284   additional hour.

285

286   ***Elevated plus maze***

287   An 8-radial arm maze for mice (Ugo Basile) was placed within the empty water tank and raised 60 cm

288   from the tank base. Four of the eight arms were kept open to form a plus shape; two of the arms had

289   walls while the other two (opposite one another) were without walls. Each mouse was individually

290   released in the centre of the maze and video tracked for 10 minutes.

291

292   ***Spontaneous alternation for spatial working memory***

293   An 8-radial arm maze for mice (Ugo Basile) was placed within the circular confines of the tank used for

294   the water maze. Four out the eight arms (with walls) were kept open to form a cross. The entrances

295   to the other four arms were closed. Each mouse was individually released in the centre of the maze

296   and video tracked for 10 minutes. The sequence of arm entries was scored. A correct alternation was

297   considered when a mouse made no repetition in four entries (Mohler et al., 2007).

298

299   ***Water maze for spatial reference memory***

300   Experimentally naïve mice were used and an inter-trial interval of 120 s over 4 daily trials was

301   employed. The pool was filled daily with fresh water, which was made opaque by the use of UHT milk.

15

302

303 Stage 1 Habituation: each mouse was placed on a 20 cm diameter platform located in the centre of a

304 180 cm diameter pool filled with opaque water (28°C) and was allowed to observe the environment

305 for two minutes. The pool was surrounded by curtains which did not allow the distal visual cues to be

306 seen. Water level was ~1 cm above the top of the platform. Each mouse then received 3 consecutive

307 trials (different starting points) where it was left free to swim in the pool for a maximum of 2 minutes

308 and then placed on the platform and left there for 30 sec.

309 Stage 2 (days 2 and 3) Visual Cue: The platform was placed in the centre of the pool and a visible

310 object was placed upon it (yellow TV toy 6 x 6 x 5 cm). Each mouse received 4 consecutive trials

311 (different cardinal starting points) where it was left free to swim in the pool for a maximum of 2

312 minutes. Water level was ~ 1 cm above the platform surface. Water was kept at 26°C. The pool was

313 surrounded by curtains which did not allow the distal visual cues to be seen.

314 Stage 3 (days 4 to 7) Training: Curtains were removed. Water was kept at 26°C. The platform was

315 placed in the centre of the South-East or North-West quadrant and kept constant for any given

316 mouse. Water level was ~ 1 cm above the platform surface. Each mouse received 4 trials (different

317 starting points) where it was left free to swim in the pool for a maximum of 2 minutes and then left

318 on the platform for 30 sec.

319 Stage 4 (day 8) Precision Testing: The platform was reduced from 20 cm to 10 cm in diameter to test

320 for more specific memory of the location of the escape platform. All other parameters as per Stage 3.

321 Stage 5 (day 9) 24 hrs delay Probe trial: Water was kept at 26°C. The platform was removed and distal

322 spatial cues were present as per previous the stage. Each mouse received a single 120 sec trial.

323 Starting point was distal to the location of the platform during training; e.g. if platform was South-East

324 starting point was North.

325

326   *Water maze reversal learning protocol for cognitive flexibility*

327   Stage 1 Habituation: As described above but two days were given instead of one.

328   Stage 2 (days 3 and 4) Visual Cue: As described above.

329   Stage 3 (days 5 to 7) Training: As described above.

330   Stage 4 (days 8 and 9) Reversal Learning: The platform was placed in the quadrant opposite to that

331   used during Training. All other parameters as per Stage 3.

332   Stage 5 (day 10) 24 hrs delay Probe trial: As described above. Starting point was distal to the location

333   of the platform during Reversal Learning stage (Stage 4).

334   Mice used for this experiment were experimentally naïve with respect to the water maze, but had

335   undergone open field ± novel object, the elevated plus maze and spontaneous alternation.

336

337   Behavioural tests were video-tracked and analysed using AnyMaze 4.99 video tracking system. All the

338   behavioural experiments were conducted blind to genotype.

339

340   **RNA-Seq:**

341   Hippocampal RNA was prepared from experimentally naïve mice of 3 – 4 months of age. Samples

342   were prepared and analysed blind to the two genotypes and two housing conditions. The hippocampi

343   from contemporaneous mice were used for western blotting for GluA1, GluA2, EGR1 and Arc/Arg3.1

344   as described above.

345

346   *RNA extraction and library preparation*

347 Hippocampi were extracted and then rapidly homogenized in Trizol (Invitrogen, #15596018). Total

348 RNA was precipitated using isopropanol following the manufacturer's protocol and treated with

349 DNaseI. RNA quality was checked using Nanodrop and a Qubit 4 fluorimeter (Invitrogen).

350

351 mRNA libraries were prepared using the TruSeqv2 (Illumina, #RS-122-2001) LS protocol in-house by

352 the School of Life Sciences Genomics Facility. Briefly, poly-A mRNA was pulled down using poly-T

353 magnetic beads, fragmented, and primed with random hexamers before first-strand synthesis.

354 Following second-strand synthesis, blunt end repair was performed with a 3' to 5' exonuclease, and 3'

355 ends adenylated. Adaptors were then ligated to the cDNA. All 24 library samples were quality checked

356 on a 2100 bioanalyser (Agilent) and assayed on a Qubit 4 fluorimeter (Invitrogen) before being

357 multiplexed 6 samples to a lane and sequenced at 150bp paired end on an Illumina HiSeq 4000. An

358 average of 41.76M reads per sample were obtained (Extended Data Table 7-1 for Figure 7).

359

360 **Analysis Pipeline**

361 *Quality Control and Trimming:* Samples were de-multiplexed and the raw fastq files quality checked

362 using FastQC (v0.11.3) (Andrews, 2010). Adaptor contamination was removed using Skewer (v0.2.2)

363 (Jiang et al., 2014), with Illumina TruSeq v2 adapter lists, including reverse complements and

364 theoretical PCR product. Fastq files were also trimmed if the mean quality of bases dropped below 10

365 (4bp window) and only reads >50bp were kept. Adapter contamination removal was confirmed using

366 FastQC. Paired fastq files for each sample (forward and reverse) were aligned to the mouse genome

367 (GRCm38) using STAR aligner (v2.5) (Dobin et al., 2013), and annotated (GRCm38.87). Average read

368 alignment was 92.29% after exclusion of the 2 samples that failed quality control (Extended Data

369 Table 7-1 for Figure 7). Aligned BAM files were then loaded into IGV (v2.3.65) (Robinson et al., 2011)

370 and compared at the MSK1 gene locus, to check for a mismatch in the kinase domain of the MSK1

371 gene introduced as a point mutation into the kinase dead mutants (Corrêa et al., 2012). QC metrics

372 were calculated for each sample using SeqMonk (Andrews, 2018), examining high probe read counts

373 across ribosomal RNA and mitochondrial genes, and observing how many reads fell within genes and

374 exons. HtSeq (v0.6.1p1) (Anders et al., 2015) was then used to quantify read counts for individual

375 genes, using default parameters, specifying unstranded reads and only unique read alignment. A

376 minimum average PHRED quality score of 10 was necessary for reads to be counted.

377

378 Sample 34B contained a large percentage (~33%) of reads mapping to ribosomal RNA (rRNA).

379 Additionally, sample 34B contained roughly 1% of the read number of sample 35A, indicating poor

380 amplification of the cDNA library, and contained unacceptable sequence duplication levels. Ribosomal

381 (rRNA) contamination (~25% of reads) was also observed in sample 33B. Both sample 34B and 33B

382 were removed from further analysis based on poor quality control metrics.

383

384 Intra-group sample variation was observed to be quite high for some samples, and this interfered with

385 obtaining good quality distinct expression. Therefore, several more samples were removed for each

386 group, based on how well they correlated with other within-group samples. After alignment QC, and

387 after removing samples displaying poor intra-group correlation, a new sample table was made for

388 testing differential gene expression (Table 7-2). Differential gene expression lists for each condition

389 comparison are included in Extended Data Table 7-3 for Figure 7.

390

391 Principal component analysis was conducted in R (3.5.0) (RTeam, 2018) using the DEseq2 package

392 (v1.20.0) (Love et al., 2014). Differential gene expression statistical comparisons were conducted

393 using the Wald test statistic with a Benjamini-Hochberg-corrected p value cut-off of ≤ 0.05, and a log2

394 fold-change cut-off of 0.38 (corresponds to a 1.3-fold increase/decrease) used to define significant

395 differentially expressed genes. Gene ontology enrichment analysis was performed using the topGO

396 package (v2.32.0) (Alexa and Rahnenfuhrer, 2006) using the "classic" algorithm (Alexa and

397 Rahnenfuhrer, 2006) and Fisher's exact test for enrichment scoring against the ontology

398 org.Mm.eg.db (v3.6.0) (Carlson, 2018). Multiple-testing correction was carried out using Benjamini-

399 Hochberg correction, and GO-terms were considered significant at a corrected p-value ≤ 0.01. Unless

400 explicitly stated, default parameters were used for all tools and scripts used for these analyses and

401 will be provided upon request.

402

403 **Statistical Analysis**

404 Statistics were computed by IBM SPSS 25 using two-tailed one or two-way analysis of variance

405 (ANOVA) with genotype and housing condition as the two between group factors and day of training,

406 time-point or stimulus strength as within factor as appropriate, with simple main effects or main

407 effects as the post-hoc comparison. In the absence of significant housing x genotype interactions,

408 planned comparisons regarding the effects of genotype or housing were conducted, in keeping with

409 the views expressed in expert treatments of statistics (Faraway, 2005; Kutner et al., 2005; Howell,

410 2010; Wei et al., 2012; Laerd, 2017). The level of significance was taken to be p < 0.05. Data are

411 reported as mean ± SEM and bar graphs display individual data points.

