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The kinase function of MSK1 regulates BDNF signaling to CREB and basal synaptic transmission, but is not required for hippocampal long-term potentiation or spatial memory

MSK1, spatial memory and LTP

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Abbreviated title: MSK1, spatial memory and LTP

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Abstract

The later stages of long-term potentiation *in vitro* and spatial memory *in vivo* are believed to depend upon gene transcription. Accordingly, considerable attempts have been made to identify both the mechanisms by which transcription is regulated and indeed the gene products themselves. Previous studies have shown that deletion of one regulator of transcription, the mitogen- and stress-activated kinase 1 (MSK1), causes an impairment of spatial memory. Given the ability of MSK1 to regulate gene expression via the phosphorylation of CREB at serine 133, MSK1 is a plausible candidate as a prime regulator of transcription underpinning synaptic plasticity and learning and memory. Indeed, prior work has revealed the necessity for MSK1 in homeostatic and experience-dependent synaptic plasticity. However, using a knock-in kinase-dead mouse mutant of MSK1 the current study demonstrates that, whilst the kinase function of MSK1 is important in regulating the phosphorylation of CREB at serine 133 and basal synaptic transmission in hippocampal area CA1, it is not required for mGluR-dependent long-term depression, two forms of long-term potentiation or several forms of spatial learning in the water maze. These data indicate that other functions of MSK1, such as a structural role for the whole enzyme, may explain previous observations of a role for MSK1 in learning and memory.

78 **Significance Statement**

79 The nuclear kinase MSK1 has been identified as a possible link between cell-surface
80 neurotransmitter receptors and the gene expression necessary for long-term memory: by coupling
81 the activation of BDNF receptors to the regulation of transcription via the phosphorylation of
82 CREB, MSK1 unites a neurotrophin heavily implicated in synaptic plasticity with changes in gene
83 expression. Using a kinase-dead MSK1 mouse mutant we show that, whilst MSK1 is necessary for
84 CREB phosphorylation and the regulation of basal synaptic transmission, it is not required for
85 mGluR-dependent long-term depression, long-term potentiation or several forms of spatial
86 reference memory. MSK1 may instead play a homeostatic role in the CNS that allows synapses to
87 adapt to prevailing synaptic or sensory experience.

88

Introduction

The intracellular mechanisms that link the activation of cell surface neurotransmitter receptors to the genomic changes promoting neuronal morphological and functional adaptations has been a topic of considerable interest. This interest has arisen from the potential to both identify signaling pathways responsible for these changes, and to use this knowledge to intervene pharmacologically in a host of developmental, neurological and psychiatric conditions (Guzman-Karlsson *et al.*, 2014; Lynch *et al.*, 2014).

In this regard, the nuclear kinase mitogen- and stress-activated kinase (MSK) (Deak *et al.*, 1998) is well-placed to couple activity at the cell surface to changes in gene expression. Of the two isoforms of MSK (MSK1 and MSK2), MSK1 is most highly expressed in brain (Arthur *et al.*, 2004) and is activated in response to neurotrophins, including brain-derived neurotrophic factor (BDNF) (Arthur, 2008; Frenguelli & Corrêa, 2012; Reyskens & Arthur, 2016). The stimulation of BDNF TrkB receptors results in the activation of a number of signaling pathways including the MAPK cascade, and in particular of ERK1/2 and p38 (Minichiello, 2009; Panja & Bramham, 2013). These enzymes translocate to the nucleus and are responsible for the direct activation of MSK1 via phosphorylation of key residues on the C-terminal kinase domain, which then phosphorylates residues on the N-terminal kinase domain (McCoy *et al.*, 2005). Once activated, the N-terminal kinase domain of MSK1 phosphorylates cAMP response element binding protein (CREB) at S133 (Deak *et al.*, 1998; Arthur, 2008; Frenguelli & Corrêa, 2012; Reyskens & Arthur, 2016). From observations in *Aplysia* (Dash *et al.*, 1990), *Drosophila* (Yin *et al.*, 1994) and mice (Bourtchuladze *et al.*, 1994), CREB has emerged as an evolutionarily-conserved mechanism by which neurons convert activity into persistent modifications of synaptic function and the formation and stabilization of memories (Sakamoto *et al.*, 2011; Kandel, 2012; Kida & Serita, 2014).

Accordingly, by linking BDNF to CREB-dependent gene transcription, MSK1 is positioned to respond to the many experiential and synaptic stimuli that provoke the release of BDNF and to convert these stimuli into long-term structural, functional and cognitive adaptations. Indeed, prior work using MSK single or double knockout mice has suggested that MSKs regulate neurogenesis (Choi *et al.*, 2012; Karelina *et al.*, 2012; Karelina *et al.*, 2015) and BDNF-induced CREB phosphorylation (Arthur *et al.*, 2004). These observations, as well as a role for MSK1 in post-translational modifications of histones (Chwang *et al.*, 2007; Chandramohan *et al.*, 2008), and in the regulation of the plasticity-related protein Arc/Arg3.1 (Shepherd & Bear, 2011; Corrêa *et al.*, 2012), may explain the reported deficits in MSK1 knockout mice in: spatial learning in the water maze; fear conditioning (Chwang *et al.*, 2007); the display of behavioral immobility in the forced swim test (Chandramohan *et al.*, 2008); learning in the Barnes maze, and discrimination in novel object recognition (Karelina *et al.*, 2012).

Whilst these knockout studies suggest an important role of MSK1 in various aspects of learning and memory, this role may involve other aspects of MSK1 beyond its kinase function. For example, it has been reported that MSK1 forms a structural complex with ERK1/2 and the glucocorticoid receptor which is necessary for transcription of the immediate early genes c-Fos and Egr-1 (Gutierrez-Mecinas *et al.*, 2011). Thus, the phenotype of knockouts of MSK1 does not directly discriminate between structural and kinase roles for MSK1 in synaptic and cognitive function. In order to address this issue an MSK1 mutant has been generated in which the kinase activity of MSK1 is selectively inactivated, but one in which protein levels of MSK1 remain close to wild-type levels (Corrêa *et al.*, 2012). This kinase-dead mutation involves the knock-in substitution of an alanine for the aspartate at position 194 in the DFG motif of the N-terminal kinase domain of the endogenous MSK1 gene. Mutations in the DFG motif are commonly used to abolish the kinase activity of enzymes (Moran *et al.*, 1988; Vijayan *et al.*, 2015) and confirmation that this results in a

kinase-dead version of MSK1 (MSK1 KD) was previously evidenced by the inability of MSK1 KD to phosphorylate peptide substrates, even when over-expressed in cell lines (Deak *et al.*, 1998; McCoy *et al.*, 2005; Corrêa *et al.*, 2012).

Using this MSK1 KD mutant we show that, whilst there is reduction in BDNF stimulation of CREB phosphorylation and a deficit in basal synaptic transmission in hippocampal area CA1, there is no change in paired-pulse facilitation, metabotropic glutamate receptor-dependent long-term depression (mGluR LTD), or tetanus- or theta-burst-induced long-term potentiation (LTP). Moreover, we observe no overt deficits in various water maze paradigms. These observations suggest that, under conditions of typical rearing of experimental rodents, the kinase activity of MSK1 is not necessary for several forms of synaptic plasticity and spatial learning, but may instead be required for homeostatic adaptation to prevailing synaptic activity.

Materials and Methods

Animals

A kinase dead MSK1 mouse was generated by mutating Asp194 to Ala (D194A) in the DFG motif in the endogenous MSK1 gene (Corrêa *et al.*, 2012). The knock-in was produced by TaconicArtemis GmbH using standard targeting methods in C57/Bl6 ES cells. Routine genotyping was carried out by PCR using the primers 5'-CGGCCATGTGGTGCTGACAGC-3' and 5'-GGGTCAGAGGCCTGCACTAGG-3', which gives 378 bp and 529 bp products for wild-type and targeted alleles, respectively. Western blots of MSK1 protein (n = 4 per genotype) revealed that MSK1 was expressed in MSK1 KD mice at approximately 65 % of the levels found in wild-type mice (data not shown).

Unless otherwise stated, all mice were kept in individually ventilated cages (Tecniplast Blue Line 1284L) with a sawdust substrate, paper shavings and at least one cardboard play tunnel with water and food provided *ad libitum*. Mice were kept in social groups where possible with a maximum of 5 mice per cage and maintained on a 12/12 light dark cycle with lights on at 0700. The care and accommodation of all the animals held in the facility comply with the standards set out in relevant codes of practice for the housing and care of animals used for scientific purposes.

Extracellular recordings: Male C57/BL6 WT and MSK1 KD mice (2 to 5 months old) were killed by cervical dislocation in accordance with appropriate animal welfare legislation. Sagittal brain slices (400 µm) were prepared and extracellular recordings were made from stratum radiatum in area CA1 at 32-33 °C from slices that were either submerged in aCSF or, in the case of the Actinomycin-D experiments, held at a humidified and oxygenated air/aCSF interface. A two-pathway stimulation protocol was adopted with each electrode placed either side of the recording electrode. Each pathway was stimulated in an alternating fashion with either 60 s (submerged slices) or 90 s (interface slices) between stimuli to a given pathway. A prolonged interval between

stimuli avoids activity-dependent fatigue of LTP (Fonseca *et al.*, 2006a; Villers *et al.*, 2012). That the stimulated fiber pathways were convergent but independent was confirmed with a cross-pathway paired-pulse facilitation protocol (50 ms inter-pulse interval) that showed no facilitation across pathways.

Stimulus input/output curves were generated over a range of electrical stimuli from 10 – 300 μA (0.1 ms in duration) using a Digitimer DS3 constant current isolated stimulator. Paired-pulse facilitation was measured over a range of 50 – 350 ms inter-pulse interval.

