Arc expression regulates long-term potentiation magnitude and metaplasticity in area CA1 of the hippocampus in ArcKR mice.

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Abstract

Expression of the immediate early gene Arc/Arg3.1 (Arc), a key mediator of synaptic plasticity, is enhanced by neural activity and then reduced by proteasome-dependent degradation. We have previously shown that disruption of Arc degradation, in an Arc knock-in mouse (ArcKR), where the predominant Arc ubiquitination sites were mutated, reduced the threshold to induce, and also enhanced, the strength of Group I metabotropic glutamate receptor-mediated long-term depression (DHPG-LTD). Here we have investigated if ArcKR expression changes long-term potentiation (LTP) in CA1 area of the hippocampus. As previously reported, there was no change in basal synaptic transmission at Schaffer collateral/commissural-CA1 (SC-CA1) synapses in ArcKR versus wild-type (WT) mice. There was however a significant increase in the amplitude of synaptically-induced (with low frequency paired-pulse stimulation) LTD in ArcKR mice. Theta burst stimulation-evoked LTP at SC-CA1 synapses was significantly reduced in ArcKR versus WT mice (after 2 hours). Group 1 mGluR priming of LTP was abolished in ArckR mice, which could also potentially contribute to a depression of LTP. Although high frequency-stimulation (HFS)-induced LTP was not significantly different in ArcKR compared to WT mice (after 1 hour) there was a phenotype in environmentally enriched mice, with the ratio of LTP to short-term potentiation (STP) significantly reduced in ArcKR mice. These findings support the hypothesis that Arc ubiquitination supports the induction and expression of LTP, likely via limiting Arc-dependent removal of AMPA receptors at synapses.

Introduction

Activity-regulated cytoskeleton associated protein (Arc/Arg3.1) is one of the major molecular players in cognition, as it is required for protein-synthesis dependent synaptic plasticity and learning and memory (Park *et al.* , 2008; Waung *et al.* , 2008; Bramham *et al.* , 2010; Shepherd & Bear, 2011). Arc plays a key role in determining synaptic strength through promoting endocytosis of AMPA-type glutamate receptors (AMPARs) during mGluR-LTD which has been most studied in area CA1 of the hippocampus (Chowdhury *et al.* , 2006; Park *et al.* , 2008; DaSilva *et al.* , 2016; Wall & Correa, 2018).

One key feature of Arc protein expression in area CA1 of the hippocampus, is its transient nature. Following increased network activity or exposure to a novel environment Arc expression increases and then rapidly declines (Guzowski *et al.* , 2000; Ramirez-Amaya *et al.* , 2005; Miyashita *et al.* , 2009). The regulation of Arc protein induction occurs at the level of mRNA transcription, mRNA trafficking, and protein translation (Bramham *et al.* , 2008; Korb & Finkbeiner, 2011). Arc protein expression is then reduced by rapid proteasomal degradation (Rao *et al.* , 2006; Greer *et al.* , 2010) following ubiquitination by the RING domain ubiquitin ligase Triad3A/RNF216 (Mabb *et al.* , 2014). However, not all of Arc expression is transient, as a basal level of Arc appears to be retained at synapses, where Arc interacts with several proteins in the post-synaptic density (PSD) scaffold (Fiuza *et al.* , 2017) and reviewed in (Zhang & Bramham, 2020).