412

413

414

20

RESULTS

MSK1 is necessary for the full extent of experience-dependent enhancement of cognition

To confirm that our enrichment protocol had tangible effects on animal behaviour, we initially assessed the influence of enrichment on locomotor function and anxiety, the latter of which in particular is sensitive to enrichment (Rogers et al., 2019). WT and MSK1 KD mice raised from birth in standard housing behaved similarly when exposed to an open field arena (Figure 1A, B) and the elevated plus maze (Figure 1C, D). In contrast to their counterparts raised in standard housing, enriched animals of both genotypes displayed reduced locomotor activity in the open field and in response to the introduction of a novel object (Figure 1A, B), and travelled further in the open arms of the elevated plus maze, indicative of reduced anxiety (Figure 1C, D). These observations confirm the effectiveness of the enrichment protocol in influencing behaviour, the absence of gross sensorimotor impairments in the MSK1 KD mutant mice that could confound subsequent investigations, for example, due to the high levels of MSK1 in the striatum and cerebellum (Heffron and Mandell, 2005), and the ability of MSK1 KD mice to display some benefits of enrichment.

To assess hippocampus-dependent forms of learning and memory, we began by testing spatial working memory using a spontaneous alternation task (Figure 2A). While standard housed mice of both genotypes performed at comparable levels (Figure 2B), there was a significant effect of enrichment that was reflected in significantly improved performance in WT mice, but not in the MSK1 KD animals (Figure 2B).

To extend these observations to spatial reference memory, the four groups of mice were tested in the Morris water maze. Both MSK1 KD and WT mice benefitted from enrichment in learning the position

438 of the platform more rapidly than their standard housed counterparts, who performed comparably,

439 as described previously (Daumas et al., 2017)(Figure 2C). Similarly, when the 20 cm platform was

440 replaced with a 10 cm platform on Day 5 of training to test the accuracy of learning the location of the

441 platform, enriched mice of both genotypes were better able to locate the smaller diameter platform

442 than standard housed mice, with the more selective searching of the enriched mice obvious from heat

443 maps of trajectories obtained on Day 5 (Figure 2D). These data suggest that MSK1 KD mice can display

444 some cognitive benefits of enrichment. However, these benefits were not lasting over time: during

445 the probe trial 24 hr later, enriched WT mice spent significantly more time in the training quadrant

446 than their standard housed counterparts, while no enrichment-induced improvement was observed

447 in the MSK1 KD mice (Figure 2E, F). MSK1 is therefore required for the full extent of enrichment-

448 induced persistence of spatial reference memory.

449

450 To further probe the requirement for MSK1 in the cognition-enhancing effects of enrichment, new

451 experiments with four additional groups of water maze-naïve animals were conducted using the

452 Lipp/Wolfer protocol for cognitive flexibility (Lipp and Wolfer, 1998). After training to asymptotic

453 levels by day 3, the location of the escape platform was moved to the opposite quadrant and

454 performance assessed on days 4 (R1) and 5 (R2) (Figure 3A). As expected, switching the location of the

455 platform to the opposite quadrant resulted in longer escape latencies across all groups on the first

456 exposure on reversal day 1 (Figure 3A, R1). However, by the second day of platform reversal (R2), the

457 enriched WT mice escaped more quickly than standard housed WT mice. This pattern was not seen in

458 enriched MSK1 KD mice, with latency to platform and heat maps of their swimming trajectory

459 observed to be comparable to that of standard housed WT mice (Figure 3A, B). This suggests a

460 requirement for MSK1 in the cognitive flexibility required for reversal learning.

461

462 Moreover, the failure to retain information over 24 hours was again shown by MSK1 mutant mice, but

463 now concerning the new location for escape: in the probe trial given 24 hrs later (Figure 3C, D), both

464 standard housed and enriched WT mice showed clear preference to navigate to the most recent

465 (reversed) location of the platform. In contrast, MSK1 mutant mice either displayed no preference

466 between the new and the old escape locations or, in the case of the enriched MSK1 KD mice, even a

467 preference for the former location of the platform used for training on days 1 – 3 (Figure 3C, D).

468

469 MSK1 KD mice that had experienced enrichment seemed to fare worse on both probe trial tests for

470 memory persistence. To examine this in greater detail we considered the amount of time spent in the

471 appropriate quadrant for both the small platform experiment (Figure 2C-F; target quadrant) and the

472 reversal learning experiment (Fig 3A-D; reversal quadrant). To compensate for differences in

473 performance across these two trials, we normalised performance across all groups to the mean of the

474 percentage time spent in the appropriate quadrant by standard housed WT mice. Bringing these two

475 data sets together in this way increases the power of the observations and allows more robust

476 conclusions to be drawn as to the performance of enriched MSK1 KD mice. This analysis (Figure 3E)

477 showed a significant genotype x housing interaction with enhanced memory ability in enriched WT

478 mice compared to enriched MSK1 KD mice. These observations indicate that enrichment improves

479 both spatial working and reference memory in WT mice, and that both the persistence of memory

480 and reversal learning, an index of cognitive flexibility, requires the kinase activity of MSK1.

481

482 ***Environmental enrichment induces synaptic homeostasis in an MSK1-dependent manner***

23

483   To establish the extent to which these cognitive impairments were reflected at the cellular level, we

484   performed electrophysiological recordings of basal excitatory synaptic transmission from area CA1

485   (Figure 4). We have previously reported an enhancement in mEPSC amplitude after enrichment in

486   WT, but not MSK1 KD mice (Corrêa et al., 2012; Lalo et al., 2018). We also reported that mEPSCs in

487   MSK1 KD mice were ~10% smaller than those recorded from WT mice (Corrêa et al., 2012; Lalo et al.,

488   2018), and more recently showed that this translated into smaller evoked field excitatory potentials

489   (fEPSP) in area CA1 of MSK1 KD hippocampal slices (Daumas et al., 2017). Thus, in order to establish

490   whether i) environmental enrichment also enhanced synaptic transmission at the population fEPSP

491   level in WT animals and ii) whether the basal fEPSP deficit in synaptic transmission in MSK1 KD mice

492   persisted or was ameliorated after enrichment, we constructed input/output curves of stimulation

493   strength *vs.* the slope of the fEPSP in slices taken from animals raised under standard housing or

494   environmentally-enriched housing (Figure 4A).

495

496   Consistent with previous observations under standard housing conditions (Daumas et al., 2017), basal

497   synaptic transmission in MSK1 KD slices was substantially reduced compared to that observed in WT

498   slices (Figure 4A). This was not due to an impairment in the recruitment of presynaptic axons as the

499   fibre volley amplitudes (Figure 4A) did not differ between these two groups, nor indeed between the

500   groups having underwent enrichment. To establish whether the reduced synaptic transmission

501   reflected differences in the expression of glutamate AMPA receptors, which are responsible for the

502   majority of excitatory synaptic transmission at CA1 synapses, we conducted western blots for GluA1

503   and GluA2, the two primary AMPAR subunits contributing to synaptic transmission in area CA1

504   (Tsuzuki et al., 2001; Lu et al., 2009; Renner et al., 2017; Terashima et al., 2017; Diering and Huganir,

505   2018). No differences in GluA1 or GluA2 expression were observed under standard housing conditions

506   that could explain the deficit in synaptic transmission observed in MSK1 KD mice (Figure 4B).

507

508   The deficit in basal synaptic transmission in MSK1 KD mutant mice compared to WT mice persisted in

509   animals raised in enriched conditions (Figure 4A). Moreover, enrichment had no discernible effect on

510   basal synaptic transmission in the MSK1 KD mutants; the input-output curves of standard housed and

511   enriched MSK1 KD mice essentially overlapped. In contrast, synaptic transmission in slices from

512   enriched WT mice was weaker over the entire range of stimulus strengths, but did not reach statistical

513   significance. While enrichment had no effect on GluA1 expression (Figure 4B), there was an

514   enrichment x genotype interaction for GluA2 levels such that there was an apparent increase and

515   decrease in WT and MSK1 KD mice, respectively. These changes in GluA2 expression do not directly

516   translate to the observed effects of enrichment on synaptic transmission, which saw decreases and no

517   change in WT and MSK1 KD mice, respectively, but suggest that both experience and MSK1 can

518   influence the expression of glutamate AMPA receptor subunits.

519

520   One potential explanation for the observation of a tendency to reduced synaptic transmission in WT

521   mice is that there has been a reduction in the probability of evoked glutamate release, such that on

522   average there are fewer synapses and postsynaptic AMPA receptors activated per stimulus. To test

523   this we constructed paired-pulse facilitation (PPF) profiles over the inter-pulse interval of 50 – 350 ms

524   (Figure 4C) as an index of the initial probability of glutamate release (Jackman and Regehr, 2017).

525   Under standard housing conditions, and as reported previously (Daumas et al., 2017), the PPF profile

526   of MSK1 KD mutant and WT slices were not different from one another. This suggests that MSK1 does

527   not play a role in regulating transmitter release under standard housing conditions.

528

529 Similar to the lack of effect on basal synaptic transmission (Figure 4A), raising MSK1 KD mice in

530 enriched conditions had no effect on the PPF profile compared to their standard housed counterparts.

531 However, WT mice raised in enriched housing showed a clear, consistent and significant *enhancement*

532 of PPF across the entire PPF range (Figure 4C). Since PPF is inversely proportional to the initial

533 probability of neurotransmitter release, this enhancement of PPF in WT slices likely reflects a

534 reduction in the probability of glutamate release, and may explain the consistent decrease in fEPSP

535 strength observed in enriched WT mice (Figure 4A). As such, these observations of changes in the

536 probability of release in WT, but not MSK1 mutant hippocampal slices may be an adaptive, MSK1-

537 dependent homeostatic response to the increase in miniature excitatory postsynaptic currents we

538 previously reported in enriched WT mice (Corrêa et al., 2012), potentially to limit network excitability.