LTP was induced using two protocols: a tetanus of 100 stimuli at 100 Hz, and a theta-burst stimulation (TBS) protocol at test intensity (1 mV fEPSP amplitude in submerged slices; 3 mV fEPSP amplitude in interface slices). Bursts consisted of 4 stimuli at 100 Hz. Each train was composed of 10 bursts separated by 200 ms. Trains were repeated 3 times with an interval of 20 seconds. That TBS resulted in transcription-dependent LTP was tested in two series of experiments (in interface chambers) using either 40 μM Actinomycin-D (Act-D; in 0.08 % DMSO) applied for the duration of the experiment (> 30 min before TBS and at least 80 mins after TBS), or 25 μM Act-D (0.05 % DMSO) given 15 min before and until 15 min after TBS. An additional series of interleaved experiments were performed in DMSO, which was present throughout the experiments at the level (0.08%) found in the 40 μM Act-D experiments.

Metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD) was induced in hippocampal slices via the application of the Group I mGluR agonist DHPG (100 μM; 10 min) in the presence of both the $GABA_A$ receptor antagonist picrotoxin (50 μM) and the NMDA receptor glycine site antagonist L689,560 (5 μM).

Stimulation and recording parameters, as well as the analysis of evoked fEPSPs, were under the control of WinLTP data acquisition software. Experiments were interleaved and performed blind to the genotype of the mice, which was revealed only after the experiments had been analysed and confirmed with post-hoc genotyping.

Immunohistochemistry for CREB phosphorylation at serine 133.

After cutting, hippocampal slices (300 µm) were suspended on a mesh located within a 50 ml beaker (up to four slices/beaker) for 3 hrs in oxygenated circulating aCSF at 34 °C. Slices were then either treated with 50 ng/ml recombinant human BDNF (Cell Guidance Systems; GFH1) or forskolin (50 µM; Sigma Aldrich; F6886) for 10 min or left untreated to serve as parallel time controls. Treated and untreated slices were rapidly immersed in 4 % paraformaldehyde in phosphate-buffered saline (PBS; pH 7.4) and fixed overnight. The slices were washed three times in PBS and then incubated at room temperature in a solution containing both 10 % goat serum, to prevent nonspecific antibody binding, and 0.4 % Triton X-100 in PBS to permeabilize slices. After three washes in PBS the slices were incubated for 4 hours at room temperature with a phospho-CREB primary antibody targeting serine 133 (S133; rabbit mAb; Cell Signalling; #9198) diluted 1:400 in 10 % donor goat serum and 0.4 % Triton X-100 in PBS. The slices were then washed twice in PBS and incubated for 1.5 hours at room temperature in Alexa Fluor 488 goat anti-rabbit antibody (Molecular Probes; #A-11008) diluted 1:800 in 10 % donor goat serum and 0.4 % Triton X-100 in PBS. The slices were then washed again in PBS three times. The slices were viewed and images were acquired blind to the genotype using identical settings with Zeiss ZEN2 software on an LSM 880 laser confocal-scanning system coupled to a Zeiss inverted microscope. Images were taken using a 40x oil immersion objective. Averages of four scans were collected for each image. The mean pixel intensity in the CA1 cell body region was determined using ImageJ 1.46r. The pixel intensity corresponding to individual CA1 neurons (identified by a threshold mask) was averaged

on a per slice basis and comparisons were made between treated and untreated slices in terms of absolute fluorescence (Arbitrary Units; AU), measured using identical acquisition parameters, and the % change pixel intensity in BDNF- or forskolin-treated slices over the corresponding untreated controls. Immunofluorescence data was acquired from a total of 11 wild-type and 13 MSK1 KD mice from which between 1 and 5 slices each were used for the imaging studies for each experimental condition (control, BDNF and forskolin). On average approximately 70 neurons were analysed per slice across the various experimental conditions. The specificity of the phospho-CREB primary antibody was confirmed by testing the antibody in hippocampal slices prepared from a conditional CREB S133 mutant mouse in which S133 is mutated to alanine (CREB S133A)(Wingate *et al.*, 2009). No fluorescent signal was detected in CA1 pyramidal neurons (data not shown).

Behavior

Male MSK1 KD mice (n = 12) and control littermates (n = 12), aged 15 weeks at the start of the analysis, received daily handling during the last week of a quarantine period, and were subject to a total of 5 weeks of experimentation. During this period, they were group housed, had *ad libitum* access to food and water, and were maintained on a light/dark cycle of 14:10 hr with lights on at 08.00 hr. All mice were trained and tested “blind” for genotype.

The watermaze

The open-field watermaze is 2 m in diameter and was housed in a large laboratory room with prominent extramaze cues. The water was filled and drained daily, maintained at 25±2°C, and made opaque by the addition of 500 ml of liquid latex. Each mouse was gently placed into the water facing the side-walls at one of the four pre-planned start positions (North, South, East and West). If a mouse failed to reach the platform after 90 sec (during training) or 60 sec (probe tests) had elapsed, it was guided by hand to the escape platform. Once on the platform, each mouse was

allowed to remain for 30 sec. After 30 sec, the mouse was taken away from the platform using a paint roller and put under a heat lamp until the next trial. The swimming path of the mice was tracked using the Actimetrics Watermaze software (Coulbourn Electronics).

Visible cue task in the water maze

Curtains were drawn around the pool to exclude the extra-maze cues. Escape from the water was via a single moveable platform of 20 cm diameter located 1.5 cm beneath the water surface. The platform was made visible by placing upon it a red/black plastic ball (about 20 cm in height). Mice were tested in groups of no more than 6. Each mouse was given 3 days of training (4 trials/day; t = 90s). Each cardinal point was used once as the starting position on each day. The inter-trial interval was 10 min and the platform location was changed across trials.

Spatial reference memory task

Curtains were absent for the rest of the spatial training, in order for mice to use extra-maze cues to build up a spatial representation of the room. The escape platform, still beneath the water surface, was now uncued and remained at the same spatial location throughout the spatial reference memory (SRM) training, forcing the animals to use a spatial strategy to retrieve the platform location. The animals were subjected to 5 days of training with 4 trials/day, and an inter-trial interval of 10 min. As in the cue task, mice were released from each cardinal point in a random order. Twenty-four hours after the last trial a probe test (PT1) was conducted in which the platform was removed and the animal placed in the pool to swim for 60s.

Serial spatial memory task

The animals were then subjected to serial spatial learning, a series of 5 spatial reference memory tasks trained to a criterion of performance as described by Chen et al., (2000) and Daumas et al.,

(2008). Each task constituted a separate spatial problem, with all 5 problems taking place in the same water maze in the same experimental room (starting 24-48h after SRM completion). The platform location was varied from placement on an inner "virtual" ring (1m diameter) or an outer ring (1.5m diameter). In this way, the location differed between problems but remained the same within each day of training and each spatial problem, until the chosen criterion was reached. The animals had a maximum of 32 trials to acquire a spatial problem task, but if they reached the criterion of an average escape latency of <20 sec on 3 consecutive trials, the training for the given spatial problem was stopped. The training for the next spatial problem began two days later. There was a maximum of 8 trials per day, with an inter-trial interval of 10 min. If an animal did not reach the platform within 90 sec, it was directed to the platform using a small paint roller, and allowed to rest for 30 sec.

In order to assess the strength of memory for each platform location, a probe test was conducted. To study short-term memory the probe test was given 10 min after reaching criterion on each of the 5 problems. To study long-term memory the probe test was given 24h after reaching criterion. A 13 cm diameter Atlantis platform was used to avoid extinction between probe tests. This platform, which was unavailable to the mice during the test, is software-driven to rise after the 60s of the probe trial had elapsed. The animals had 30 additional seconds to find the platform and if they did not, were guided to the platform with a paint roller and left there for 30s.

Massed spatial water maze task

A week after the end of the serial spatial task, mice were submitted to 4 consecutive sessions of 4 trials, with an inter-session delay of 15 minutes during which they were returned to their home cage. The Atlantis platform was submerged 0.5 cm beneath the surface of the water. Animals were introduced in the maze from different starting points and allowed to swim freely or until they

reached the platform. Mice failing to find the platform within 90 s were gently guided to the platform and left on it for 30s. The starting positions were determined in a pseudorandom order, and the sequence of starting locations was randomized such as each of them was used 4 times during the four training sessions.

Ten minutes, 24 hours and 7 days after the last training session mice were submitted to a probe test using the Atlantis platform, which rose after the 60s of the probe trial had elapsed, after which mice were allowed to search for it for an additional 30s.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 22 using the tests described in Table 1. Statistical significance was set at $p < 0.05$.

Results

MSK1 is required for BDNF-dependent phosphorylation of CREB S133

To confirm the kinase-dead nature of the mutation in the MSK1 gene in the MSK1 KD mutant mice, we conducted a series of experiments utilizing acutely-prepared hippocampal slices taken from wild-type and MSK1 KD mice of an age similar to those used for the electrophysiology and behavioral studies. Incubation of wild-type hippocampal slices with BDNF (50 ng/ml; 10 min) resulted in a significant increase in CA1 immunoreactivity for phosphorylated CREB at S133 (79.2 ± 5.1 arbitrary units (AU); 21 slices from 8 mice[(a)]) compared to parallel control, untreated slices from the same animal (59.3 ± 4.8 AU; 15 slices from 8 mice; $p = 0.004$[(a)]; Figure 1A, upper panels). There was little or no increase in CREB phosphorylation in slices taken from MSK1 KD mice (63.4 ± 4.7 vs 56.7 ± 3.4 AU in BDNF; both 20 slices from 10 mice; $p = 0.29$[(a)]; Figure 1A, lower panels). There was no difference in basal pCREB fluorescence between the two genotypes ($p = 0.55$[(a)]). A comparison of the BDNF-induced change in immunofluorescence showed a significant BDNF-dependent increase in CREB phosphorylation in wild-type mice (139.5 ± 8.4 %, n = 21 slices from 8 mice[(b)]), but no overall change in pCREB immunoreactivity in the CA1 region of slices taken from MSK1 KD mice (95.3 ± 7.9 %, n = 20 slices from 10 mice; $t_{39} = 3.83$; $p = 0.0004$[(b)]; Figure 1B). That CREB could be phosphorylated in MSK1 KD mice was demonstrated by the incubation of slices from wild-type and MSK1 KD mice in the adenylate cyclase activator forskolin (50 µM; 10 min; data not shown). Basal phosphoCREB immunofluorescence was similar across both genotypes (66.2 ± 8.9 AU for wild-type; 7 slices from 5 mice; vs 78.8 ± 7.8 AU for MSK1 KD slices; 15 slices from 6 mice; $p = 0.37$[(c)]) and rose to 100.5 ± 9.8 AU and 104.9 ± 8.2 AU after forskolin application in wild-type and MSK1 KD slices, respectively (n = 8 slices from 5 wild-type mice and 16 slices from 6 MSK1 KD mice; $p = 0.003$[(c)]). Expressed as a percentage, forskolin application increased phosphoCREB immunofluorescence to a similar extent in wild-type slices (148.1 ± 12.7 %; n = 8 slices from 5 mice) and MSK1 KD slices (137.3 ± 9.0 %; n = 16 slices from 6 mice).

