Supplementary figure legends

Supplementary fig 1 Immunohistochemical and histochemical staining of brain sections shows grossly normal morphology and no disruption of MAGUK or NMDA receptor subunit expression patterns in the hippocampus of hemizygous mutant mice. **(a)** Whole hippocampus. **(b)** Area CA1 at high magnification. All sections are counterstained blue with haemotoxylin except the Nissl images, which are stained with cresyl violet only. Scale bars: 50 μm.

Supplementary fig 2 Loss of SAP102 does not affect hippocampal cell density. **(a)** Dashed rectangles indicate regions of CA1 and dentate gyrus (DG) in which cell numbers were quantified. Scale bar: 500 mm. **(b)** Cell numbers in CA1. (i) Cells were quantified by counting the number of Nissl-stained cell bodies between the dorsal and ventral boundaries of the CA1 pyramidal layer at 50 mm-intervals along the most dorsal part of the layer. Representative sections are shown. Scale bars: 100 mm. (ii) No difference in cell numbers were observed between wt and hemizygous animals ($F = 1.23$, $p = 0.33$, $n = 6$). **(c)** Cell numbers in the dentate gyrus. (i) Cells were quantified using the same counting method at the caudal end of the lower arm of the pyramidal layer of the dentate gyrus. Scale bars: 50 mm. (ii) No difference between wt and hemizygous cell numbers was observed ($F = 0.36$, $p = 0.58$, $n = 6$).

Supplementary fig 3 No alteration in motor coordination or anxiety levels in SAP102 mutant mice. **(a)** Rotorod test for motor coordination. The mean ±

SEM latency to fall from the beam at 4, 16, or 32 rpm on each of three days. **(b-g)** Open field test for anxiety: **(b)** Mean latency to enter the centre portion of the open field (±SEM), and **(c)** the mean time (±SEM) spent in the centre square during the 15-minute trial. **(d)** the mean number of stretch attend postures that were observed (± SEM) and **(e)** the time spent freezing and the time spent immobile (±SEM). **(f)** Mean number of line crosses (±SEM) observed. **(g)** Rearing. Mean number of rears that were observed (±SEM). **(h-j)** Elevated plus maze test for anxiety: **(h)** mean time spent in the open arms of the maze (±SEM). **(j)** the mean number of head dips observed in the 5 minute trial. **(k)** the mean number of stretch attend postures observed and **(l)** the mean distance travelled (±SEM).

Supplementary table 1. Primary antibodies. Antibody uses are WB – western blot; IHC – immunohistochemistry; IP – immunoprecipitation. Non-commercial suppliers are Seth Grant, Wellcome Trust Sanger Institute, Cambridge, UK; Masahiko Watanabe, Department of Anatomy, Hokkaido University School of Medicine, Sapporo, Japan and Frank Dunn-Moore, School of Biology, University of St Andrews, St Andrews, UK.

Antigen	**Supplier**	**Antibody type**	**Working dilution or concentration**
NR1	S. Grant	sheep	5 μg per IP
NR1	Upstate	mouse monoclonal	1:1000 (WB)
NR1	Chemicon	mouse monoclonal	5 μg/ml (IHC)
NR2A	Upstate	rabbit polyclonal	1:500 (WB), 5 μg/ml (IHC)
NR2B	BD Biosciences	mouse monoclonal	1:250 (WB), 5 μg/ml (IHC)
GluR1	Upstate	rabbit polyclonal	1:670 (WB)
GluR2	Zymed	mouse monoclonal	1:300 (WB)
GluR6/7	Upstate	rabbit polyclonal	1:500 (WB)
Kv1.4	Upstate	mouse monoclonal	1:667 (WB)
ErbB4	Santa Cruz	rabbit polyclonal	1:200 (WB)
SAP102	Synaptic Systems	rabbit polyclonal	1:5000 (WB)
SAP102	M. Watanabe	rabbit polyclonal	5μg/ml (IHC)
PSD-95	BD Biosciences	mouse monoclonal	1:500 (WB)
PSD-95	Zymed	rabbit polyclonal	10 μg/ml (IHC)
PSD-93	Synaptic Systems	rabbit polyclonal	1:1000 (WB)
PSD-93	Watanabe	rabbit polyclonal	4μg/ml (IHC)
SAP97	BD Biosciences	mouse monoclonal	1:1000 (WB)
SynGAP	Upstate	rabbit polyclonal	1:2000 (WB)
Sec8	BD Biosciences	mouse monoclonal	1:1000 (WB)
Pyk2	BD Biosciences	mouse monoclonal	1:1000 (WB)
Kalirin	Upstate	rabbit polyclonal	1:250 (WB)
Stargazin	Calbiochem	rabbit polyclonal	1:200 (WB)