539

540 An alternative explanation, that the differences in synaptic transmission between genotypes and after

541 enrichment in WT mice reflect corresponding decreases in the number of dendritic spines, are not

542 supported by spine density measurements made from apical dendrites in Golgi-impregnated CA1

543 neurons (Figure 5). As reported previously (Corrêa et al., 2012), CA1 apical dendrite spine density was

544 significantly higher in MSK1 KD mice than in WT mice (Figure 5A, B), with a trend towards greater

545 spine density after enrichment in both genotypes.

546

547 ***The experience-dependent enhancement of the dynamic range of synapses requires MSK1***

548 These measures of basal synaptic transmission, the probability of glutamate release, GluA subunit

549 expression and spine density point to subtle cellular effects of enrichment that would not be expected

550 to contribute appreciably to either the observed enhancement of cognition in WT mice, or the

551   inability of MSK1 KD mice to display the full cognitive benefits of enrichment. Accordingly, to probe

552   the potential cellular basis of the enrichment- and MSK1-dependent enhancement of spatial learning

553   and memory, we performed electrophysiological recordings of synaptic plasticity in area CA1 of

554   hippocampal slices prepared from standard housed and enriched WT and MSK1 KD mutant mice.

555

556   Since there is widespread agreement that activity-dependent changes in the efficacy of synaptic

557   transmission, which have been observed after enrichment (Ohline and Abraham, 2019), underlie the

558   ability of animals to learn and remember (Takeuchi et al., 2014), we predicted that: a) enrichment

559   would enhance the ability of synapses to display activity-dependent modifications of synaptic

560   strength, and b), given the impairment of cognition observed in MSK1 mutant mice, any enrichment-

561   induced synaptic enhancement would require MSK1, and hence be absent in the MSK1 mutants. We

562   thus performed dual-pathway long-term potentiation (LTP) and long-term depression (LTD)

563   experiments from area CA1 in hippocampal slices from standard housed and enriched WT and MSK1

564   KD mice, where one pathway served as a time control, and the other pathway was subjected to

565   plasticity-inducing stimulation. Consistent with our hypotheses, both LTP (Figure 6A) and LTD (Figure

566   6B) were significantly enhanced in the CA1 region of hippocampal slices prepared from WT mice that

567   had received enrichment. In stark contrast, neither LTP nor LTD were affected by enrichment in the

568   MSK1 mutant mice, where the extent of synaptic plasticity was comparable to that obtained from

569   standard housed mice of both genotypes (Figure 6A, B).

570

571   These observations indicate that MSK1 is required for the bidirectional expression of the enhanced

572   plasticity associated with environmental enrichment, i.e. an expansion of the dynamic range of

573   synapses, which occurred in the absence of appreciable changes in basal synaptic transmission or

574   dendritic spine density (Figures 4 and 5), and in experiments where baseline fEPSPs were carefully

575   stimulus-matched for amplitude to take into account differences in basal synaptic transmission

576   between MSK1 KD mutants and WT mice (see Materials and Methods: *Hippocampal slice preparation*

577   *and extracellular recordings*).

578

579   To quantify the extent of the enrichment- and MSK1-dependent expansion of the synaptic dynamic

580   range, we compared, from the weakest to the strongest, the range of synaptic strengths recorded in

581   the LTD and LTP experiments. On this basis we calculated a ~28% increase in the synaptic dynamic

582   range in WT animals, but essentially no change in synaptic strength in response to enrichment in

583   MSK1 KD mice (~-5 %). The bidirectional enhancement of synaptic strength in enriched WT animals

584   can be appreciated in a plot of the cumulative distribution of individual LTD and LTP values for each of

585   the experiments in each of the four groups of animals (Figure 6C). A complementary comparison

586   examined the net change from baseline (100 %) in each of the LTP and LTD experiments (Figure 6D)

587   across both genotype and housing conditions. This analysis revealed that enrichment selectively

588   enhanced the dynamic range of synapses in WT mice, and thus demonstrates that the kinase activity

589   of MSK1 is necessary for the experience-dependent bidirectional expansion of synaptic strength.

590

591   ***Experience influences gene expression in an MSK1-dependent manner***

592   Given the dependence of persistent changes in synaptic function and cognition on gene expression

593   (Alberini and Kandel, 2014), and the importance of MSK1 in regulating transcription (Reyskens and

594   Arthur, 2016), including for key plasticity-related proteins such as Arc/Arg3.1 (Hunter et al., 2017), we

595   addressed the molecular mechanisms downstream of MSK1 by examining patterns of gene expression

596   in WT mice in which MSK1 was active, and in kinase-dead animals in which it was not. RNA-Seq was

597   performed on hippocampal tissue obtained from the four groups of mice. A principal component

598   analysis (Figure 7A, B) revealed that a striking 73% of the variance in gene expression was captured by

599   the first principal component, corresponding to housing type, with both WT and MSK1 KD standard

600   housed mice clustering together (Figure 7B). The enriched WT group was readily distinguishable from

601   these two groups, with members clustered tightly together. In contrast, the enriched MSK1 KD mice

602   were distinct from both standard housed mice of both genotypes and enriched WT mice (Figure 7B).

603   Thus, enrichment had an effect on gene expression in MSK1 KD mice, but it appeared to be an

604   uncoordinated or random response to enrichment, in stark contrast to the tightly regulated

605   transcriptomic response in WT mice.

606

607   Differential gene expression analysis revealed that, while there were only three differentially-

608   expressed genes between standard housed WT and MSK1 KD mice (Figure 7C, Extended Data Tables

609   7-3, 7-4 for Figure 7), enrichment affected the regulation of 261 unique genes in WT mice (Figure 7C,

610   D; Extended Data Table 7-5 for Figure 7). In contrast, only 42 genes showed selective regulation by

611   enrichment in the MSK1 KD mice (Figure 7C, D; Extended Data Table 7-6 for Figure 7), with an

612   additional 214 genes regulated by enrichment in both WT and MSK1 KD mice (Figure 7C, D; Extended

613   Data Table 7-7 for Figure 7). These data indicate that the majority of genes affected by enrichment

614   are regulated by MSK1.

615

616   To identify the function of the genes regulated by enrichment, we performed Gene Ontology (GO)

617   analysis on genes differentially expressed in response to enrichment (Figure 7D, E, F). The 214 MSK1-

618   independent genes were distributed amongst 153 GO categories (Figure 7E; Extended Data Table 7-8

619   for Figure 7), which included cell adhesion, extracellular matrix and structure organisation and,

620 notably, the regulation of MAPK signalling, of which MSK1 is an integral part. A similar GO analysis of

621 the 261 unique MSK1-dependent genes revealed a restricted distribution amongst only 10 GO

622 categories (Figure 7F; Extended Data Table 7-9 for Figure 7), with 7 of these being unique to the

623 MSK1-dependent genes. These unique categories encompassed microtubule bundle formation, cilium

624 organization, assembly and movement, as well as ciliary axoneme assembly and collagen fibril

625 organisation.

626

627 *MSK1 is necessary for an experience-dependent homeostatic downregulation of plasticity gene*

628 *expression*

629 An examination of specific genes regulated by both MSK1 and enrichment revealed 11 genes (Figure

630 8A, B; Extended Data Table 8-10 for Figure 8) that were significantly differently expressed between

631 enriched WT and MSK1 KD mutant mice. In particular, two genes regulated by neurotrophins and the

632 MAPK cascade were strongly downregulated in enriched WT mice: Sprouty4 (Spry4) (Cabrita and

633 Christofori, 2008) and early growth response protein 1 (Egr1/Zif268/NGFI-A) (Veyrac et al., 2014;

634 Duclot and Kabbaj, 2017). This unexpected downregulation of Egr1, which has repeatedly been shown

635 to be elevated acutely in response to enrichment, plasticity- and learning and memory-inducing

636 stimuli (Pinaud, 2004; Veyrac et al., 2014; Duclot and Kabbaj, 2017), prompted a curated investigation

637 of genes relevant to the activation of MSK1. This analysis (Figure 9A) revealed a striking and

638 unexpected enrichment-induced downregulation of the MSK1 signalling cascade, including of MSK1

639 itself (Figure 9B), but not of the related MSK2 isoform (Figure 9C). In addition to downregulation of

640 Egr1, downregulation was also observed of the MSK1 substrate CREB, and of the key plasticity-related

641 protein Arc/Arg3.1 (Epstein and Finkbeiner, 2018), which we have previously shown was regulated in

642 an MSK1-dependent manner during both homeostatic synaptic plasticity (Corrêa et al., 2012) and in

643   response to BDNF (Hunter et al., 2017). That this enrichment-induced reduction of MSK1 gene

644   expression had tangible effects on Egr1 and Arc/Arg3.1 protein expression was confirmed by western

645   blots from contemporaneous hippocampal tissue, which showed strong enrichment-induced

646   downregulation of Egr1 and Arc/Arg3.1 protein levels exclusively in WT mice (Figure 9D, E). These

647   data indicate that MSK1 orchestrates an experience-dependent homeostatic downregulation of key

648   plasticity-related proteins.

649

650 **DISCUSSION**

651 The molecular pathway that links the external environment to the genomic response that underpins

652 experience-dependent neuronal and cognitive adaptations has remained elusive, but is important to

653 identify given the potential for pharmacological manipulation to remediate the cognitive deficits

654 associated with congenital, acquired and age-related cognitive impairment (Guerrieri et al., 2017;

655 Consorti et al., 2019). While both BDNF and a range of plasticity-related proteins have been

656 implicated in the neuronal response to enrichment (Cowansage et al., 2010; Sale et al., 2014; Rogers

657 et al., 2019), the mechanism that allows experience to couple BDNF to plasticity-related proteins has

658 yet to be described.