The regulation of basal synaptic transmission, but not the probability of transmitter release, mGluR-dependent long-term depression or long-term potentiation, is dependent upon the kinase activity of MSK1.

To establish the role of MSK1 in synaptic function we performed electrophysiological experiments in area CA1 of hippocampal slices prepared from MSK1 KD and wild-type mice.

The construction of stimulus input/output curves (10 – 300 μA) revealed a mild reduction in excitatory synaptic transmission in the MSK1 KD mutants (n = 12 mice; 17 pathways) when compared to wild-type animals (n = 18 mice; 27 pathways). This was apparent when the fEPSP slope was compared to the presynaptic fiber volley (Figure 2A), or when the slope of the fEPSP was plotted as a function of the stimulus strength (Figure 2B): There was a main effect of genotype ($p = 0.032$[(d)]) and a significant interaction between genotype and stimulus intensity ($p = 0.0001$[(d)]) at the higher stimulus strengths (200 – 300 μA; p: 0.048; 0.035 and 0.033, respectively[(e)]). However, there was no appreciable difference in the size of the presynaptic fiber volley between mutant and wild-type mice $p = 0.23$[(f)]) nor was there a fiber volley genotype x stimulus intensity interaction ($p = 0.42$[(g)]; Figure 2C). To rule out differences in GABAergic inhibition between mutant and wild-type animals, input/output curves were constructed from slices taken from a different set of animals in the presence of the $GABA_A$ receptor antagonist picrotoxin (50 μM; 5 pathways from 4 wild-type mice and 7 pathways from 5 MSK1 KD mice). The deficit in synaptic transmission at higher stimulus strengths (200 - 300 μA) in slices from MSK1 KD mice persisted during antagonism of $GABA_A$ receptors ($p = 0.001$) at 200, 250 and 300 μA ($p = 0.048$; 0.035 and 0.040, respectively[(h)]) (Figure 2D).

362 To test whether this deficit in basal synaptic transmission had a presynaptic component, we
363 measured the facilitation of synaptic transmission associated with delivering pairs of electrical
364 stimuli at 50 to 350 ms intervals. Such paired-pulse facilitation is taken as an index of the initial
365 probability of transmitter release. There was no difference in paired-pulse facilitation between
366 mutant (22 pathways from 19 mice) and wild-type mice (21 pathways from 18 mice), with the
367 paired-pulse facilitation profile over the 50 – 350 ms range essentially overlapping in the two
368 genotypes (Figure 3A). Thus, as far as can be determined with paired-pulse facilitation and the
369 similar magnitudes of the presynaptic fiber volley, the deficit in basal synaptic transmission in the
370 MSK1 KD mice is not presynaptic and instead appears likely to have a postsynaptic locus,
371 potentially via a reduction in synaptic AMPA-type glutamate receptors.
372
373 To test whether this impairment in basal synaptic transmission reflected a long-term depression
374 (LTD)-type state, we examined the LTD induced by the activation of Group I (GI) metabotropic
375 glutamate receptors (mGluR). This form of LTD requires the induction of Arc/Arg3.1 (Waung *et al.*,
376 2008) and a deficit in Arc/Arg3.1 regulation has been observed in MSK1 KD neurons in response to
377 activity deprivation (Corrêa *et al.*, 2012), which may explain the failure to induce bidirectional
378 homeostatic plasticity in MSK1 KD mutant neurons. Accordingly, we applied the GI mGluR agonist
379 DHPG (100 µM; 10 min) to hippocampal slices from both MSK1 KD mutant and wild-type slices and
380 measured fEPSPs from area CA1. DHPG caused a ~ 40 % inhibition of the fEPSP 60 min after the
381 onset of DHPG application that was no different between MSK1 KD and wild-type slices[(i)] (Figure
382 3B).
383
384 To establish instead whether long-term potentiation (LTP), which also has a requirement for
385 Arc/Arg3.1 (Plath *et al.*, 2006) would be affected by the MSK1 KD mutation, we performed dual-
386 pathway LTP experiments in both mutant and wild-type slices and used both tetanus and theta-

burst stimulation (TBS) paradigms as they have been reported to recruit different intracellular signaling pathways. In particular, in contrast to single tetanus LTP, TBS is reported to recruit both ERK1/2- (Winder *et al.*, 1999), and transcription-dependent LTP (Nguyen & Kandel, 1997) and where a deficit in slices from MSK1 KD mice might be expected to be observed.

Given the difference in basal synaptic transmission between the MSK1 KD and wild-type mice (Figure 2), and to avoid bias associated with differential postsynaptic depolarization associated with LTP-inducing high frequency stimulation, basal fEPSP amplitude was set to 1 mV across the two genotypes. This protocol has been adopted previously with mutant mice displaying impaired basal synaptic transmission (Patterson *et al.*, 1996). It should be noted that whilst such stimulus-matching is important in allowing comparisons regarding relative enhancements of synaptic transmission after high frequency stimulation, it does so only at a fixed stimulus strength. Thus, in the absence of full input-output curves before and after LTP, it is conceivable that MSK1 KD mice would still display a deficit in basal synaptic transmission after LTP when compared to wild-type mice.

Both tetanus and TBS induced robust LTP which persisted for the duration of the experiment (180 minutes post-high frequency stimulation). There was no obvious difference between either the initial potentiation or the potentiation at 180 minutes between the two genotypes or stimulation protocols (Figure 3C, D). For wild-type mice tetanus-induced LTP measured over the last ten minutes of the experiment was 127.1 ± 9.9 % (n = 9), while after TBS it was 137.6 ± 10.9 % (both relative to the preceding baseline; n = 7); and in the MSK1 KD mutants the corresponding values were: tetanus-induced LTP at 132.6 ± 6.4 (n = 8) and theta-burst induced LTP at 130.9 ± 6.1 % (n = 7). Neither tetanus- nor TBS-induced LTP differed significantly across the genotypes ($p = 0.53$ and p

411 = 0.61[j], respectively). Accordingly, it would seem that the kinase activity of MSK1 is not required
412 for LTP, at least over the time course (3 hrs) of the present experiments.
413
414 To confirm that the late LTP evoked required gene transcription, we performed a series of
415 experiments using the transcription inhibitor Actinomycin-D (Act-D) during TBS-induced LTP
416 (Nguyen & Kandel, 1997). In these experiments slices were maintained in an interface chamber, in
417 which a deficit in basal synaptic transmission was still observed in slices from MSK1 KD mice (data
418 not shown). Due to the larger fEPSPs evoked from slices in interface chambers (Reid *et al.*, 1988;
419 Croning & Haddad, 1998; Bortolotto *et al.*, 2011), basal fEPSP amplitudes in both genotypes were
420 set at 3 mV.
421
422 When applied at 40 µM for the duration of the experiment (n = 5; 4 wild-type and 1 MSK1 KD
423 mice) Act-D resulted in a return of LTP to baseline values within 80 mins after TBS compared to
424 interleaved DMSO control experiments (n = 5; 3 wild-type and 2 MSK1 KD mice[k]) where the LTP
425 was maintained at ~130 % of control (Figure 3E). However, a ~15 % decrease in the control
426 pathway was observed with this protocol that could have accounted, at least in part, for the
427 observed decrementing LTP. We therefore conducted an additional series of experiments where a
428 lower concentration of Act-D (25 µM; n = 9; 3 wild-type mice and 4 MSK1 KD mice; Figure 3E) was
429 applied for 15 mins before and until 15 mins after TBS. This lower concentration and shorter
430 duration of Act-D had a similar inhibitory effect on TBS-induced LTP, with a return to baseline at
431 approximately 80 mins, but with less of an influence (< 10 %) on the control pathway, and which
432 was similar to that recorded in the DMSO control experiments. Since the above experiments
433 combined slices from both wild-type and MSK1 KD slices, and showed that the effects of 25 and 40
434 µM Act-D on LTP were similar, we segregated the Act-D data on the basis of genotype (7 slices
435 from 7 wild-type mice and 7 slices from 5 MSK1 KD mice). This analysis showed that TBS-induced

LTP in both wild-type and MSK1 KD slices was equally sensitive to Act-D (Figure 3F). These data confirm that LTP induced by TBS requires gene transcription for its maintenance[k,l] (Nguyen & Kandel, 1997) in both wild-type and MSK1 KD mice and further indicate that transcription regulated by MSK1 is not required.