To determine the importance of the Arc removal process in spatial learning behaviour and synaptic plasticity, a mutant mouse line (ArcKR) was generated in which mutations were introduced within Exon 1 of the Arc gene. When encoded, the introduction of the two point mutations replace lysine to arginine at positions 268 and at 269 (Wall *et al.* , 2018). These sites have been previously shown to be ubiquitinated by Triad3A (Greer *et al.* , 2010; Mabb *et al.* , 2014). Hippocampal neurons isolated from mice bearing the mutation of the Triad3A-dependent sites (ArcKR) showed markedly increased Arc protein 30 minutes after activation of Group I metabotropic glutamate receptors (GI-mGluR), with the agonist DHPG, consistent with diminished degradation of Arc protein (Wall *et al.* , 2018). In addition to these effects on Arc expression, ArcKR mice displayed impaired cognitive flexibility, which was coupled with elevated levels of Arc protein expression in the hippocampus, a reduced threshold to induce GI-mGluR-mediated long-term depression (GI-mGluR-LTD) induced by the GI agonist DHPG (DHPG-LTD), and enhanced DHPG-LTD amplitude (Mabb & Ehlers, 2018; Wall *et al.* , 2018).

Although the role of Arc in mGluR-LTD is consistent with its action in facilitating the internalisation of synaptic AMPARs (Waung *et al.* , 2008; Wall & Correa, 2018; Wilkerson *et al.* , 2018), the mechanism by which Arc regulates potentiation, particularly long term potentiation (LTP) is less clear. For example, an LTP-inducing stimulus in the hippocampus increased *Arc* mRNA levels, transporting*Arc* mRNA to activated synapses (Lyford *et al.* , 1995; Steward *et al.* , 1998; Steward & Worley, 2001a; b). Arc has also been implicated in F-actin stability, which is responsible for dendritic spine maintenance and plasticity. Knocking down Arc expression blocks LTP in the dentate gyrus by reducing F-actin formation and cofilin phosphorylation (Messaoudi *et al.* , 2007) . The genetic knockout of *Arc* was also shown to be associated with impaired LTP at SC-CA1 synapses in vitro and in the perforant pathway in vivo (Plath *et al.* , 2006). Arc is also involved in inverse synaptic tagging during LTP induction, where Arc mediates endocytosis of AMPARs at inactive synapses that recently experienced strong stimulation (Okuno*et al.* , 2012; Okuno *et al.* , 2018).

A recent study has directly addressed the role of Arc in LTP at SC-CA1 synapses using two different *Arc* knock-out (Arc KO) mouse lines and a conditional KO floxed line (Arc cKO), for both *in vitro* and*in vivo* LTP analysis (Kyrke-Smith*et al.* , 2021). It was found that Arc was not required for the maintenance of high frequency stimulation (HFS)-induced LTP. In contrast, theta burst stimulation (TBS)-induced LTP had an enhanced amplitude in Arc KO mice. This effect on LTP was not observed in the conditional Arc KO mouse line. It was therefore suggested that deletion of Arc may have developmental compensatory effects, which leads to the indirect enhancement of LTP, rather than being a direct effect of Arc removal (Kyrke-Smith *et al.* , 2021). Kyrke-Smith et al (2021) also demonstrated that the deletion of Arc had no effect on the threshold for LTP induction at SC-CA1 synapses. Here we investigated whether expression of ArcKR leads to an LTP phenotype in area CA1 of the hippocampus.

2. Methods

All animal care and experimental procedures were reviewed and approved by the institutional animal welfare and ethical review body (AWERB) at the University of Warwick or in accordance with the National Institutes of Health Guidelines for the Use of Animals. Experiments were performed under the appropriate project licenses with local and national ethical approval. Animals were kept in standard housing with littermates, provided with food and water *ad libitum* and maintained on a 12:12 (light-dark) cycle. The ArcKR knock-in mice were originally produced by the Ingenious Targeting Laboratory (Ronkonkoma, NY). Gene targeting was performed in iTL IC1 (C57BL/6) ES cells to introduce 2-point mutations within Exon 1 of the *Arc* gene that when encoded substitutes lysine to arginine at positions 268 and 269 (Wall *et al.* , 2018).