659

660 *MSK1 orchestrates an experience-dependent genomic homeostasis*

661 We have shown previously that mice lacking the kinase activity of MSK1, an enzyme regulated by

662 BDNF (Arthur et al., 2004; Daumas et al., 2017; Hunter et al., 2017), fail to upregulate miniature

663 excitatory synaptic currents in response to either enrichment in vivo, or activity deprivation in vitro

664 (Corrêa et al., 2012; Lalo et al., 2018). This selective mutation of the MSK1 gene obviates concerns

665 regarding a structural role for MSK1 (Gutierrez-Mecinas et al., 2011) that may contribute to the basal

666 (Chwang et al., 2007; Choi et al., 2012; Karelina et al., 2012) and enrichment-induced (Karelina et al.,

667 2012) deficits in learning and memory and neurogenesis observed in mice in which MSK1 has been

668 constitutively deleted. Instead, the MSK1 kinase dead (KD) mutation allows the influence of

669 experience to be examined against an intact basal synaptic plasticity and cognitive repertoire

670 (Daumas et al., 2017).

671

672 Using mice lacking the kinase activity of MSK1, we have presented evidence from several

673 independent, but convergent lines of investigation, from genes to behaviour, that MSK1 is a strong

674 candidate for acting as an important link between the environment and the genome. While we have

675 used male mice, which predominate in environmental enrichment research (Simpson and Kelly, 2011;

676 Girbovan and Plamondon, 2013), it is unlikely that our observations would not generalise to females

677 given the reported increases in BDNF production and MAPK activity in female rodents after

678 enrichment (Bakos et al., 2009; Ramirez-Rodriguez et al., 2014). However, this should be empirically

679 tested in subsequent studies.

680

681 Through comparing WT and MSK1 KD mice, we have shown that the kinase activity of MSK1 has an

682 obligatory role in the regulation of the transcriptional response to experience; the modulation of the

683 dynamic range of synapses; the persistence of memory, and cognitive flexibility. We propose that

684 these effects are causally linked. A coordinated and MSK1-dependent pattern of gene expression

685 likely facilitates the growth and development of the enriched brain, with Sprouty4 and genes

686 regulating the primary cilium being especially targeted. Sprouty4 exerts an inhibitory influence on the

687 actions of neurotrophins (Cabrita and Christofori, 2008; Alsina et al., 2012) and on axonal outgrowth

688 (Hausott et al., 2012). The experience-dependent downregulation of Sprouty4 would thus be

689 expected to remove this inhibition allowing greater influence of neurotrophins on neuronal structure

690 and function. The primary cilium is a cellular organelle that protrudes from the surface of virtually all

691 mammalian cells, including neurons, and has important signalling properties (Nachury and Mick,

692 2019). The cilium plays an important role in dendritic arborisation (Guadiana et al., 2013), neuronal

693 development and neurogenesis (Guemez-Gamboa et al., 2014; Valente et al., 2014; Lepanto et al.,

694 2016), and has been implicated in the maturation of neuronal circuits, synaptic plasticity and learning

695 and memory (Kumamoto et al., 2012; Rhee et al., 2016). These, and other, MSK1-dependent genes

696 are therefore likely to contribute to the frequently observed changes in brain structure and function

697 that support experience-dependent enhanced cognition (Rosenzweig and Leiman, 1968; Rosenzweig

698 and Bennett, 1996; Kolb and Whishaw, 1998). Moreover, once having facilitated the functional and

699 structural response to enrichment, MSK1 orchestrates a genomic homeostatic scaling characterised

700 by the downregulation of the transcription factor EGR1 and the plasticity-related protein Arc/Arg3.1.

701 The function of this unexpected downregulation, which is also observed in upstream MSK1-activating

702 kinases such as ERK2 and P38, and indeed of MSK1 itself, may be to stabilise and preserve the

703 neuronal networks, synaptic plasticity and cognitive enhancement arising from experience. Thus, the

704 normal pattern of MSK1-dependent gene expression can be homeostatically down-tuned in response

705 to enrichment in WT mice, but this adaptive ability is lost in the MSK1 KD mice, likely resulting in the

706 genomic, synaptic and cognitive impairments seen in the mutants.

707

708 ***MSK1 regulates the dynamic range of synapses and is required for the full expression of experience-***

709 ***dependent enhancement of cognition.***

710 In parallel, experience augments the dynamic range of hippocampal synapses in an MSK1-dependent

711 manner through the enhancement of both LTP and LTD. MSK1 thus enables synapses to both store

712 more information, and potentially be more responsive to prevailing synaptic and neuronal activity.

713 This may manifest as the ability to rapidly switch from one learned behaviour, potentially by both

714 weakening established neuronal circuits in an LTD-like manner, and mastering another, and for

715 longer, through LTP-like strengthening of new networks. Hippocampus-dependent behavioural

716 correlates of these forms of synaptic plasticity, reversal learning and the persistence of spatial

717 memory, were both impaired in mice lacking the kinase activity of MSK1. This suggests that the MSK1-

718   dependent expansion of the dynamic range of synapses increases the information capacity of

719   synapses, underpins the experience-dependent enhancement of cognition and thus provides a

720   plausible mechanism for the consistent improvements in cognition repeatedly observed in response

721   to enrichment since their first description in the 1940s by Donald Hebb (Hebb, 1947, 1949). Equally,

722   the increased number of dendritic spines in MSK1 KD mice observed here and in a previous study

723   (Correa et al., 2012) suggest that MSK1 influences spine density, either constitutively as a regulator of

724   gene expression, or in an activity-dependent manner in response to synaptic activity. This

725   dysregulation of spine number in the MSK1 KD mutant may contribute in particular to the cognitive

726   impairments seen in MSK1 KD mice after enrichment, and has parallels with the greater spine density

727   and impaired cognition observed in both human autism spectrum disorder and animal models of

728   autism (Coley and Gao, 2018; Nakai et al., 2018). MSK1 may thus coordinate the neuronal

729   mechanisms and networks supporting synaptic structure, function, plasticity and cognition through

730   the regulation of gene expression. After enrichment, the MSK1-driven genomic downregulation of

731   plasticity-related proteins leads to a reduced baseline against which activity-dependent elevations in

732   their transcription may proportionally have greater synaptic and cognitive impact.

733

734   ***MSK1 is a key regulator of experience- and activity-dependent genomic, synaptic and cognitive***

735   ***plasticity.***

736   The recruitment of MSK1 during exposure to a complex environment underpins a range of adaptive

737   genomic, molecular and synaptic responses that contribute appreciably to the experience-dependent

738   enhancement of cognition. Moreover, by initiating the downregulation of key plasticity-related genes,

739   MSK1 plays a pivotal role in ensuring the stability of the new and improved experience-dependent

740   genomic, neuronal and cognitive landscape, and one that is potentially primed to respond more

741 effectively to new challenges. MSK1, from regulating homeostatic synaptic scaling in vitro (Corrêa et

742 al., 2012) to initiating genomic homeostasis in response to experience in vivo, thus represents a key

743 molecular sensor linking the environment and prevailing synaptic activity to the genome and the

744 ensuing adaptive neuronal and cognitive response.

745

746

747 **Author contributions**

748 LP performed and analysed the electrophysiological studies and prepared RNA; LM performed and

749 analysed the behavioural studies, prepared Golgi sections of brain tissue and supervised spine

750 analysis; DDC performed the RNA-Seq analysis; PR conducted the western blots; MT analysed spine

751 density; DH guided the RNA-Seq analysis; JSCA provided the MSK1 KD mice and expertise on

752 molecular analyses; BGF obtained funding, conceived and oversaw the study. LP, LM, DDC, PR, DH,

753 JSCA, BGF contributed to the writing of the manuscript. All authors approved the final version of the

754 manuscript.

755

REFERENCES

Alberini CM, Kandel ER (2014) The regulation of transcription in memory consolidation. Cold Spring
Harb Perspect Biol 7:a021741.

Alexa A, Rahnenfuhrer J (2006) topGO: Enrichment Analysis for Gene Ontology. R package version
2.32.0.

Alsina FC, Irala D, Fontanet PA, Hita FJ, Ledda F, Paratcha G (2012) Sprouty4 is an endogenous
negative modulator of TrkA signaling and neuronal differentiation induced by NGF. PLoS One
7:e32087.

Anders S, Pyl PT, Huber W (2015) HTSeq--a Python framework to work with high-throughput
sequencing data. Bioinformatics 31:166-169.

Anderson WW, Collingridge GL (2007) Capabilities of the WinLTP data acquisition program extending
beyond basic LTP experimental functions. JNeurosciMethods 162:346-356.

Andrews, S. (2010) FastQC: a quality control tool for high throughput sequence data
http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Andrews, S. (2018) SeqMonk: A tool to visualise and analyse high throughput mapped sequence data
https://www.bioinformatics.babraham.ac.uk/projects/seqmonk/

Aronoff E, Hillyer R, Leon M (2016) Environmental Enrichment Therapy for Autism: Outcomes with
Increased Access. Neural Plast 2016:2734915.

Arthur JS, Fong AL, Dwyer JM, Davare M, Reese E, Obrietan K, Impey S (2004) Mitogen- and stress-
activated protein kinase 1 mediates cAMP response element-binding protein phosphorylation
and activation by neurotrophins. JNeurosci 24:4324-4332.

777 Bakos J, Hlavacova N, Rajman M, Ondicova K, Koros C, Kitraki E, Steinbusch HW, Jezova D (2009)

778   Enriched environment influences hormonal status and hippocampal brain derived

779   neurotrophic factor in a sex dependent manner. Neuroscience 164:788-797.