The kinase activity of MSK1 is not required for spatial memory tasks in the watermaze

Given previous reports that MSK1 is required for a number of learning tasks (Chwang *et al.*, 2007; Chandramohan *et al.*, 2008; Karelina *et al.*, 2012), we examined MSK1 KD mice in a variety of hippocampal-dependent water maze training protocols (Figures 4, 5 and 6). In the cued version of the water maze (Figure 4A) mice of both genotypes improved their performance over the three days of training with a decline in escape latency ($p < 0.0001$[m]); there was no significant effect due to the genotype ($p = 0.40$[n]), nor a significant interaction between genotype and day ($p = 0.19$[o]). These data indicate that the MSK1 KD mutant does not display gross motor or sensory deficits.

In the spaced trials hidden platform version of the water maze (Figure 4B), mice of both genotypes improved their performance over the five days of training ($p< 0.0001$[p]); there was no significant effect due to the genotype ($p = 0.69$[q]), nor a significant interaction between genotype and day ($p = 0.46$[r]). In a probe trial given 24 hours later (Figure 4C), both mutant and wild-type mice displayed selective searching in the training quadrant (42 and 38 % for WT and MSK1 KD, respectively) compared to chance level (25 %; $p = 0.002$[s] for both) demonstrating memory retention in both genotypes. The time spent in the training quadrant did not differ between genotypes ($p = 0.24$[t]).

In a further iteration of the water maze that maximizes the opportunity for interference, mice were subjected to a serial spatial learning task in which a new platform location was used across a

461 series of sessions (Figure 5A), and the number of trials required to identify the new location of the
462 platform taken as a measure of learning. This test proved to be exceptionally sensitive in a study of
463 age-related changes of performance in human mutant APP mice (Chen *et al.*, 2000). As before, the
464 wild-type and MSK1 mutant mice performed similarly in the acquisition phase (Figure 5B): an RM-
465 ANOVA showed that mice of both genotypes improved their performance in relation to the task
466 order presentation ($p < 0.0001^{(u)}$; *i.e.* the number of trials required for spatial problem 1 > spatial
467 problem 2 & 3 > spatial problem 4 & 5). It also showed that there was no effect due to the
468 genotype ($p = 0.57^{(v)}$), nor any significant interaction between spatial problem and genotype ($p =$
469 $0.91^{(w)}$).
470
471 In a probe test trial given 10 min after the last training session for each platform location (Figure
472 5C), the performance of the mutant and wild-type was similar: the average performance was
473 above the chance level (12.5 %; $p = 0.002^{(x)}$ for both WT and MSK1-KD). An RM-ANOVA showed
474 that there was no effect due to genotype ($p = 0.19^{(y)}$), nor any significant interaction between task
475 and genotype ($p = 0.16^{(z)}$). The apparent better performance by WT mice on the first spatial
476 problem did not reach statistical significance ($p = 0.067^{(aa)}$).
477
478 Similarly, mutant and wild-type mice behaved no differently from each other in a probe test given
479 24 hours after the training sessions (Figure 5D): the average performance was above the chance
480 level (12.5 %; $p = 0.002^{(ab)}$ for both WT and MSK1-KD). An RM-ANOVA showed that there was no
481 effect due to genotype ($p = 0.88^{(ac)}$), nor any significant interaction between task and genotype (p
482 $= 0.95^{(ad)}$).
483
484 Massed training tends to give rise to a weaker memory trace that fails to show lasting retention, a
485 level of performance that may be more sensitive for revealing a subtle phenotype. To examine the

decay of memory over time, both genotypes were therefore exposed to massed training in which four water maze trials were given per session with a 15 min interval between the 4 sessions (Figure 6A). Mice of both genotypes showed a steady decline in escape latency over the four sessions of training ($p = 0.025^{(ae)}$); there was no significant effect due to the genotype ($p = 0.64^{(af)}$), nor a significant interaction between genotype and day ($p = 0.39^{(ag)}$; Figure 6B).

Mice were then tested for memory of the location of the platform 10 min, 24 hr and 7 d later (Figure 6C). At the 10 min probe test only, mice of both genotypes spent more time in the platform quadrant than the chance level (25 %; $p = 0.015$ and $0.005^{(ah)}$ for WT and MSK1 KD, respectively), but the percentages did not differ between genotypes ($p = 0.31^{(ai)}$). Forgetting was observed in both groups over 24 hr and 7 days.

These data indicate that, whilst there is a deficit in BDNF signalling to CREB and basal synaptic transmission in the MSK1 KD mouse, this does not translate into an impairment of mGluR-LTD, LTP or in long-term hippocampal-dependent spatial learning in the water maze. Moreover, intact long-term learning and memory in the water maze is consistent with intact LTP in the hippocampus in the mutant mice.

Discussion

Our main findings are that mice harboring a knock-in kinase dead mutation of MSK1 display a deficit in BDNF-dependent phosphorylation of CREB and of basal synaptic transmission, but not of paired-pulse facilitation, mGluR LTD or LTP induced by either tetanic or theta-burst stimulation. In addition, MSK1 mutant mice showed no impairment in various tests of spatial memory in the water maze. These data suggest that the kinase activity of MSK1 is not directly required for several forms of synaptic plasticity or spatial learning and memory, at least under standard housing conditions. Instead, our observations of changes in basal synaptic transmission, and thus the long-term regulation of synaptic strength, support observations of a deficit in homeostatic and experience-dependent plasticity in MSK1 KD mutants (Corrêa *et al.*, 2012).

MSK1 as a CREB S133 kinase

The immunofluorescence associated with the phosphorylation of CREB at S133 was similar under basal conditions between MSK1 KD and wild-type mice. This suggests that under normal circumstances the many CREB kinases, which include CaMKs, PKA and RSKs (Flavell & Greenberg, 2008; Sakamoto *et al.*, 2011; Kida & Serita, 2014) ensure an appropriate level of CREB S133 phosphorylation, and presumably the maintenance of CREB-dependent transcription. The wide variety of CREB S133 kinases and their recruitment in an activity-dependent manner also potentially explains the minimal synaptic and behavioral phenotype of the MSK1 KD mutant mouse. Thus, there may only be specific circumstances in which MSK1 is recruited as a CREB S133 kinase, for example in response to environmental enrichment (Corrêa *et al.*, 2012; Karelina *et al.*, 2012) or, interestingly, to molecules such as BDNF (Arthur *et al.*, 2004), which is believed to play an important role in mediating the beneficial effects of enrichment on neuronal structure, synaptic function and cognition (Cowansage *et al.*, 2010; Bekinschtein *et al.*, 2011).

Accordingly, stimulation of hippocampal slices with BDNF resulted in an increase in phosphorylation of CREB S133 in CA1 neurons of wild-type mice, but not in MSK1 KD mice. These observations confirm the long-held view of MSK1 as a CREB kinase (Deak *et al.*, 1998; Reyskens & Arthur, 2016) and, in particular, the observations made in primary neuronal cell culture of MSK1 and MSK2 single and double knockouts of the necessity for MSK1 in neurotrophin-mediated phosphorylation of CREB (Arthur *et al.*, 2004). However, as others have shown, MSK1 is also downstream of other signalling cascades that culminate in CREB phosphorylation, including that of the cAMP/PKA pathway (Frodin *et al.*, 1994; Delghandi *et al.*, 2005). Whilst we observed no obvious deficit in forskolin-stimulated CREB phosphorylation in slices from MSK1 KD mice, which may reflect a dominant contribution of PKA under our experimental conditions, others have reported that the Ca^{2+}-stimulated adenylyl cyclase/PKA pathway recruits MSK1 in a subset of CA1 neurons in response to contextual fear conditioning (Sindreu *et al.*, 2007). Since the number of CA1 neurons in our analysis of CREB phosphorylation were similar across genotypes and basal and stimulated conditions (~70 cells/slice), we do not see evidence of subsets of responding neurons. This may reflect the bath application of BDNF and forskolin, as opposed to the pathway-specificity of a behavioral stimulus.

Role of MSK1 in synaptic transmission and plasticity

Despite there being a number of studies describing various roles for MSK1 in neuronal and cognitive function, there have been no reports to date of synaptic plasticity in MSK1 mutant mice. The observations we have made here suggest that the kinase activity of MSK1 is necessary to regulate basal synaptic transmission as MSK1 KD mice showed smaller CA1 fEPSPs in response to stimulation of the afferent Schaffer pathway, a phenotype they share with BDNF knockout mice (Patterson *et al.*, 1996). In the MSK1 KD mutants, this impairment was not due to reduced

553 excitability or number of presynaptic fibres, since the amplitude of the presynaptic fibre volley was
554 no different from wild-type animals. In addition, this deficit did not reflect either an increase in
555 inhibitory GABAergic synaptic transmission, as the difference between mutant and wild-type slices
556 persisted in the presence of a $GABA_A$ receptor antagonist, or reduced probability of transmitter
557 release since the paired-pulse facilitation profile was no different between mutant and wild-type
558 slices. Instead, one possibility is that this deficit reflects reduced numbers of postsynaptic AMPA
559 receptors, which mediate the majority of excitatory synaptic transmission at these synapses.
560 Indeed, in whole-cell recordings from MSK1 KD and wild-type neurons, a ~10-15 % reduction in the
561 amplitude of miniature excitatory postsynaptic currents (mEPSCs) in hippocampal slices from
562 animals of a similar age was observed (Corrêa *et al.*, 2012). This observation is in contrast to the
563 situation in cultured neurons prepared from neonatal animals where mEPSCs are larger in neurons
564 prepared from MSK1 KD mice, likely due to the increased cell surface expression of GluA1 (but not
565 GluA2) (Corrêa *et al.*, 2012). This suggests either that the influence of MSK1 on synaptic strength
566 may be developmentally regulated, or that homeostatic synaptic changes induced by the
567 preparation of cultured neurons and acute brain slices do not occur in the MSK1 KD mutants.
568
569 The mechanism underlying the deficit in synaptic transmission is not clear, but could potentially
570 involve the reduced BDNF-dependent stimulation of CREB phosphorylation in hippocampal slices
571 we observed. Such a deficit in the ERK signalling cascade could give rise to deficits in the induction
572 of the immediate early gene Arc/Arg3.1 (Korb & Finkbeiner, 2011). Arc/Arg3.1 has been implicated
573 in regulating synaptic strength via its association with the endocytotic proteins dynamin and
574 endophilin (Chowdhury *et al.*, 2006). Indeed, MSK1 KD mutants failed to downregulate Arc/Arg3.1
575 during prolonged (24 hrs) activity deprivation *in vitro,* and, likely as a consequence, the expected
576 increase in mEPSC amplitude was not observed (Corrêa *et al.*, 2012). Similarly, the larger spines
577 reported in MSK1 KD neurons (Corrêa *et al.*, 2012) has parallels with the increase number of larger

spines in the Arc/Arg3.1 knockout (Peebles *et al.*, 2010). However, mGluR LTD, which requires Arc/Arg3.1 for its induction (Waung *et al.*, 2008), was not affected by the loss of MSK1 kinase activity suggesting that there are alternative means by which Arc/Arg3.1 can be regulated to support mGluR LTD.