2.1 Preparation of hippocampal slices

Parasagittal slices of hippocampus (400 μm) were prepared from 2–3-month-old male and female wildtype (WT) C57BL/6 and ArcKR mice (Wall *et al.* , 2018). Mice were kept on a 12-hour light-dark cycle with slices made 90 minutes after entering the light cycle. In accordance with the U.K. Animals (Scientific Procedures) Act (1986), mice were killed by cervical dislocation and then decapitated. The brain was removed, cut down

the mid-line and the two sides of the brain stuck down to a base plate. Slices were cut around the midline with a Microm HM 650V microslicer in cold (2-4°C) high Mg^{2+}, low Ca^{2+}artificial CSF (aCSF), composed of (mM): 127 NaCl, 1.9 KCl, 8 $MgCl_2$, 0.5 $CaCl_2$, 1.2 KH_2PO_4, 26 $NaHCO_3$, 10 D-glucose (pH 7.4 when bubbled with 95% O_2 and 5% CO_2, 300 mOSM). Slices were stored at 34°C bubbled with 95% O_2 and 5% CO_2 for 1-6 hours in aCSF (1 mM $MgCl_2$, 2 mM $CaCl_2$) before use.

2.2 Extracellular recording of synaptic transmission and plasticity

A slice was transferred to the recording chamber, submerged in aCSF and perfused at 4-6 ml/min (32°C). The slice was placed on a grid allowing perfusion above and below the tissue and all tubing (Tygon) was gas tight (to prevent loss of oxygen). To record field excitatory postsynaptic potentials (fEPSPs), an aCSF-filled microelectrode was placed on the surface of stratum radiatum in CA1. A bipolar concentric stimulating electrode (FHC) controlled by an isolated pulse stimulator model 2100 (AM Systems, WA) was used to evoke fEPSPs at the Schaffer collateral–commissural pathway. Field (f)EPSPs were evoked every 30 s (0.03 Hz). Stimulus input/output curves for fEPSPs were generated using stimulus strength of 1-5 V for all slices (stimulus duration 200 μs). For the synaptic plasticity experiments, the stimulus strength was set to produce a fEPSP slope ˜ 40 % of the maximum response and a 20-minute baseline was recorded before plasticity induction. Paired-pulse facilitation was measured over an interval range of 20 to 500 ms. Recordings of mGluR-LTD were made in the presence of 50 μM picrotoxin to block $GABA_A$ receptors (Sigma) and the NMDA receptor antagonist L-689,560 (trans-2-carboxy-5,7-dichloro-4-phenylaminocarbonylamino-1,2,3,4- tetrahydroquinoline; 5 μM; Tocris) with area CA3 removed from the slices. To induce mGluR-LTD, a paired-pulse low frequency protocol was used (PP-LFS) consisting of 900 paired pulses (50 ms interval) delivered at 1 Hz (Oliet *et al.* , 1997). Long-term potentiation was induced by two protocols: high frequency stimulation (HFS 100 stimuli in 1s, 100 Hz) and theta burst stimulation (TBS, 4 trains separated by 20s with train consisting of 10 bursts of 4 pulses at 100 Hz, separated by 200 ms as in Kyrke-Smith et al., 2021). For LTP priming the CA3 region was removed from slices and 20 μM (RS)-3,5-DHPG (3,5-dihydroxyphenylglycine, Tocris) was applied for 10 minutes and then washed for 20 minutes after which LTP was induced by HFS. Signals were filtered at 3 kHz and digitised on-line (10 kHz) with a Micro CED (Mark 2) interface controlled by Spike software (Vs 6.1, Cambridge Electronic Design, Cambridge UK). The fEPSP slope was measured from a 1 ms linear region following the fibre volley.