780 Cabrita MA, Christofori G (2008) Sprouty proteins, masterminds of receptor tyrosine kinase signaling.

781   Angiogenesis 11:53-62.

782 Carlson M (2018) org.Mm.eg.db: Genome wide annotation for Mouse. R package version 3.6.0.

783 Coley AA, Gao WJ (2018) PSD95: A synaptic protein implicated in schizophrenia or autism? Prog

784   Neuropsychopharmacol Biol Psychiatry 82:187-194.

785 Consorti A, Sansevero G, Torelli C, Berardi N, Sale A (2019) From Basic Visual Science to

786   Neurodevelopmental Disorders: The Voyage of Environmental Enrichment-Like Stimulation.

787   Neural Plast 2019:5653180.

788 Correa SA, Hunter CJ, Palygin O, Wauters SC, Martin KJ, McKenzie C, McKelvey K, Morris RG,

789   Pankratov Y, Arthur JS, Frenguelli BG (2012) MSK1 regulates homeostatic and experience-

790   dependent synaptic plasticity. J Neurosci 32:13039-13051.

791 Corrêa SA, Hunter CJ, Palygin O, Wauters SC, Martin KJ, McKenzie C, McKelvey K, Morris RG,

792   Pankratov Y, Arthur JS, Frenguelli BG (2012) MSK1 regulates homeostatic and experience-

793   dependent synaptic plasticity. Journal of Neuroscience 32:13039-13051.

794 Cowansage KK, Ledoux JE, Monfils MH (2010) Brain-derived neurotrophic factor: a dynamic

795   gatekeeper of neural plasticity. CurrMolPharmacol 3:12-29.

796 D'Amato FR, Zanettini C, Sgobio C, Sarli C, Carone V, Moles A, mmassari-Teule M (2010) Intensification

797   of maternal care by double-mothering boosts cognitive function and hippocampal morphology

798   in the adult offspring. Hippocampus 21:298-308.

799   Daumas S, Hunter CJ, Mistry RB, Morè L, Privitera L, Cooper DD, Reyskens KM, Flynn HT, Morris RG,

800   Arthur JS, Frenguelli BG (2017) The kinase function of MSK1 regulates BDNF signaling to CREB

801   and basal synaptic transmission, but is not required for hippocampal long-term potentiation or

802   spatial memory. eNeuro 4.

803   de la Tremblaye PB, Cheng JP, Bondi CO, Kline AE (2019) Environmental enrichment, alone or in

804   combination with various pharmacotherapies, confers marked benefits after traumatic brain

805   injury. Neuropharmacology 145:13-24.

806   Diering GH, Huganir RL (2018) The AMPA Receptor Code of Synaptic Plasticity. Neuron 100:314-329.

807   Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR (2013)

808   STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29:15-21.

809   Duclot F, Kabbaj M (2017) The Role of Early Growth Response 1 (EGR1) in Brain Plasticity and

810   Neuropsychiatric Disorders. Front Behav Neurosci 11:35.

811   Epstein I, Finkbeiner S (2018) The Arc of cognition: Signaling cascades regulating Arc and implications

812   for cognitive function and disease. Semin Cell Dev Biol 77:63-72.

813   Farah MJ (2018) Socioeconomic status and the brain: prospects for neuroscience-informed policy. Nat

814   Rev Neurosci 19:428-438.

815   Faraway JJ (2005) Linear models with R. Boca Raton: Chapman & Hall/CRC.

816   Girbovan C, Plamondon H (2013) Environmental enrichment in female rodents: considerations in the

817   effects on behavior and biochemical markers. Behav Brain Res 253:178-190.

818   Guadiana SM, Semple-Rowland S, Daroszewski D, Madorsky I, Breunig JJ, Mykytyn K, Sarkisian MR

819   (2013) Arborization of dendrites by developing neocortical neurons is dependent on primary

820   cilia and type 3 adenylyl cyclase. J Neurosci 33:2626-2638.

821   Gubert C, Hannan AJ (2019) Environmental enrichment as an experience-dependent modulator of

822   social plasticity and cognition. Brain Res 1717:1-14.

823   Guemez-Gamboa A, Coufal NG, Gleeson JG (2014) Primary cilia in the developing and mature brain.

824   Neuron 82:511-521.

825   Guerrieri D, Moon HY, van Praag H (2017) Exercise in a Pill: The Latest on Exercise-Mimetics. Brain

826   Plast 2:153-169.

827   Gutierrez-Mecinas M, Trollope AF, Collins A, Morfett H, Hesketh SA, Kersante F, Reul JM (2011) Long-

828   lasting behavioral responses to stress involve a direct interaction of glucocorticoid receptors

829   with ERK1/2-MSK1-Elk-1 signaling. ProcNatlAcadSciUSA 108:13806-13811.

830   Hausott B, Vallant N, Schlick B, Auer M, Nimmervoll B, Obermair GJ, Schwarzer C, Dai F, Brand-Saberi

831   B, Klimaschewski L (2012) Sprouty2 and -4 regulate axon outgrowth by hippocampal neurons.

832   Hippocampus 22:434-441.

833   Hebb DO (1947) The effects of early experience on problem-solving at maturity. American

834   Psychologist 2:306-307.

835   Hebb DO (1949) The Organization of Behaviour. New York: John Wiley.

836   Heffron D, Mandell JW (2005) Differential localization of MAPK-activated protein kinases RSK1 and

837   MSK1 in mouse brain. Brain ResMolBrain Res 136:134-141.

838   Hirase H, Shinohara Y (2014) Transformation of cortical and hippocampal neural circuit by

839   environmental enrichment. Neuroscience 280:282-298.

840   Howell DC (2010) Statistical methods for psychology, 7th Edition. Australia Belmont, CA: Thomson

841   Wadsworth.

842   Hunter CJ, Remenyi J, Correa SA, Privitera L, Reyskens KM, Martin KJ, Toth R, Frenguelli BG, Arthur JS

843   (2017) MSK1 regulates transcriptional induction of Arc/Arg3.1 in response to neurotrophins.

844   FEBS Open Bio.

845   Jackman SL, Regehr WG (2017) The Mechanisms and Functions of Synaptic Facilitation. Neuron

846   94:447-464.

847   Jax (2009) A Jackson Laboratory Resource Manual: Breeding strategies for maintaining colonies of

848   laboratory mice https://www.research.uci.edu/forms/docs/iacuc/JAX-breeding-strategies.pdf

849   Jiang H, Lei R, Ding SW, Zhu S (2014) Skewer: a fast and accurate adapter trimmer for next-generation

850   sequencing paired-end reads. BMC Bioinformatics 15:182.

851   Kolb B, Whishaw IQ (1998) Brain plasticity and behavior. Annu Rev Psychol 49:43-64.

852   Kumamoto N, Gu Y, Wang J, Janoschka S, Takemaru K, Levine J, Ge S (2012) A role for primary cilia in

853   glutamatergic synaptic integration of adult-born neurons. Nat Neurosci 15:399-405, S391.

854   Kutner MH, Nachtsheim CJ, Neter J, Li W (2005) Applied Linear Statistical Models, 5 Edition. Boston:

855   McGraw-Hill Irwin.

856   Laerd Statistics (2017) Two-way ANOVA using SPSS Statistics. Statistical tutorials and software guides.

857   https://statistics.laerd.com/

858   Lalo U, Bogdanov A, Moss GW, Frenguelli BG, Pankratov Y (2018) Role for astroglia-derived BDNF and

859   MSK1 in homeostatic synaptic plasticity. Neuroglia 1:14.

860   Lepanto P, Badano JL, Zolessi FR (2016) Neuron's little helper: The role of primary cilia in

861   neurogenesis. Neurogenesis (Austin) 3:e1253363.

862   Lipp HP, Wolfer DP (1998) Genetically modified mice and cognition. Curr Opin Neurobiol 8:272-280.

863   Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq

864   data with DESeq2. Genome Biol 15:550.

865   Lu W, Shi Y, Jackson AC, Bjorgan K, During MJ, Sprengel R, Seeburg PH, Nicoll RA (2009) Subunit

866   composition of synaptic AMPA receptors revealed by a single-cell genetic approach. Neuron

867   62:254-268.

868   Miguel PM, Pereira LO, Silveira PP, Meaney MJ (2019) Early environmental influences on the

869   development of children's brain structure and function. Dev Med Child Neurol 61:1127-1133.

870   Mo C, Hannan AJ, Renoir T (2015) Environmental factors as modulators of neurodegeneration:

871   insights from gene-environment interactions in Huntington's disease. Neurosci Biobehav Rev

872   52:178-192.

873   Mohler EG, Shacham S, Noiman S, Lezoualc'h F, Robert S, Gastineau M, Rutkowski J, Marantz Y,

874   Dumuis A, Bockaert J, Gold PE, Ragozzino ME (2007) VRX-03011, a novel 5-HT4 agonist,

875   enhances memory and hippocampal acetylcholine efflux. Neuropharmacology 53:563-573.

876   Nachury MV, Mick DU (2019) Establishing and regulating the composition of cilia for signal

877   transduction. Nat Rev Mol Cell Biol 20:389-405.

878   Nakai N, Takumi T, Nakai J, Sato M (2018) Common Defects of Spine Dynamics and Circuit Function in

879   Neurodevelopmental Disorders: A Systematic Review of Findings From in Vivo Optical Imaging

880   of Mouse Models. Front Neurosci 12:412.

881   Ohline SM, Abraham WC (2019) Environmental enrichment effects on synaptic and cellular physiology

882   of hippocampal neurons. Neuropharmacology 145:3-12.

883   Pinaud R (2004) Experience-dependent immediate early gene expression in the adult central nervous

884   system: evidence from enriched-environment studies. Int J Neurosci 114:321-333.