The LTP induction protocols used in this study evoked long-lasting (3 hrs) potentiation of synaptic transmission in slices from both wild-type and MSK1 KD mice. Such longevity has been previously reported for TBS-induced LTP (Nguyen & Kandel, 1997), but is not considered to be a feature of LTP induced by a single tetanus. However, it is clear from a number of studies that long-lasting LTP can be induced by a single tetanus (Bortolotto & Collingridge, 2000; Fonseca *et al.*, 2004; Capron *et al.*, 2006; Fonseca *et al.*, 2006a; Fonseca *et al.*, 2006b; Sajikumar *et al.*, 2008; Villers *et al.*, 2012), although there is debate as to whether or when this requires protein synthesis, as there is for TBS-induced LTP (Lynch *et al.*, 2015). Such factors that may influence the persistence of LTP after a tetanus include the strength (Tsokas *et al.*, 2005) or the frequency of basal synaptic transmission, with less frequent stimulation, as seen in alternating two pathway experiments such as ours, prolonging the magnitude and duration of the tetanus-induced LTP (Fonseca *et al.*, 2006a; Villers *et al.*, 2012).

Given the persistence of the LTP seen in our experiments, the lack of effect of the MSK1 KD mutation is at first glance somewhat surprising given that: 1) MSK1 phosphorylates CREB; 2) CREB has been implicated in synaptic plasticity, albeit with some controversy (Sakamoto *et al.*, 2011); 3) the original CREB knockout mouse showed a deficit in LTP over a period of 90 mins (Bourtchuladze *et al.*, 1994); 4) that the MAPK/ERK pathway may be recruited preferentially by TBS (Winder *et al.*, 1999) and 5) that the ensuing LTP is sensitive to inhibition of transcription (Nguyen & Kandel, 1997).

One potential, if unlikely, explanation is that the mutation of the MSK1 gene has allowed an additional form of LTP to be evoked, in response to both TBS and tetanic stimulation, that displays identical magnitude, kinetics and duration (to 3 hrs) to that evoked in slices from wild-type mice. It is thus superficially indistinguishable, to the point that it too is dependent upon transcription, at least for TBS-induced LTP. This will remain a possibility until a full biophysical and pharmacological characterization of LTP in the MSK1 KD slices is performed.

A more parsimonious interpretation of the lack of an effect of the MSK1 KD mutation on LTP may arise from the work using residue-specific mutations of the BDNF TrkB receptor: an LTP deficit, over a similar time-course to the present experiments (3 hrs), was only observed in mice with a mutation in TrkB Y816, which signals to PLCγ, CaM kinase IV and CREB, and not mutations in TrkB Y515, which signals via Shc and the MAPK cascade, downstream of which is MSK1 (Korte *et al.*, 2000; Minichiello *et al.*, 2002).

MSK1 and spatial learning

Previous reports have shown that MSK single or double knockouts are impaired on the water maze, fear conditioning, the Barnes maze and in the display of behavioral immobility in the forced-swim test (Chwang *et al.*, 2007; Chandramohan *et al.*, 2008; Frenguelli & Corrêa, 2012; Karelina *et al.*, 2012; Reyskens & Arthur, 2016). However, in our studies of MSK1 KD mice, we observed no deficit in various water maze paradigms. This is consistent with the intact LTP we recorded, but is at odds with previous work in other laboratories using MSK1 knockouts. One potential explanation is that, especially in the context of the previous water maze studies (Chwang *et al.*, 2007), subtle differences in the way the test is conducted or the animals housed could influence the signalling

pathways recruited. Indeed, other paradigms such as fear conditioning or forced swimming could recruit MSK1-dependent signalling pathways and underpin learning in those models.

An alternative explanation is that that MSK1 subserves two functions – one as a kinase and the other as a scaffolding partner in a signalling complex. The latter possibility has been made all the more likely by the observation that MSK1 forms a complex with the glucocorticoid receptor and ERK1/2 for optimal phosphorylation (serine 10) and acetylation (lysine 14; via Elk-1) of histone H3. This complex resulted in the induction of c-Fos and Egr-1 during a 15 min forced swim test (Gutierrez-Mecinas *et al.*, 2011). Loss of the entire MSK1 protein in the knockout may thus compromise this scaffolding role as well as the kinase pathway, and its effect may be more pronounced in tests associated with increased stress, for example fear conditioning, forced swimming and the use of aversive motivation in the Barnes maze. Thus, whereas the MSK1 KO studies have to date been interpreted as relating to the loss of kinase activity, our use of the kinase dead (KD) mutation raises questions about this interpretation.

Conclusions

Mice with an inactivating kinase-dead knock-in mutation in the N-terminal kinase domain MSK1 show normal mGluR-LTD, tetanus and theta-burst LTP and were unaffected in spatial memory tasks in the water maze. However, these mice display a deficit in basal synaptic transmission and in BDNF-mediated phosphorylation of CREB. These data suggest that MSK1 may be important in regulating long-term, adaptive neuronal properties, rather than the acute response to experience. This is consistent with previous observations that demonstrate the necessity for MSK1 in homeostatic synaptic scaling *in vitro* and environmental enrichment-induced synaptic plasticity *in vivo* (Corrêa *et al.*, 2012).

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852 **Table 1: Results Statistics Summary**

ID	Measure	Distribution	Type of test	F or t; p value	DoF	95% CI WT Lower; Upper	95% CI KD Lower; Upper
a	BDNF/CREB phosphorylation	Normal	Two-way ANOVA				
			Genotype x Treatment effect	**8.31; 0.005**	1,72	Control:	Control:
			Simple Main Effects			49.05; 69.62	53.60; 73.22
			Control: WT vs. KD	0.36; 0.55	1,33		
			BDNF: WT vs. KD	**13.08; 0.001**	1,39	BDNF:	BDNF:
			WT: Control vs. BDNF	**8.74; 0.004**	1,34	68.55; 89.88	49.65; 63.81
			KD: Control vs. BDNF	1.12; 0.29	1,38		
b	BDNF/CREB phosphorylation increase %	Normal	Independent Samples T-test (2-tailed)	**3.83; 0.0004**	39	101.76; 188.51	51.78; 142.31
c	Forskolin/CREB phosphorylation	Normal	Two-way ANOVA			Control: 62.19; 87.44	
			Treatment effect	**10.25; 0.003**	1,42	Forskolin: 90.53; 116.33	
			Genotype effect	0.82; 0.37	1,42	67.52; 101.47	79.91; 104.65
			Control: WT vs. KD	0.85; 0.36	1,20	Control:	Control:
			Forskolin: WT vs. KD	0.11; 0.74	1,22	44.30; 88.07	62.02; 95.65
			WT: Control vs. Forskolin	**4.91; 0.032**	1,13	Forskolin:	Forskolin:
			KD: Control vs. Forskolin	**5.86; 0.020**	1,29	77.31; 123.72	87.50; 122.27
d	Input/Output fEPSP Overall	Normal	One-way RM-ANOVA				
			Genotype effect	**4.93; 0.032**	1,42	-1.45; -1.02	-1.08; -0.75
e	Input/Output fEPSP Higher stimulus strengths	Normal	One-way RM-ANOVA				
			Genotype x Stimulus-intensity effect	**4.00; 0.0001**	7,294	NA	NA
			Simple Main Effects for 200;	**4.15; 0.048**	1,42	-2.39; -1.65	-1.80; -1.22
			250;	**4.75; 0.035**	1,42	-3.07; -1.96	-2.07; -1.36

ID	Measure	Distribution	Type of test	F or t; p value	DoF	95% CI WT Lower; Upper	95% CI KD Lower; Upper
			300 µA	**4.84; 0.033**	1,42	-3.43; -2.17	-1.08; -0.74
f	Input/Output Fiber volley	Normal	One-way RM-ANOVA Genotype effect	1.51; 0.23	1,42	-0.35; -0.26	-0.32; -0.22
g	Input/Output Fiber volley	Normal	One-way RM-ANOVA Genotype x Stimulus-intensity effect	1.01; 0.42	7,294	NA	NA
h	Input/Output fEPSP Higher stimulus strengths	Normal	One-way RM-ANOVA Genotype x Stimulus intensity effect	**4.32; 0.001**	7,70	NA	NA
			Simple Main Effects for 200;	**5.09; 0.048**	1,10	-3.95; -2.14	-2.77; -1.26
			250;	**5.92; 0.035**	1,10	-4.30; -2.09	-2.73; -1.34
			300 µA	**5.55; 0.040**	1,10	-4.55; -1.92	-2.70; -1.39
i	LTD DHPG	Normal	One-way RM-ANOVA Genotype effect	0.065; 0.81	1,23	50.46; 74.69	50.93; 70.24
			RM-ANOVA Genotype x minute effect	0.94; 0.49	9,207	NA	NA
j	Tetanus and TBS-induced LTP	Normal	One-way RM-ANOVA				
			Genotype effect, Tetanus	0.41; 0.53	1,15	103.48; 147.98	118.94; 147.56
			Genotype effect, TBS	0.27; 0.61	1,12	110.41; 165.52	115.84; 146.72
k	LTP Actinomycin-D	Normal	One-way RM-ANOVA				
			Drug effect	**33.33; 0.000**	2,13		
			WT: DMSO vs. Act-25	**28.46; 0.000**	1,12	DMSO: 124.50; 142.50	
			WT: DMSO vs. Act-40	**28.46; 0.000**	1,8	Act-D 25µM: 103.16; 112.18	
			KD: DMSO vs. Act-25	**10.61; 0.001**	1,12	Act-D 40µM: 98.13; 108.36	
			KD: DMSO vs. Act-40	**10.61; 0.004**	1,8		