NrCAM	F. Gunn-Moore	rabbit polyclonal	1:1000 (WB)
MAP2B	BD Biosciences	mouse monoclonal	1 μg/ml (IHC)

Supplementary materials and methods

Neuroanatomy

All mice were treated in accordance to guidelines determined through the UK Animals (Scientific Procedures) Act, 1986 and all procedures were approved through the British Home Office Inspectorate and the University of Edinburgh Ethics Review board.Mice were anaesthetised with a lethal dose of sodium pentobarbitone, perfused with aldehydes and the brain dissected, post-fixed and sliced into 5 μm sections. Immunohistochemistry was performed on an automated Ventana Discovery machine according to the manufacturer's instructions. Primary antibodies were against SAP102 and PSD-95 (gifts from M. Watanabe), PSD-95 (10 μg/ml) from Zymed, NR1 (5 μg/ml) from Chemicon, NR2A (5 μg/ml) from Upstate, NR2B (5 μg/ml) and MAP2B (1 μg/ml) from BD Biosciences. Secondary antibodies were biotin-conjugated rabbit anti-mouse (Dako Cytomation) or biotin-conjugated donkey anti-rabbit (Jackson). Standard procedures were used for mounting and coverslipping.

Phosphorylation screen

Hippocampal protein extracts were prepared by homogenising dissected hippocampi in 20 mM MOPS pH 7.4, 2 mM EDTA, 5 mM EGTA, 0.5% Triton X-100, 30 mM NaF, 40 mM β-glycerophosphate, 20 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1mM PMSF, 3mM

benzamidine, 5 μM pepstatin and 10 μM leupeptin and quantifying the protein concentrations using a BCA assay (Pierce). Extracts were subjected to the KPSS-1.3 phosphorylation screen (Kinexus Bioinformatics Corporation) which uses phospho-specific antibodies to quantify phosphorylation states of the following proteins (phospho-sites shown in parentheses): ADD1 (S724), ADD3 (S693), B23 (S4), CDK1/2 (T14/Y15), CREB1 (S133), ERK1 (T202/Y204), ERK2 (T185/Y187), GSK3□ (S21, 279), GSK3β (S9, Y216), JNK (T183/Y185), Jun (S73), MEK1 (S217/S221), MEK3 (S218), MEK6 (S207), MSK1 (S375), NR1 (S896), p38α MAPK (T180/Y182), PKBα (T308), S473), PKCα (S657), PKCα/β2 (T638/T641) PKCδ (T505) PKCε (S729), PKR1 (T414), Raf1 (S259), Rb (S773, S800/S804), RSK1/2 (T359/T365), S6Kα (T412), Smad1/5/9 (S463/S465/S428/S430), Src (Y423, Y543) STAT1 (Y701), STAT3 (S727) and STAT5A (Y694).

Behavioural Test Battery

Rotorod

Apparatus

A fixed-speed rotorod (IITC Life Science, Series 8 Model 755, Harvard Apparatus UK) was used with the following settings to achieve 60 s trials, with the 3.17 cm rod set to spin at 2, 4, 16 or 32 rpm. Passive rotations, as defined by the mouse hanging onto the bar for a complete revolution, were also recorded.