885   Ramirez-Rodriguez G, Ocana-Fernandez MA, Vega-Rivera NM, Torres-Perez OM, Gomez-Sanchez A,

886   Estrada-Camarena E, Ortiz-Lopez L (2014) Environmental enrichment induces neuroplastic

887   changes in middle age female Balb/c mice and increases the hippocampal levels of BDNF, p-

888   Akt and p-MAPK1/2. Neuroscience 260:158-170.

889   Renner MC, Albers EH, Gutierrez-Castellanos N, Reinders NR, van Huijstee AN, Xiong H, Lodder TR,

890   Kessels HW (2017) Synaptic plasticity through activation of GluA3-containing AMPA-receptors.

891   Elife 6.

892   Reyskens KM, Arthur JS (2016) Emerging Roles of the Mitogen and Stress Activated Kinases MSK1 and

893   MSK2. Front Cell Dev Biol 4:56.

894   Rhee S, Kirschen GW, Gu Y, Ge S (2016) Depletion of primary cilia from mature dentate granule cells

895   impairs hippocampus-dependent contextual memory. Sci Rep 6:34370.

896   Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP (2011)

897   Integrative genomics viewer. Nat Biotechnol 29:24-26.

898   Rogers J, Renoir T, Hannan AJ (2019) Gene-environment interactions informing therapeutic

899   approaches to cognitive and affective disorders. Neuropharmacology 145:37-48.

900   Rosenzweig MR, Leiman AL (1968) Brain functions. AnnuRevPsychol 19:55-98.

901   Rosenzweig MR, Bennett EL (1996) Psychobiology of plasticity: effects of training and experience on

902   brain and behavior. BehavBrain Res 78:57-65.

903   RTeam (2018) R: A language and environment for statistical computing. www.R-project.org

904   Sale A, Berardi N, Maffei L (2014) Environment and brain plasticity: towards an endogenous

905   pharmacotherapy. Physiol Rev 94:189-234.

906   Simpson J, Kelly JP (2011) The impact of environmental enrichment in laboratory rats--behavioural

907   and neurochemical aspects. BehavBrain Res 222:246-264.

908   Sindreu CB, Scheiner ZS, Storm DR (2007) Ca2+ -stimulated adenylyl cyclases regulate ERK-dependent

909   activation of MSK1 during fear conditioning. Neuron 53:79-89.

910   Stairs DJ, Bardo MT (2009) Neurobehavioral effects of environmental enrichment and drug abuse

911   vulnerability. PharmacolBiochemBehav 92:377-382.

912   Takeuchi T, Duszkiewicz AJ, Morris RG (2014) The synaptic plasticity and memory hypothesis:

913   encoding, storage and persistence. Philos Trans R Soc Lond B Biol Sci 369:20130288.

914   Terashima A, Suh YH, Isaac JTRJNR (2017) The AMPA Receptor Subunit GluA1 is Required for CA1

915   Hippocampal Long-Term Potentiation but is not Essential for Synaptic Transmission.

916   Tsuzuki K, Lambolez B, Rossier J, Ozawa S (2001) Absolute quantification of AMPA receptor subunit

917   mRNAs in single hippocampal neurons. J Neurochem 77:1650-1659.

918   Valente EM, Rosti RO, Gibbs E, Gleeson JG (2014) Primary cilia in neurodevelopmental disorders. Nat

919   Rev Neurol 10:27-36.

920   Veyrac A, Besnard A, Caboche J, Davis S, Laroche S (2014) The transcription factor Zif268/Egr1, brain

921   plasticity, and memory. Prog Mol Biol Transl Sci 122:89-129.

922   von Bernhardi R, Bernhardi LE, Eugenin J (2017) What Is Neural Plasticity? Adv Exp Med Biol 1015:1-

923   15.

924   Wassouf Z, Schulze-Hentrich JM (2019) Alpha-synuclein at the nexus of genes and environment: the

925   impact of environmental enrichment and stress on brain health and disease. J Neurochem

926   150:591-604.

927   Wei J, Carroll RJ, Harden KK, Wu G (2012) Comparisons of treatment means when factors do not

928   interact in two-factorial studies. Amino Acids 42:2031-2035.

929   Wolf LW, LaRegina MC, Tolbert DL (1996) A behavioral study of the development of hereditary

930   cerebellar ataxia in the shaker rat mutant. Behav Brain Res 75:67-81.

931   Woo CC, Leon M (2013) Environmental enrichment as an effective treatment for autism: a

932   randomized controlled trial. Behav Neurosci 127:487-497.

933   Woo CC, Donnelly JH, Steinberg-Epstein R, Leon M (2015) Environmental enrichment as a therapy for

934   autism: A clinical trial replication and extension. Behav Neurosci 129:412-422.

935

936   **FIGURE LEGENDS**

937   **Figure 1. Environmental enrichment influences exploration and anxiety independently of MSK1.**

938   **A** Mice (n = 16 per group) of both genotypes (WT and MSK1 KD) and housing condition (standard

939   housed (SH) and enriched (EE)) were placed in an open field for 60 mins (left half of graph) after which

940   a 50 ml plastic tube was introduced into the arena (right half of graph; novel object). Animal

941   locomotion was tracked for the entire period. During the first 60 minutes a RM-ANOVA showed an

942   effect of time for all groups ($F_{(5,300)}$ = 149.89, p = 0.001) indicating a reduction in activity over this

943   period, and also a housing effect ($F_{(1,60)}$ = 12.10, p = 0.001) indicating that standard housed mice

944   were more active regardless of their genotype. During the second 60 minutes of the test (after the

945   introduction of the novel object) a RM-ANOVA showed an effect of time for all groups ($F_{(5,300)}$ =

946   220.46, p = 0.001) indicating a reduction in activity over time, and also a housing effect ($F_{(1,60)}$ =

947   10.31, p = 0.002) showing that standard housed mice remained more active in response to the

948   introduction of a novel object regardless of their genotype. Data are presented as mean ± SEM. Data

949   points have been offset for clarity. **B** Heat maps of activity before and after introduction of the novel

950   object for each of the four groups of animals. **C** Mice (n = 16 per group) were placed on the central

951   area of an elevated plus maze and allowed to explore for 10 mins during which time their activity was

952   video tracked. Enriched mice of either genotype travelled further in the open arms compared to mice

953   housed under standard conditions ($F_{(1,60)}$ = 11.21 p = 0.001). Bars show the mean ± SEM and

954   individual data points. **D** Occupancy heat maps for each of the groups, with the closed arms of the

955   elevated plus maze running vertically.

956   **Figure 2. The kinase activity of MSK1 is necessary for enrichment-induced memory persistence**

957   **A** In a spontaneous alternation test of spatial working memory, mice (n = 15 to 16 per group) were

958   exposed for 10 mins to a radial maze (with walls) in which four arms were open to the mice. The mice

959   scored correctly if four different arms were visited before a return visit occurred to any one of the

960   arms. **B** The percentage of correct alternations was higher for enriched mice (EE) regardless of

961   genotype (housing effect: F(1,58) = 8.15; p = 0.006). While there was no significant genotype x

962   housing interaction, the difference between enrichment and standard housed (SH) mice was greater

963   in the WT, which showed a significant improvement (F(1,58) = 6.41; p = 0.014), compared to the

964   MSK1 KD (KD) that showed no improvement (F(1,58) = 2.22; p = 0.14). Bar graph depicts mean ± SEM

965   and individual data points. **C** Mice (n = 10 to 14 per group) were trained on the water maze over five

966   consecutive days. All groups showed learning of the platform location over the first stage of training

967   (4 days; D1-D4) with a 20 cm platform. A RM-ANOVA, with genotype and housing as factors showed a

968   significant effect of session (F(3,129) = 36.16; p = 0.000) on the latency to reach the escape platform.

969   It also showed a main effect of housing (F(1,43) = 4.94; p = 0.032) where enriched mice of both

970   genotypes performed better than standard housed mice, with no difference in performance between

971   standard housed mice. On the second stage of the training (1 day; D5), in which a 10 cm platform was

972   employed to test for more precise spatial reference memory, the ANOVA on the latency to reach the

973   escape platform with between factors as genotype and housing again showed an effect of housing

974   with better performance in enriched mice (F(1,43) = 8.45; p = 0.006). Data shown as mean ± SEM.

975   Data points are offset for clarity. **D** Heat maps of trajectories for all four groups. The platform was

976   located in the south east quadrant. More selective searching (greater occupancy of the training

977   quadrant) can be seen in the enriched mice. **E** On the probe trial 24 hours after D5, enriched WT mice

978   showed the best retention for the spatial location of the escape platform, whereas the enriched MSK1

979   KD mice showed the worst performance. The ANOVA on the total time spent on the target quadrant

980   (where the platform had been previously) showed a significant interaction between genotype and

981   housing (F(1,43) = 6.65; p = 0.013) and the simple main effect showed a significant difference

982   between enriched WT mice and enriched MSK1 KD mice (F(1,43) = 4.84; p = 0.033). Bar graph depicts

983   mean ± SEM and individual data points. **F** The retention deficit in the enriched MSK1 KD mice was

984   observed across the entire probe trial duration where they spent consistently less time in the

985   appropriate quadrant compared to other groups. Data shown as mean ± SEM. Data points are offset

986   for clarity.

987   **Figure 3. The kinase activity of MSK1 is necessary for enrichment-dependent cognitive flexibility**

988   **and long-term memory**

989   **A** All groups (n = 12 or 13 per group) showed learning over the first stage of training on the Morris

990   water maze (3 days; D1 – D3). A RM-ANOVA with genotype and housing as between factors showed a

991   significant effect of session (F(2,92) = 71.91; p = 0.000) on the latency to reach the escape platform. It

992   also showed a main effect of housing (F(1,46) = 7.76; p = 0.008) where the enriched mice performed

993   better than standard housed mice. On the reversal learning stage (2 days; R1 – R2) a RM-ANOVA on

994   the latency to reach the new escape platform location showed an effect of session (F(1,46) = 16.15; p

995   = 0.000), genotype (F(1,46) = 15.66; p = 0.000) and housing (F(1,46) = 6.59; p = 0.014) indicating that

996   all groups learned over the two days, but WT mice performed better than MSK1 KD mutant mice, and

997   enriched mice better than standard housed mice. There was no significant interaction between

998   genotype and housing, but on the first day of reversal learning (R1) there was a significant difference

999   between standard housed WT mice and standard housed MSK1 KD mice (F(1,46) = 5.54; p = 0.023).