ID	Measure	Distribution	Type of test	F or t; p value	DoF	95% CI WT Lower; Upper	95% CI KD Lower; Upper
l	LTP Actinomycin-D	Normal	One-way RM-ANOVA Genotype effect	0.82; 0.46	1,13	101.49; 124.51	104.82; 122.46
m	Cued learning watermaze	Normal	One-way RM-ANOVA Day of training effect	**52.69; 0.0001**	2,44	day 1: 23.07; 36.76 day 3: 6.72; 12.12	
n	Cued learning watermaze	Normal	One-way RM-ANOVA Genotype effect	0.74; 0.40	1,22	11.16; 21.45	12.30; 27.47
o	Cued learning watermaze	Normal	One-way RM-ANOVA Genotype x Day effect	1.72; 0.19	2,44	NA	NA
p	Spaced trials task	Normal	One-way RM-ANOVA Day of training effect	**18.66; 0.0001**	4,92	day 1: 31.18; 46.61 day 5: 11.60; 18.70	
q	Spaced trials task	Normal	One-way RM-ANOVA Genotype effect	0.16; 0.69	1,23	19.53; 24.10	16.89; 24.97
r	Spaced trials task	Normal	One-way RM-ANOVA Genotype x Day effect	0.91; 0.46	4,92	NA	NA
s	Spaced trials task probe trial	Normal	One-sample Wilcoxon Signed Rank Test (WT and KD)	**NA; 0.002**	NA	36.74; 47.36	31.92; 43.49
t	Spaced trials task probe trial	Normal	Independent Samples T-test (2-tailed)	-1.20; 0.24	23	as above	as above
u	Serial spatial learning task	Normal	One-way RM-ANOVA Task order effect	**6.48; 0.0001**	4,88	Spatial problem 1: 6.12; 10.88 Spatial problem 5: 3.87; 5.71	
v	Serial spatial learning task	Normal	One-way RM-ANOVA Genotype effect	0.33; 0.57	1,22	4.85; 7.41	4.62; 6.78
w	Serial spatial learning task	Normal	One-way RM-ANOVA Genotype x Task effect	0.014; 0.91	4,88	NA	NA
x	Serial spatial	Normal	One-sample Wilcoxon Signed				

ID	Measure	Distribution	Type of test	F or t; p value	DoF	95% CI WT Lower; Upper	95% CI KD Lower; Upper
	learning task 10 min probe trial		Rank Test (WT and KD) Average for spatial pr. 1 to 5	**NA; 0.002**	NA	23.15; 29.26	18.37; 27.42
y	Serial spatial learning task 10 min probe trial	Normal	One-way RM-ANOVA Genotype effect	1.80; 0.19	1,22	as above	as above
z	Serial spatial learning task 10 min probe trial	Normal	One-way RM-ANOVA Genotype x Task effect	1.71; 0.16	4,88	NA	NA
aa	Serial spatial learning task 10 min probe trial	Normal	Independent Samples T-test (2-tailed) 1st spatial problem	1.925; 0.067	22	23.32; 43.94	15.48; 29.82
ab	Serial spatial learning task 24 hr probe trial	Normal	One-sample Wilcoxon Signed Rank Test (WT and KD)	**NA; 0.002**	NA	17.48; 23.93	18.23; 22.60
ac	Serial spatial learning task 24 hr probe trial	Normal	One-way RM-ANOVA Genotype effect	0.02; 0.88	1,22	as above	as above
ad	Serial spatial learning task 24 hr probe trial	Normal	One-way RM-ANOVA Genotype x Task effect	0.18; 0.95	4,88	NA	NA
ae	Massed training spatial learning	Normal	One-way RM-ANOVA Session effect	**3.33; 0.025**	3,66	session 1: 18.89; 27.56 session 4: 11.37; 20.80	
af	Massed training spatial learning	Normal	One-way RM-ANOVA Genotype effect	0.22; 0.64	1,22	13.19; 21.95	11.37; 26.53
ag	Massed training	Normal	One-way RM-ANOVA				

ID	Measure	Distribution	Type of test	F or t; p value	DoF	95% CI WT Lower; Upper	95% CI KD Lower; Upper
	spatial learning		Genotype x Session effect	0.76; 0.39	3,66	NA	NA
ah	Massed training spatial learning probe trial at 10 min	Normal	One-sample Wilcoxon Signed Rank Test: WT; KD;	**NA; 0.015** **NA; 0.005**	NA NA	26.82; 38.13	30.68; 41.66
ai	Massed training spatial learning probe trial at 10 min	Normal	One-way ANOVA Genotype effect	1.06; 0.31	1,22	as above	as above

857 **Figure Legends**

858 *Figure 1. MSK1 is necessary for BDNF-dependent CREB phosphorylation in area CA1.*

859 A) Treatment of wild-type hippocampal slices (upper panels) with BDNF (50 ng/ml; 10 mins)
860 resulted in a robust increase in CREB phosphorylation in CA1 neurons, compared to control
861 untreated slices, as indicated by changes in immunofluorescence associated with a monoclonal
862 antibody directed at the phosphorylation of CREB S133. In contrast, BDNF induced little or no
863 effect on CREB phosphorylation in slices taken from MSK1 KD mice (lower panels). B) Box and
864 whisker plot showing mean (open square), median, 25 and 75 percentile (box) and ± 1 standard
865 deviation (whiskers) of data from 21 BDNF-treated slices from 8 wild-type mice and 20 BDNF-
866 treated slices from 10 MSK1 KD mice. The specificity of the antibody for the phosphorylation of
867 CREB S133 in CA1 pyramidal neurons was confirmed using a mouse expressing a CREB Serine133-
868 alanine point mutation (data not shown).

869

870 *Figure 2. MSK1 KD mice display a deficit in basal synaptic transmission which is not due to*
871 *increased GABAergic inhibition.*

872 Basal synaptic transmission was measured in the CA1 region of hippocampal slices from wild-type
873 (black squares) or MSK1 KD (white circles) mice. A) plot of fibre volley amplitude vs. fEPSP slope
874 demonstrates that MSK1 KD mice show reduced synaptic transmission at higher stimulus
875 strengths. Inset are representative fEPSPs at 10 – 300 µA stimulus strengths for both genotypes (n
876 = 27 pathways from 18 wild-type mice and 17 pathways from 12 MSK1 KD mice). This deficit is also
877 observed when fEPSP slope is plotted against stimulus strength (B; *** $p = 0.0001$; genotype x
878 stimulus strength interaction). In contrast, a plot of fibre volley amplitude against stimulus
879 strength (C) showed no significant difference between the genotypes. D) The deficit in fEPSP was

maintained when the experiments were repeated in the presence of 50 µM of the $GABA_A$ receptor antagonist picrotoxin (n = 5 pathways from 4 wild-type mice and 7 pathways from 5 MSK1 KD mice; ** p = 0. 001; genotype x stimulus strength interaction). Error bars represent SEM

Figure 3. Electrophysiological characterization of MSK1 KD mice shows no deficit in paired-pulse facilitation, mGluR LTD or LTP.

A) Paired-pulse facilitation in area CA1 was measured in hippocampal slices prepared from adult wild-type (black squares; 21 pathways from 18 animals) or MSK1 KD (white circles, 22 pathways from 19 animals) mice. Representative families of paired fEPSPs taken over the 50 – 250 ms inter-stimulus interval range are shown in the upper panel, and quantification of the paired pulse ratios in the lower panel. Data are expressed as mean and error bars (where visible) represent the SEM. B) mGluR LTD was induced in area CA1 of hippocampal slices from wild-type (black squares) and MSK1 KD (white circles) mice. The Group I mGluR agonist DHPG (100 µM) was applied from time 0 to 10 min (as indicated by the black bar on the graph) in the presence of both picrotoxin (50 µM) and the NMDA receptor glycine site antagonist L689,560 (5 µM). Inset are representative fEPSPs taken 10 min before and 50 min after the end of DHPG application in wild-type and MSK1 KD slices (n = 15 and 10 slices from 11 wild-type and 8 MSK1 KD mice, respectively). Data are expressed as mean and error bars represent the SEM. C-D) Tetanus- (C) and theta-burst stimulation-induced LTP (D) were measured in the area CA1 of hippocampal slices from adult mice. Inset are representative fEPSPs taken 10 min before and 120 min after induction of LTP (at time zero) in the stimulated (left) and control (right) pathways for each genotype. Data are expressed as mean and error bars represent the SEM. For tetanus-induced LTP, data are taken from single slices from 9 wild-type and 8 MSK1 KD mice while for theta-burst-induced LTP, individual slices from 7 wild-type and 7 MSK1 KD mice were used. E) TBS-LTP is sensitive to the transcription inhibitor actinomycin-D (Act-

D). Graphs show the effects of DMSO vehicle (0.08 %) applied throughout the experiment (black squares and dark grey bar; n = 5; 3 wild-type and 2 MSK1 KD mice); 40 µM Act-D applied for the duration of the experiment (dark grey squares and dark grey bar; n = 5; 4 wild-type and 1 MSK1 KD mice) and 25 µM Act-D (0.05 % DMSO) applied for 15 mins before and after TBS (light grey squares and light grey bar; n = 9; 3 wild-type mice and 6 slices from 4 MSK1 KD mice). Both concentrations of Act-D affect LTP similarly, but the lower concentration and shorter duration of application of 25 µM has less of an effect on the control pathway. Data are expressed as mean and error bars represent the SEM. F) The inhibitory effects of Act-D on late TBS-LTP are independent of genotype. Data are replotted from Figure 3E, but with respect to genotype instead of concentration: black squares, 7 slices from 7 wild-type mice; open circles, 7 slices from 5 MSK1 KD mice. Data are expressed as mean and error bars represent the SEM.