Procedure

Mice were run in the light portion of their light-dark cycle in a lit testing room adjacent to the vivaria. On the first day, 3 trials of 2 rpm were used to

habituate the animals to the protocol. Five mice at a time were run on the rotorod for 3 trials of at each of the 3 speeds over three consecutive days. The speed of the rod always went from the lowest to the highest speed for each day. Mice were placed on the rod and fell to activate a switch, so that latency to fall was recorded. Three-way (genotype x trial x day) mixed ANOVAs were used to assess significance of the results.

Grip-strength

Apparatus

The apparatus was one that had been built with a force displacement transducer (Grass Instruments, Mass, USA, #FT03C), being run into a Mac Lab Bridge amplifier and then a Powerlab/4SP, which fed into a computer using Powerlab ADInstruments Chart v 4.12. A bar for the mice to grasp was made from a 2 inch, 21g needle, which had been bent in the form of a trapeze bar, and this was attached to the transducer.

Procedure

A mouse picked up by its tail and the two front paws were put in the vicinity of the grasping bar. Once the mouse had grasped with both front paws, they were pulled directly back in only the horizontal plane in a continuous motion, until the mouse let go. This was done seven times for each mouse, and the high and low scores were not used, as this would control for the fact that mice may grasp with hind paws, or let go with one paw first. The remaining five trials were then used to generate a grip strength score.

Open field

Apparatus

Clean (45 cm x 28 cm x 13 cm) translucent shoebox cages were used. They were placed on the floor in a grid and the mice were then run in batches for 15 minutes. This was recorded with a Sony DCR-DVD202 recorder. Run in the light portion of the light-dark cycle in the light.

Procedure

Mice were run during the light portion of the light-dark cycle in a room lit with overhead, florescent light. A Sony DVD handicam**,** mounted on the ceiling was used to record the 15 minute exposure to the arena. To begin each session, the mouse was transported from the home cage to the arena in a small container and then placed directly into its box.

Scoring was performed at a later point with behavioural scoring software (Hindsight v 1.5). The field was divided into thirds along its length and width and the resulting inner rectangle denoted the inner zone. Data were analyzed for the following measures: frequency of rearing against the walls, frequency of rearing (unsupported), number of stretch attend postures, frequency and duration of freezing, the number of line crosses and the frequency, latency and duration that the mouse was in the inner zone.

Elevated plus maze

Apparatus

Tracksys IR EPM used with Ethovision tracking software (ver 3.0) was used with a Sony camera fitted with IR filters. The elevated maze consisted of a

black Plexiglas 'X' with four arms and a central square platform. The arms were 5 cm wide and 30 cm long. Two opposite arms had clear Perspex walls 15 cm high, and the remaining two arms were open, but had a 3 mm lip around the edge which was added to minimize the likelihood of mice falling the maze. The entire apparatus was elevated 45 cm from the floor and a digital video record was generated from with a camera suspended above the maze from tripod.

Procedure

Mice were run during the light portion of the light-dark cycle in a room lit with dim red light. Each mouse was placed onto the centre square with its head facing toward an open arm. It was then released and allowed to explore for 5 minutes, after which the mouse was removed from the maze and placed in its home cage. A trained observer sat within close proximity to a computer screen and scored head dips and stretch attend postures with the keyboard entry function within the Ethovision software. The entire maze was cleaned with alcohol-free disinfectant wipes between mice (Trigene Antiseptic Wipes, Medichem UK). After all animals had been run, data collected for each mouse (the frequency of head dips, stretch attend postures, and entries into the dark arms, light arms, and the centre square) were analyzed. The time spent exploring the open and closed arms and the centre square, as well as time spent grooming were also calculated. From these measures, the percentage of time in the open was calculated as the time spent in the open arms divided by the total time spent in the open and closed arms, excluding time spend in the centre square.