1000   On R2 there was a significant difference between enriched WT mice and enriched MSK1 KD mice

1001   (F(1,46) = 6.42; p = 0.015) and again between standard housed WT mice and standard housed MSK1

1002   KD mice (F(1,46) = 6.32; p = 0.016). Data shown as mean ± SEM. Data points are offset for clarity. **B**

1003   Occupancy plots for the second day of reversal learning (R2) which show preferential searching in the

1004   reversal quadrant (south east) by enriched WT mice. **C** On the probe trial 24 hours later, enriched WT

1005   mice showed the best goal-directed behaviour for the reverse location of the escape platform, while

1006   the enriched MSK1 KD mice again showed the worst performance. While there was no significant

1007   interaction between genotype and housing, an ANOVA on the latency to enter the reversal location of

1008   the platform showed a significant effect for genotype ($F_{(1,46)}$ = 7.51; p = 0.009) and also a significant

1009   difference between enriched WT and enriched MSK1 KD mice ($F_{(1,46)}$ = 6.39; p = 0.015). Bar graph

1010   depicts mean ± SEM and individual data points. **D** Inset are heat maps of occupancy in the water maze

1011   during the 120 s probe trial for the four groups. Enriched WT mice show activity concentrated in the

1012   reversal learning quadrant (south east). **E** A comparison of 24 hr probe trial performance across the

1013   two experiments (small platform, Figure 2E, F and reversal learning, Figure 3C, D), where performance

1014   across groups was normalised to that of WT standard housed mice, showed a significant genotype x

1015   housing interaction ($F_{(1,93)}$ = 8.49, p = 0.004), where enriched MSK1 KD mice performed significantly

1016   worse that their enriched WT counterparts ($F_{(1,93)}$ = 11.48, p = 0.001) who in turn performed better

1017   than standard housed WT mice ($F_{(1,93)}$ = 5.08, p = 0.027). Data from 23-27 mice per group. Broken

1018   line indicates level of performance when normalised to the respective WTSH mean.

1019   **Figure 4. Enrichment has minimal effects on basal synaptic transmission, but differentially affects**

1020   **GluA2 expression and reduces the probability of neurotransmitter release in an MSK1-dependent**

1021   **manner.**

1022   **A** MSK1 KD mice raised in standard (KD SH) and enriched (KD EE) housing displayed reduced synaptic

1023   transmission compared to WT mice (WT SH; WT EE), as previously described for standard housed WT

1024   and MSK1 KD mice (Daumas et al., 2017). A RM-ANOVA showed a significant effect of stimulus

1025   strength ($F_{(5,560)}$ = 632.11; p = 0.000) and a significant effect of genotype ($F_{(1,112)}$ = 43.99; p =

1026   0.000), where the fEPSPs were larger in the WT mice regardless of their housing condition. Insets

1027   show representative fEPSPs at increasing stimulus strengths for each genotype and housing condition.

50

1028　Data are presented as mean ± SEM. Data points have been offset for clarity. **B** Western blots of GluA1

1029　(left; open arrow) and GluA2 (right; grey arrow) expression showing two unique samples per blot from

1030　each of the four experimental groups. Quantification relative to GAPDH expression (filled arrow on

1031　blots) showed a significant genotype x housing interaction for GluA2 ($F_{(1,12)}$ = 5.71; p = 0.034). One

1032　of two blots for each is shown. WT enriched, WE; WT standard housed, WS; MSK1 KD enriched, KE;

1033　MSK1 KD standard housed, KS. The ladder blot was digitally superimposed on the blots for GluA1,

1034　GluA2 and GAPDH. **C** There was no difference in paired-pulse facilitation (PPF) between standard

1035　housed WT and MSK1 KD mice. Equally, there were no differences between standard housed and

1036　enriched MSK1 KD mice. An RM-ANOVA showed a significant difference between standard housed

1037　and enriched WT mice, with enriched WT mice showing consistently greater PPF ($F_{(6,112)}$ = 5.71; p =

1038　0.019), consistent with a reduced initial probability of neurotransmitter release. Insets show

1039　representative PPF profiles for each genotype and housing condition, normalised to the amplitude of

1040　the first of each pair of fEPSPs (broken grey line). Data are presented as mean ± SEM

1041　**Figure 5. MSK1 KD mice have greater spine density than WT mice.**

1042　**A** MSK1 KD mice had higher spine density than WT mice, consistent with observations made

1043　previously (Corrêa et al., 2012); genotype effect $F_{(1,283)}$ = 14.55, p = 0.000. Graph shows box plot of

1044　spine density distribution from 67-83 dendrites on CA1 neurons from 4 mice per condition. Box: 25-

1045　75% range; mean: horizontal line. Images show representative Golgi-stained CA1 secondary apical

1046　dendrites from across housing and genotype. Scale bars measure 10 μm. **B** Graph shows cumulative

1047　distribution of spine density across housing and genotype. Note rightward shift in spine density

1048　distribution of MSK1 KD mutants (blue colours) reflecting the greater spine density shown in **A**.

1049　**Figure 6. Experience enhances the dynamic range of synapses in an MSK1-dependent manner.**

A Robust LTP was induced by theta burst stimulation in one pathway (at time 0, arrowhead; filled symbols) while the second pathway in each experiment (open symbols) remained unstimulated. LTP was enhanced exclusively in enriched WT slices. In the first 10 mins after LTP induction an ANOVA revealed no appreciable genotype x housing interaction ($F_{(1,28)}$ = 0.93; p = 0.343), but did show a significant effect of genotype ($F_{(1,28)}$ = 4.78; p = 0.038). A post-hoc analysis revealed LTP in the enriched WT group was significantly enhanced relative to enriched MSK1 KD mice ($F_{(1,28)}$ = 5.29; p = 0.029). In the last hour of LTP (120 – 180 mins) an RM-ANOVA revealed a strong trend for a genotype x housing interaction ($F_{(1,28)}$ = 4.02; p = 0.055) in which LTP in the enriched WT group was significantly enhanced relative to enriched MSK1 KD mice ($F_{(1,28)}$ = 11.16; p = 0.001) and standard housed WT mice ($F_{(1,28)}$ = 8.37; p = 0.007). Insets are representative fEPSPs taken before (solid lines) and after (broken lines) LTP induction. Data shown as mean ± SEM. **B** At time 0 LTD was induced by delivering 900 pulses at 1 Hz to one pathway (filled symbols), while the second pathway in each experiment (open symbols) remained unstimulated. A significant genotype x housing interaction was observed ($F_{(1,24)}$ = 4.47; p = 0.045) where LTD was enhanced in enriched WT mice relative to standard housed WT ($F_{(1,24)}$ = 13.52); p = 0.001) and enriched MSK1 KD mice ($F_{(1,24)}$ = 5.80; p = 0.024). Insets are representative fEPSPs taken before (solid lines) and after (broken lines) LTD induction. Data shown as mean ± SEM. **C** Cumulative distribution of changes of synaptic strength for each LTD and LTP experiment across housing and genotype. WT enriched mice show both left- (LTD) and rightward (LTP) shifts in the range of synaptic depression and potentiation, respectively, compared to standard housed WT mice. The distributions for standard house and enriched MSK1 KD mice overlap indicating their insensitivity to enrichment. **D** The net extent of the change in synaptic strength from baseline values (100 %) in each of the LTP (LTP % - 100 %) and LTD (100 % - LTD %) experiments shows a significant interaction between housing and genotype ($F_{(1,56)}$ = 4.58; p = 0.037)

1073 and a significant effect of enrichment only in WT mice (F(1,56) = 9.79; p = 0.003), and no effect in the

1074 MSK1 KD mutants (F(1,56) = 0.10; p = 0.92), where the dynamic range of synaptic plasticity remains

1075 unchanged after enrichment. Bar graph depicts mean ± SEM and individual data points.

1076 **Figure 7. Environmental enrichment influences gene expression in an MSK1-dependent manner.**

1077 **A** Scree plot of principal component percentage variation for top 500 variance transcripts. The

1078 majority of the variance is explained by one principal component (73%), with the second principal

1079 component contributing 8% of the variance. **B** Principal component (PC) analysis of top 500

1080 transcripts by variation across samples (solid symbols). Experimental groups are distinguished by

1081 color. Each group (n = 3 to 5) is clustered around the arithmetic mean centroid (open symbol). The

1082 greatest variance (PC1; 73%) is explained by housing condition. **C** Schematic of differentially

1083 expressed gene (DEG) comparisons and number of differentially expressed genes between each

1084 genotype/housing condition combination (Tables 7-3 to 7-7). **D** Venn diagram comparing DEGs in

1085 response to enriched environment vs standard housed controls. Orange circle: DEGs in WT mice

1086 exposed to EE (475 genes; Extended Data Table 7-11 for Figure 7); Cyan circle: DEGs in MSK1 KD mice

1087 exposed to EE (256 genes; Extended Data Table 7-12 for Figure 7). **E, F** Back-to-back bar plots of

1088 significantly enriched GO Terms against Benjamini-adjusted p values (lilac bars), and the ratio of

1089 significant genes contributing to each term to the number expected in each category (yellow bars; the

1090 actual values for the number of significant and expected genes for each category are given). Broken

1091 vertical line at -2 indicates p = 0.01. **E** Significantly enriched GO Terms common to both WT and MSK1

1092 KD mice exposed to enrichment (214 genes). 14 of 152 significant categories are shown (Extended

1093 Data Table 7-8 for Figure 7). **F** Significantly enriched GO Terms unique to WT mice exposed to

1094 enrichment (261 genes). 6 of 7 unique categories out of 10 significant categories are shown (Extended

1095 Data Table 7-9 for Figure 7).

53

Figure 8. Enrichment downregulates neurotrophin signaling-related genes in a MSK1-dependent manner.