Figure 4. MSK1 KD mice display no deficit in the watermaze test for spatial reference memory.

A) The ability to locate a visible platform in a 2 m pool was assessed by a 3-day visual cue task as described in Daumas et al., (2008). Both wild-type (WT) and MSK1 KD mice improved their latency to the platform with time at an equivalent rate. B) Spatial reference memory was assessed using a standard 5-day protocol (4 spaced trials/day, 10 cm hidden platform, probe test after 24 h). No learning deficit was observed during training. C) The retention 'probe' test indicated good memory in both groups, revealed as greater time spent searching in the training quadrant. Broken line, chance level = 25%.

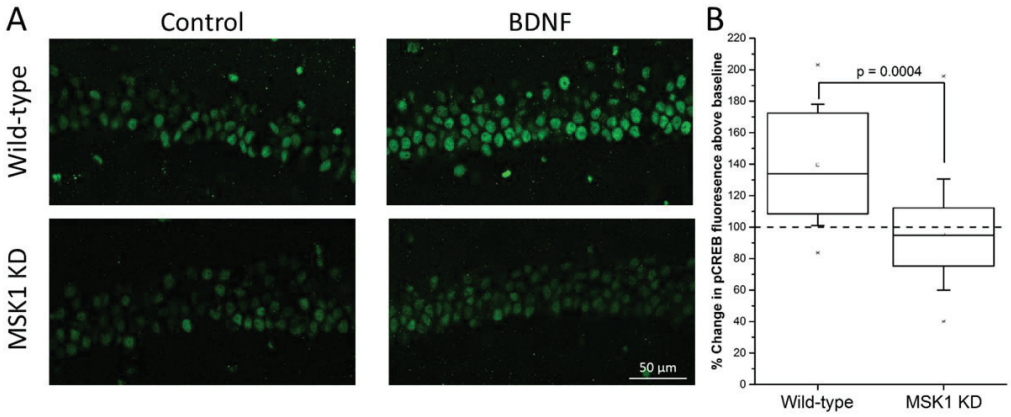
Figure 5. MSK1 KD mice display no deficit in the watermaze test for serial spatial learning.

A) A serial spatial learning task was used to assess memory flexibility as described in Daumas et al., (2008). In this task, the platform location changes between up to 8 separate locations and between days, with each location trained until the animals reached a fixed criterion of performance (latency < 20 sec over 3 trials). The measure of learning now is not latency, but the number of trials (#) required to reach criterion. B) Both groups showed similar performance. After reaching criterion for each of 5 serial locations, memory was assessed at 10 min (C) and 24 h (D). The proportion of time spent in the relevant platform zone (extended to 20 cm diameter) relative to the other 7 possible platform locations was analysed (broken line, chance level = 12.5%).

Figure 6. MSK1 KD mice display no deficit in the watermaze test for spatial reference memory with a massed training protocol.

A) In a massed training protocol (16 trials, in 4 trial blocks over 1 day), learning was comparable (B), and spatial memory assessed at 10 min, 24 h and 7 days after training (C) showed the expected and more rapid forgetting over time with no group differences between WT and MSK1 KD mice.

944 Figure 1



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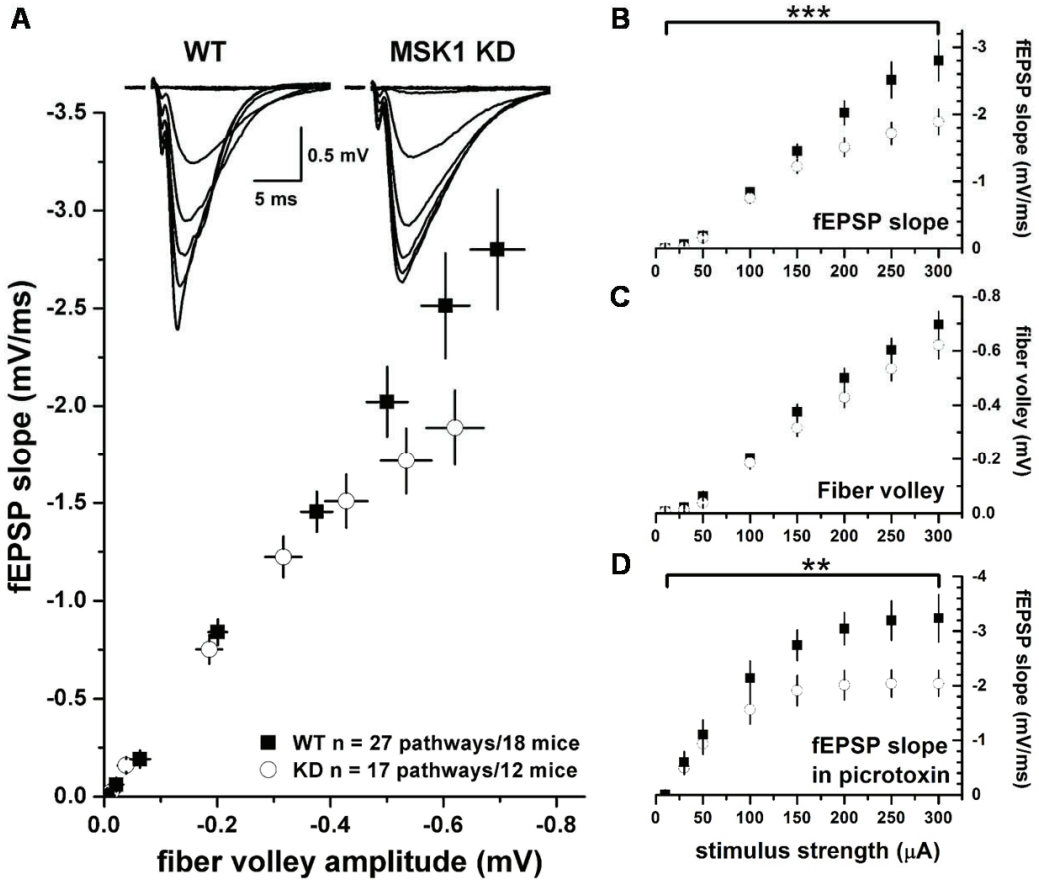
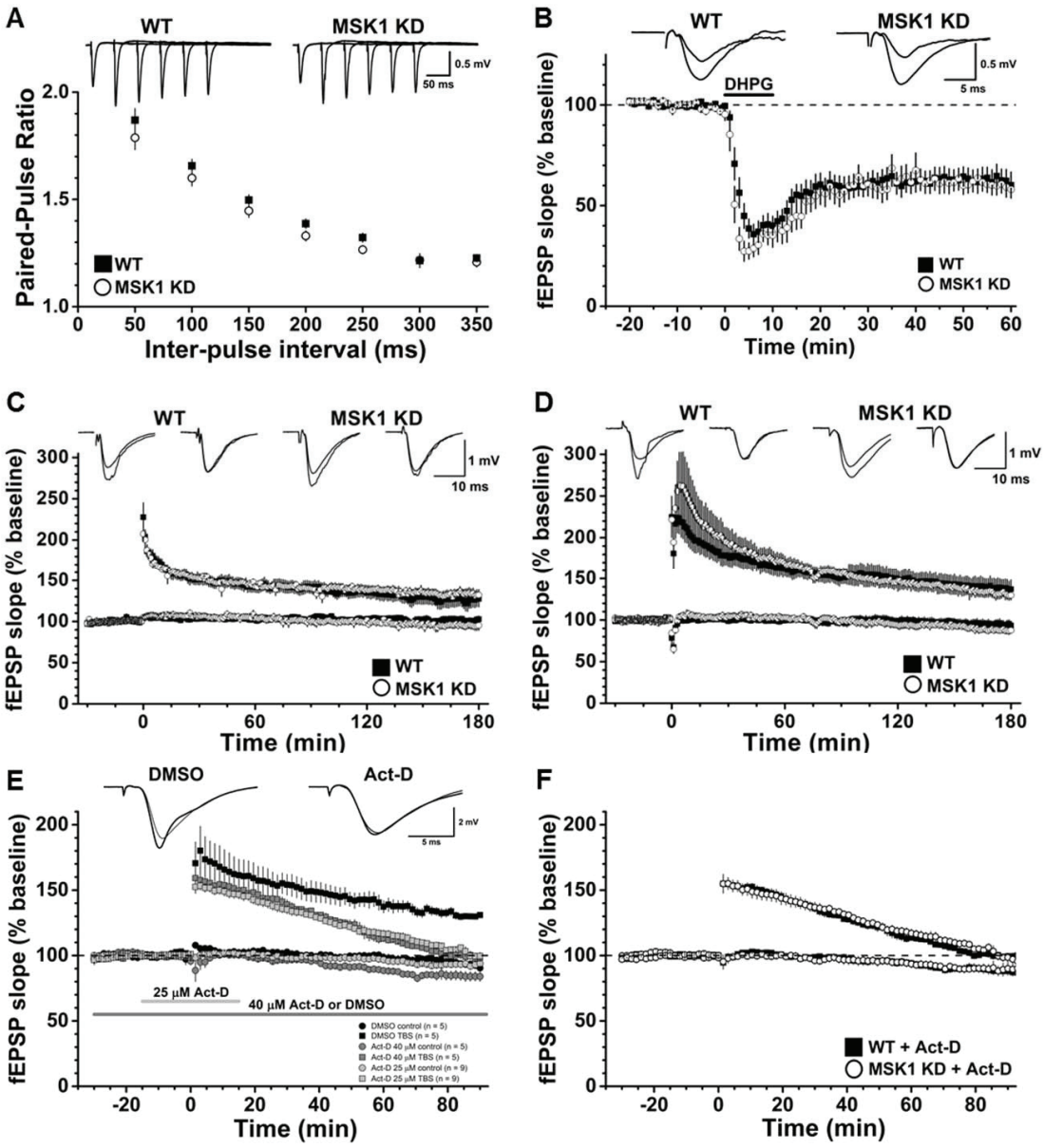
949 Figure 2