2.3 DHPG stimulation of slices to measure Arc protein expression.

For the DHPG stimulation experiments, brain slices were prepared from WT ($n = 4$) and ArcKR ($n = 4$) mice as described in the hippocampal slice preparation section. Hippocampal slices from two mice were pooled together to obtain sufficient tissue to perform an experimental repeat. Hippocampi were isolated from the surrounding tissue, area CA3 was removed, and hippocampal slices were maintained at 34°C bubbled with 95% O_2 and 5% $CO_2$for 2-3 hours in aCSF (1 mM $MgCl_2$, 2 mM $CaCl_2$) before use. Hippocampal slices for each experimental group were then incubated in aCSF (1 mM $MgCl_2$, 2 mM $CaCl_2$) containing 50 μM picrotoxin to block $GABA_A$ receptors (Tocris) and the NMDA receptor antagonist L-689,560 (5 μM; Tocris) for 30 min followed by incubation with vehicle (control), DHPG (100 μM) to induce LTD or DHPG (20 μM) to prime mGluRs. After 10 min incubation, DHPG was washed out and the slices left to rest for another 30 min before the slices from different groups were homogenized in Eppendorf vials with a pellet pestle in ice-cold solution composed of: 1 mM EDTA, 50 mM Tris-HCl (pH 7.5), 1% Triton X-100, 1 mM Sodium Orthovanadate, 50 mM Sodium Fluoride, Sodium pyrophosphate, 0.27 M Sucrose, 20% NaN_3 and protease inhibitor cocktail (Roche) and rotated for 1 h at 4°C. Homogenate was centrifuged at 13,000 g for 15 min, the supernatant collected and protein levels determined (BCA protein assay kit, Thermo Scientific). Western blotting was performed as previously described (Eales *et al.* , 2014; DaSilva *et al.* , 2016; Wall *et al.* , 2018). Membranes were probed with rabbit anti-Arc (Synaptic Systems, 1:1,000) and mouse anti- Glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Abcam, ab8245, 1:5,000) antibodies followed by goat anti-Rabbit IgG-HRP H+L (Cell Signaling, 1:10,000) and goat anti-Mouse IgG HRP LC (Jackson ImmunoResearch, 1:20,000) secondary antibodies. GAPDH was used as loading controls. Blots were imaged and analysed using the ChemiDoc^{TM} MP Imaging System (Bio-Rad) and volume intensity of each band was calculated using

the Image Lab 5.2.1 software.

2.4 Environmental enrichment

Following weaning ArcKR and WT mice were exposed to an enriched environment for 8-10 weeks. Mice were housed in larger cages (rat cages) with increased numbers of littermates per cage on a 12:12 hour light and dark cycle, with food and water ad libitum. Male and female mice were kept in separate cages. The environment of the mice was further enriched by the provision of a variety of toys including running wheels, tunnels, small structures to hide in and mezzanines as described in (Privitera *et al.* , 2020). Except for the provision of running wheels, which were permanently available to the mice, the selection and arrangement of toys was changed twice a week to ensure continued enrichment. Mice were also provided with increased amounts of nesting material to allow the expression of species typical nest building behaviour. Mice were given sesame seeds which were distributed around the cage to encourage foraging behaviour.

Drugs

Drugs were made up as stock solutions (5-10 mM) in distilled water or dimethyl sulfoxide (DMSO) and then diluted in aCSF. The concentration of DMSO did not exceed 0.1 % in the final solutions. Picrotoxin was supplied from Sigma. DHPG and L689 were supplied from Hello Bio.

Statistical Analysis

Statistical analysis was performed using Origin (Microcal). For unpaired data, the non-parametric Mann-Whitney test was used. Western blot analysis was performed using a one-way ANOVA followed by multiple comparison analysis using GraphPad Prism software. For all experiments, significance was set at p [?] 0.05. Data presented in figures are means (+- SEM).

3. Results

Basal synaptic transmission in the hippocampus of ArcKR mice is not significantly different compared to WT mice but PP-LFS mediated LTD is significantly enhanced.