A MA plot of environmentally-enriched WT vs. environmentally-enriched MSK1 KD groups. DESeq2 beta-prior transformed log2 fold-changes are plotted on the y-axis. Broken lines at ± 0.38 equate to a ± 1.3-fold change. Significantly differentially expressed genes are circled and labelled. **B** Heatmap of Z-score normalized rlog expression for genes identified as significantly different between MSK1 KD enriched (KD EE) and WT enriched (WT EE) groups (Extended Data Table 8-10 for Figure 8). Red cells indicate over-expression, blue cells indicate under-expression of a gene in a sample. Colors and labels at the top and bottom of the heatmap correspond to the sample's housing and genotype identity.

Figure 9. MSK1 orchestrates homeostatic genomic downscaling of plasticity-related pathways and proteins in response to enrichment.

A Heatmap of genes relevant to the BDNF/MAPK/MSK1 signaling cascade showing selective downregulation in samples from enriched WT mice (WT EE). For clarity the protein name is provided. For where the gene and protein name are not the same: TrkB2, Ntrk2; MEK2, Map2k2; MEK1, Map2k1; ERK1, Mapk3; ERK2, Mapk1; p38, Mapk14; MSK1, Rps6ka5; CREB, Creb1. **B, C** The expression of MSK1, but not MSK2, is selectively downregulated in response to enrichment (standard vs enriched housing; two-way ANOVA; $F_{(3,13)}$ = 29.01; p = 0.0001) in both WT ($F_{(1,13)}$ = 14.74; p = 0.002) and MSK1 KD mice ($F_{(1,13)}$ = 14.31; p = 0.002). Bar graph depicts mean ± SEM and individual data points. **D** Western blot analysis of EGR1 protein in hippocampal tissue from separate samples contemporaneous with those used for RNA-Seq. There was a significant interaction genotype x housing; $F_{(3,12)}$ = 7.17, p = 0.020 for EGR1 protein expression, which was selectively strongly downregulated in WT enriched mice ($F_{(1,12)}$ = 9.63, p = 0.009), in keeping with RNA-Seq DEG analysis (Figure 8A, B). Inset shows one of the two blots used in the analysis of EGR1 expression. WE, WT

54

1119   enriched (n = 4); WS, WT standard housed (n = 4); KE, MSK1 KD enriched (n = 4); KS, MSK1 KD

1120   standard housed (n = 4). Bar graph depicts mean ± SEM and individual data points. **E** Western blot

1121   analysis of Arc/Arg3.1 protein in hippocampal tissue from separate samples contemporaneous with

1122   those used for RNA-Seq. There was a significant interaction genotype x housing; $F_{(3,12)}$ = 9.95, p =

1123   0.008 for Arc/Arg3.1 protein expression, which was selectively strongly downregulated in WT

1124   enriched mice ($F_{(1,12)}$ = 7.64, p = 0.017). Inset shows one of the two blots used in the analysis of

1125   Arc/Arg3.1 expression. WE, WT enriched (n = 4); WS, WT standard housed (n = 4); KE, MSK1 KD

1126   enriched (n = 4); KS, MSK1 KD standard housed (n = 4). Bar graph depicts mean ± SEM and individual

1127   data points.

A

EE vs SH; P = 0.001

EE vs SH; P = 0.002

Distance (m)

N = 16/group

Open Field   Time (min)   Novel Object

B

WT SH   WT EE   KD SH   KD EE

Open Field   Novel Object   Open Field   Novel Object

WT SH

KD SH

WT EE

KD EE

C

EE vs SH; p = 0.001

Distance open/closed arms (%)

WT SH   WT EE   KD SH   KD EE

D

WT SH   WT EE

KD SH   KD EE

A

correct   incorrect

B

EE vs SH; p = 0.006

p = 0.014   p = 0.14

Correct alternations (%)

WT SH   WT EE   KD SH   KD EE

C

EE vs SH; p = 0.032

Latency to platform (s)

EE vs SH
p = 0.006

SH

EE

WT SH
WT EE
KD SH
KD EE

D1   D2   D3   D4   D5

20 cm platform   10 cm

D

D5 occupancy heat plots

WT SH   WT EE

KD SH   KD EE

E

Probe Trial
Genotype x housing; p = 0.013

p = 0.033

Time in target quadrant (%)

WT SH   WT EE   KD SH   KD EE

F

WT SH
WT EE
KD SH
KD EE

Time in target quadrant (s)

30   60   90   120

Time during probe trial (30 s bins)

A

EE vs SH; p = 0.008

EE vs SH; p = 0.014
WT vs KD; p < 0.001

WT SH
WT EE
KD SH
KD EE

Training

Reversal

B R2 occupancy (Train Plat NW)

WT SH   WT EE

KD SH   KD EE

C

WT vs KD; p = 0.009

p = 0.009

Training Platform   Reversal Platform

D Reversal probe (Train Plat NW)

WT SH   WT EE

KD SH   KD EE

E

Genotype x housing; p = 0.004

p = 0.027   p = 0.001

WT SH   WT EE   KD SH   KD EE

A

WT SH   WT EE   KD SH   KD EE

1 mV
5 ms

WT vs KD; p = 0.000

fEPSP slope (mV/ms)

Fibre volley (mV)

ns

WT SH 30 Pathways 15 Slices 8 mice
WT EE 30 Pathways 15 Slices 10 mice
KD SH 26 Pathways 13 Slices 8 mice
KD EE 30 Pathways 15 Slices 8 mice

Stimulus strength (μA)

B

GluA1   GluA2

mw   WE WS KE KS WE WS KE KS   WE WS KE KS WE WS KE KS
100
80
57
46
32
25

ns   genotype x housing; p = 0.034

GluA1/GAPDH   GluA2/GAPDH

WT SH WT EE KD SH KD EE   WT SH WT EE KD SH KD EE

C

WT SH   WT EE   KD SH   KD EE

100 ms

WT EE vs ●WT SH; p = 0.019

Paired-pulse ratio

WT SH 30 pathways 15 slices 8 mice
WT EE 30 pathways 15 slices 10 mice
KD SH 26 pathways 13 slices 8 mice
KD EE 30 pathways 15 slices 8 mice

Inter-pulse interval (ms)

WT **MSK1 KD**

Standard

Enriched

A

WT vs MSK1 KD; p = 0.000

Spine density/μm

WT SH   WT EE   KD SH   KD EE

B

Cumulative %

Spine density/μm

- - WT SH
—— WT EE
- - KD SH
—— KD EE

A

WT SH   WT EE   KD SH   KD EE

1 mV
5 ms

fEPSP slope (% baseline)

WT EE vs KD EE; p = 0.001

200

150

100

50

Time (min)

0   60   120   180

WT SH 8 slices 8 mice
WT EE 9 slices 8 mice
KD SH 7 slices 7 mice
KD EE 8 slices 8 mice

B

WT SH   WT EE   KD SH   KD EE

1 mV
5 ms

fEPSP slope (% baseline)

110

100

90

80

70

60

50

WT EE vs KD EE; p = 0.024

900 pulses @ 1 Hz

Time (min)

-20   0   20   40   60

WT SH 6 slices 6 mice
WT EE 7 slices 5 mice
KD SH 7 slices 6 mice
KD EE 8 slices 7 mice

C

Cumulative Probability

1.00

0.75

0.50

0.25

0.00

LTD | LTP

-30   -20   20 40 60 80 100

Synaptic Dynamic Range
(% of baseline)

WT SH (n = 14)
WT EE (n = 16)
KD SH (n = 14)
KD EE (n = 16)

D

genotype x housing interaction; p = 0.037

Change in synaptic plasticity (%)

100

80

60

40

20

0

p = 0.003

p = 0.92

WT SH   WT EE   KD SH   KD EE

A

High expression
in enriched WT

Low expression
in enriched WT

○ Dnaic2	○ Hist1h1c	
○ Cdhr3	○ Rarres2	
○ Egr1	○ Gm28578	
○ Vwa5b1	○ Gm11992	
○ P2rx6	○ Pih1d2	
○ Spry4		

B

A KD SH KD EE WT SH WT EE

BDNF
TrkB
Raf1
MEK2
MEK1
ERK1
ERK2
p38
MSK1
CREB
EGR1
Arc

1 3 4 5 6 7 8 32A 36A 10 11 13 14 15 16 17 33A

-2 -1 0 1 2

B MSK1

EE vs SH; p = 0.0001
p = 0.002   p = 0.002

Normalised counts
1100
1000
900
800
700
600
500
WT SH WT EE KD SH KD EE

C MSK2

ns

Normalised counts
1500
1400
1300
1200
1100
1000
900
WT SH WT EE KD SH KD EE

D EGR1
WE WS KE KS WE WS KE KS
EGR1 — 80
GAPDH — 32

genotype x housing; p = 0.020

0.6
p = 0.009
0.5
0.3
0.2
0.1
0.0
EGR1/GAPDH
WT SH WT EE KD SH KD EE

E Arc
WE WS KE KS WE WS KE KS
Arc — 45
GAPDH — 32

genotype x housing; p = 0.008

3
p = 0.017
2
1
0
Arc/GAPDH
WT SH WT EE KD SH KD EE