953 Figure 3



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956 Figure 4

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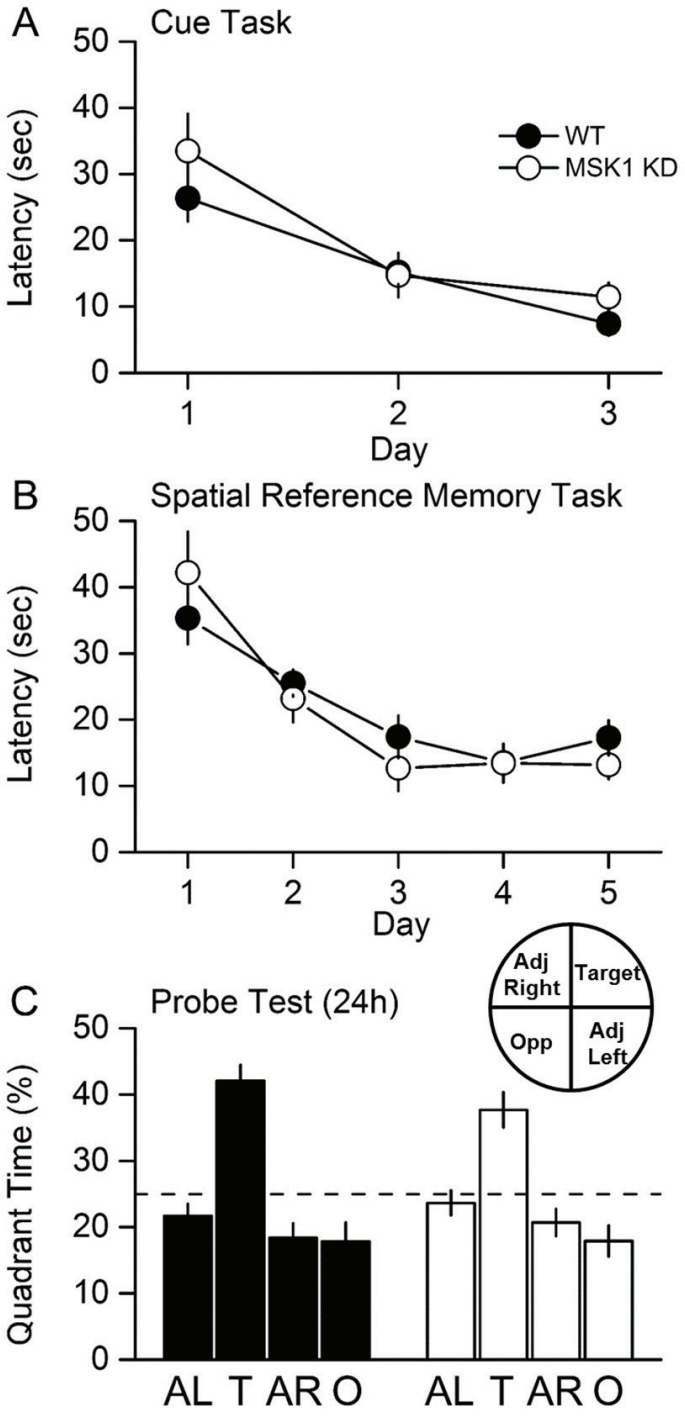

961 Figure 5

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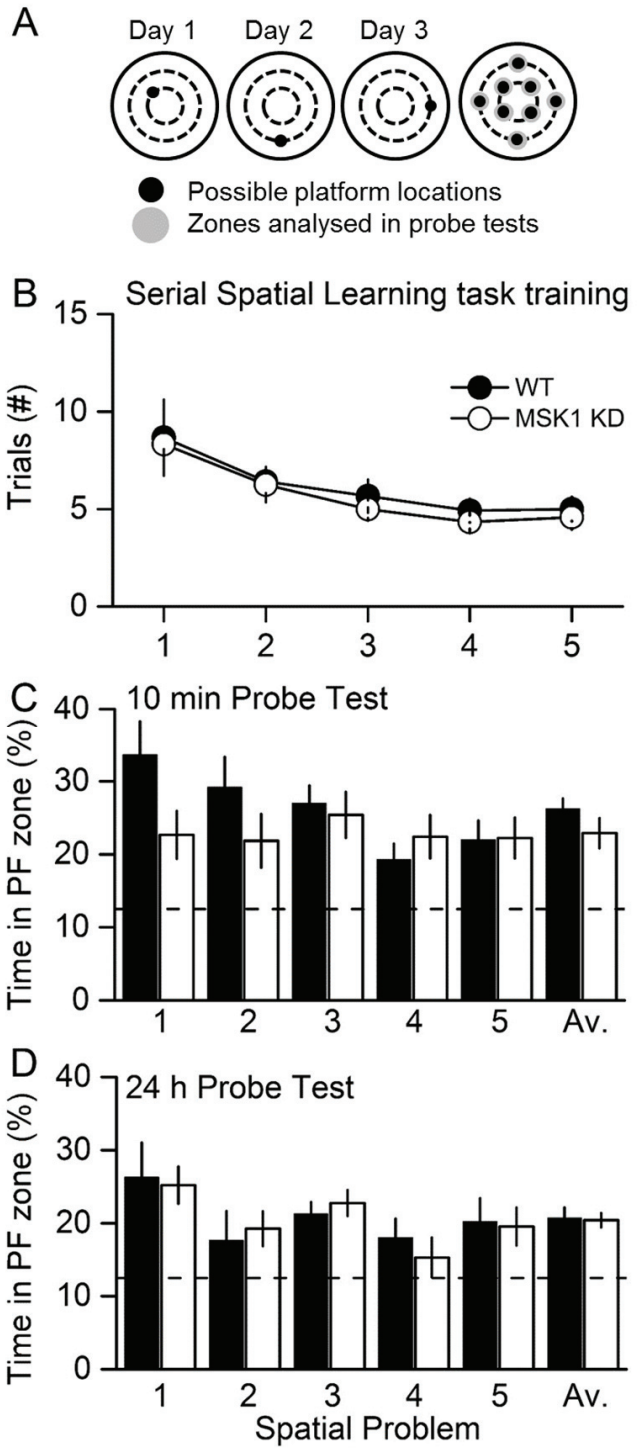

963 Figure 6

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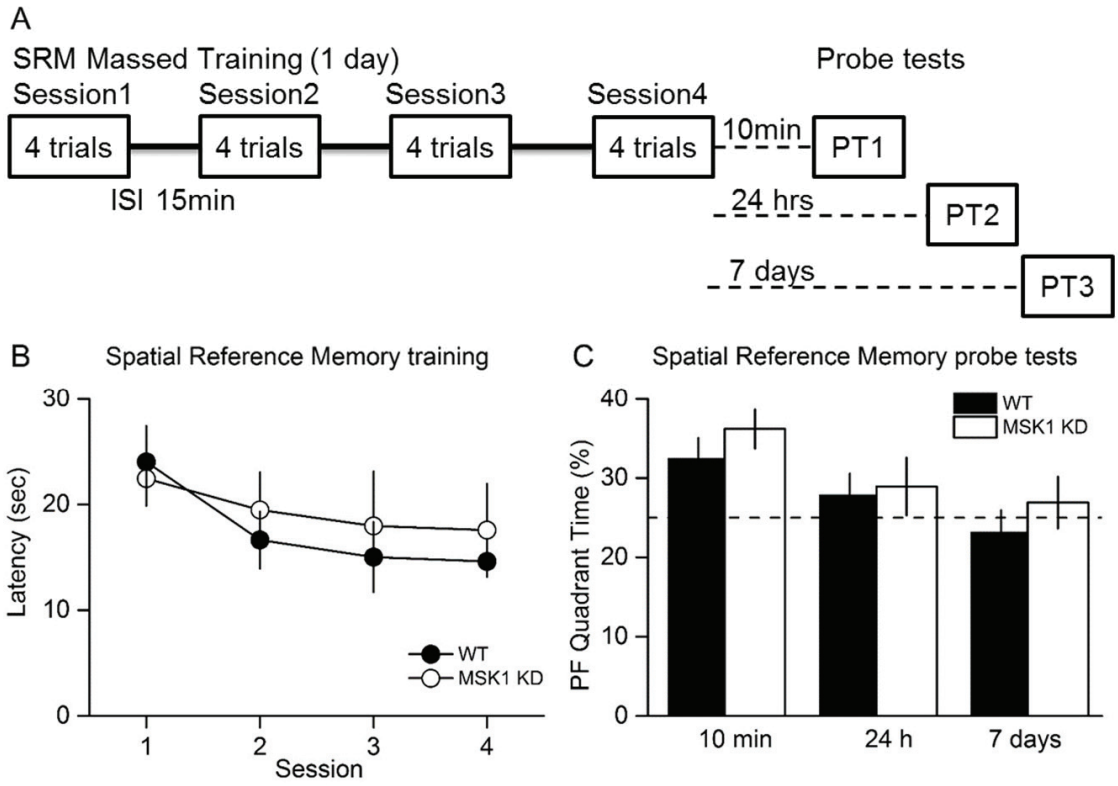

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