Before examining synaptic plasticity, we confirmed our previous observations (in 21–35-day old mice, Wall et al 2018) that basal synaptic transmission in the hippocampus of ArcKR mice is not significantly different from that in wildtype mice (at 2-3 months of age). There was no significant difference in the stimulus input/output relationships (Figure 1A) or in the degree of paired-pulse facilitation at Schaffer collateral-CA1 (SC-CA1) synapses between ArcKR and WT mice (Figure 1B). In corroboration of these findings, there was no significant difference in the expression of Arc protein in hippocampal lysates obtained from WT and ArcKR mice (at 2-3 months of age, Figure 1C). We have previously shown that mGluR-mediated long-term depression (LTD) induced with the group 1 (G1)-mGluR agonist DHPG is enhanced in the hippocampus obtained from juvenile ArcKR mice (Wall *et al.* , 2018). We investigated whether a similar enhancement can be observed in synaptically-induced LTD in 2–3-month-old mice (PP-LFS, Figure 1D-E). LTD was induced by 900-paired-pulses (50 ms interval) at 1 Hz (Oliet *et al.* , 1997) in the presence of L689,560 to block NMDA receptors. The degree of depression was significantly enhanced in slices from ArcKR mice (Figure 1D-E) consistent with previous findings using the G1-mGluR agonist DHPG to induce LTD (Wall *et al.* , 2018).

The magnitude of TBS-induced long-term potentiation is significantly reduced in ArcKR mice

Although the expression of Arc protein plays an important role in mGluR-mediated LTD (Wall *et al.* , 2018), the involvement of Arc in potentiation: (long term (LTP) and short term (STP)) is more contentious. Previous studies have shown a role for Arc in LTP, particularly in the dentate gyrus (see introduction). In contrast, (Kyrke-Smith*et al.* , 2021) showed that LTP at SC-CA1 synapses was unaffected by an inducible deletion of Arc. To further investigate the role of Arc in potentiation at SC-CA1 synapses we have compared potentiation induced in ArcKR Vs. WT mice. Since Arc protein expression is enhanced and longer lasting in ArcKR mice (Wall*et al.* , 2018), any effects of Arc on LTP might be expected to be increased in ArcKR mice. Potentiation was evoked using theta burst stimulation (TBS, 4 bursts as in (Kyrke-Smith *et al.* , 2021).

Following induction, there was no significant difference in the amplitude of STP between the genotypes (measured 2-3 minutes after induction, Fig 2A, B). However, LTP measured 2 hours after induction was significantly reduced in ArcKR mice (Fig 2A, C). This was not a consequence of a difference in synaptic activation, as there was no significant difference in the mean slope of baseline fEPSPs between ArcKR and WT mice (p = 0.409, Z = 0.825, U = 108, Mann Whitney).

ArcKR expression may regulate LTP magnitude through indirect mechanisms such as mGluR priming.

The magnitude of LTP can be regulated by many different mechanisms which could be classified as direct: such as modulating Ca^{2+}signalling, NMDA receptor opening, AMPA receptor trafficking etc, or indirect for example through metaplastic processes such as mGluR priming. mGluR priming is the low-level activation of G1-mGluRs prior to the induction of LTP leading to a significant enhancement in LTP amplitude (Cohen *et al.* , 1998; Mellentin *et al.* , 2007; Privitera *et al.* , 2019). We therefore investigated whether the priming of the mGluR signalling pathway prior to the induction of LTP is altered in ArcKR mice. To investigate this, we used the same protocol as in (Privitera *et al.* , 2019): HFS (100 Hz for 1s) to evoke LTP and 20 μM of the GI mGluR agonist DHPG to produce low-level mGluR activation. Using this LTP induction protocol, there was no significant difference in the amplitude of LTP between the genotypes after 1 hour (Figure 3A, B). To investigate mGluR priming, interleaved slices (WT and ArcKR) were exposed to DHPG (20 μM) for 10 min to prime intracellular mGluR-dependent signalling (Cohen *et al.* , 1998; Privitera *et al.* , 2019). After the DHPG was washed out for 20 min, LTP was then induced with HFS (1s burst of stimuli at 100Hz). The application of 20 μM DHPG produced a peak reduction of ˜ 40% in fEPSP slope, which was not significantly different between WT and ArcKR hippocampal slices (Figure 3C). We observed that the amplitude of LTP in WT mice was significantly enhanced compared to the LTP in ArcKR mice following application of DHPG (20 μM Fig 3C, D). Strikingly, there was no enhancement in LTP amplitude in slices obtained from ArcKR mice that had previously been exposed to DHPG (20 μM) compared to control slices (Figure 3C, D).

Although not directly comparable (as recordings were not performed using interleaved slices), the increased magnitude of LTP in control conditions was approx. 60 % for WT mice and ˜ 74% (Fig 3A, B) for ArcKR when normalized to the baseline. After priming with DHPG, in WT slices LTP magnitude had increased to ˜ 80 % but in ArcKR slices it had fallen to approx. 40% (Figure 3B, D). This apparent decrease in LTP amplitude could potentially be produced by an mGluR-mediated increase in Arc/Arg3.1 protein expression and facilitation of AMPA receptor endocytosis. To investigate this, we first examined the DHPG-induced inhibition in more detail. Although there was no difference in the peak inhibition produced by 20 μM DHPG, there did appear to be more inhibition after DHPG wash in ArcKR compared to WT slices (Figure 3E, F). Although this difference did not reach significance, there were some ArcKR slices that appeared to exhibit more DHPG-induced inhibition of the fEPSP than in WT slices (Figure 3F). In these slices, DHPG may have induced a low level of LTD via an increased accumulation of Arc expression (as the threshold for LTD induction is reduced in ArcKR mice (Wall *et al.* , 2018). These slices showed the largest fall in LTP amplitude.

To investigate this further, we incubated hippocampal slices with either vehicle (control), DHPG (100 μM) or the DHPG priming dose (20 μM) and examined levels of Arc protein 30 min after DHPG washout. Previous findings have demonstrated there is a significant increase in Arc expression in hippocampal cultures obtained from ArcKR compared to WT mice 30 min after DHPG washout (Wall*et al.* , 2018). As expected, there was a significant increase in Arc protein expression in hippocampal slices obtained from WT mice exposed to DHPG (100 μM) when compared vehicle (*p=0.01; Figure 3G, H) but no significant changes in Arc protein expression was observed between control (vehicle) and DHPG (20 μM) in both genotypes (p = 0.26). Similarly, exposure of DHPG (100 μM) caused a significant increase in expression of Arc protein levels in ArcKR when compared to control (**p=0.008; Figure 3G, H), but exposure to 20 μM DHPG had no detectable effect on Arc expression when compared to control (p=0.75; Figure 3G, H). Thus, the mechanism underlying the loss of mGluR priming in ArcKR mice remains unclear, but it may involve alterations in Arc

localisation or posttranslational modifications.

ArcKR expression modifies potentiation in environmental enriched conditions.

Several studies have shown that increasing the enrichment of the housing environment of rodents leads to enhanced synaptic plasticity and improved learning (Sale *et al.* , 2014; Fischer, 2016; Ohline & Abraham, 2019; Cooper & Frenguelli, 2021). In a recent study, LTP was markedly increased at SC-CA1 synapses in hippocampus obtained from mice exposed to environmental enrichment for 5 months, with no change in basal synaptic transmission. In these mice Arc protein expression was significantly reduced (Privitera *et al.* , 2020). Here we have investigated whether environmental enrichment has differential effects on LTP magnitude in ArcKR compared to WT mice. Firstly, LTP was induced in standard-housed mice using 3 bursts of TBS as in (Privitera *et al.* , 2020). No significant changes in LTP were observed after an hour between WT and ArcKR mice (Fig 4A, C). Before repeating the experiment in environmentally-enriched mice, we investigated if the enrichment induced any changes in basal synaptic transmission between the genotypes. There were no significant changes in the stimulus input-output relationship (Fig 4D) or the degree of paired-pulse facilitation (Fig 4E). Although there was no significant difference in LTP magnitude (induced with 3 burst of TBS, Fig 4F, G) between the genotypes, there was a significant reduction in the ratio of LTP to short-term potentiation (STP; Fig 4H). This measurement removes the variance in absolute LTP from across slices. This change in ratio reflects a decline in potentiation after STP in ArcKR, while potentiation was more stable for WT following STP (Fig 4F). This difference in LTP/STP ratio is not a consequence of differences in synaptic activation between the synapses, as the slope of baseline fEPSPs was not significantly different ($p = 0.228$, $Z = -1.206$, $U = 83$, Mann Whitney) between the genotypes. No such difference was observed in the ratio of LTP/STP in standard housed ArcKR mice (Fig 4C) and thus appears to be a consequence of the environmental enrichment.

4. Discussion

We have investigated whether expression of ArcKR, a form of Arc where the major ubiquitination sites have been mutated, and its degradation is slowed, (Mabb *et al.* , 2014; Wall *et al.* , 2018) affects synaptic plasticity in area CA1 of the hippocampus. In particular, we have investigated the effects of ArcKR expression on long-term potentiation (LTP) and whether priming of mGluR affects LTP in this mouse. In agreement with our previous study (Wall *et al.* , 2018), we found that SC-CA1 basal synaptic transmission was not significantly different in standard housed adult ArcKR mice compared to WT mice. We also showed that, like DHPG-induced- LTD (Wall*et al.* , 2018), synaptically-induced paired-pulse low frequency stimulation (PP-LFS)-induced mGluR-LTD was significantly enhanced in ArcKR mice. Corroborating the idea that transient increase in Arc expression levels facilitate PP-LFS-mediated LTD is the observation that Arc mRNA and protein expression, as well as PP-LFS mediated LTD are increased in the hippocampus in the APP/PS1 mouse at 7 months of age (Privitera *et al.* , 2022).

TBS induced LTP magnitude is reduced after 2 hours in ArcKR compared to WT mice.

We observed that the magnitude of TBS (4 bursts as in (Kyrke-Smith *et al.* , 2021) induced LTP was significantly smaller in ArcKR mice versus WT mice 2 hours following induction. This was not a consequence of differences in synaptic activation, as there was no significant difference in the baseline fEPSP slope before the induction of LTP between the genotypes. The mechanism for this reduction in LTP magnitude is currently unclear. One potential mechanism is that peak accumulation of Arc (in response to the LTP induction stimulus) is larger, and will persist for a longer duration, in the ArcKR hippocampus and this may dampen down synaptic potentiation, potentially via increased AMPAR endocytosis. Recent findings have shown that Arc interacts and disperses the protein stargazin (Zhang *et al.* , 2015) , which is located at the postsynaptic density (PSD), away from the synapse (Chen *et al.* , 2022). Thus, it could be hypothesised that if ArcKR protein accumulates at the proximities of the PSD it may move stargazin-AMPAR complexes away from the PSD to be targeted for endocytosis (Chen *et al.* , 2022). This hypothesis, of ArcKR accumulation following LTP induction, is difficult to directly test, since only a small number of hippocampal synapses are potentiated during LTP induction and thus any changes in Arc expression would only occur in specific

groups of neurons and probably be too small to measure, for example using western blotting techniques. More sensitive techniques such a the use of the Arc reporter mice could potentially be used to address this question (Eguchi & Yamaguchi, 2009)

Several studies have shown that disrupting LTP in the CA1 area of the hippocampus leads to deficiency in spatial learning (reviewed in (Lynch, 2004; Kandel *et al.* , 2014; Abraham *et al.* , 2019)). Thus, it may be predicted that spatial memories and learning would be disrupted in the ArcKR mouse. Previous experiments in younger animals showed no such deficits in the acquisition of spatial memories ((Wall *et al.* , 2018) but significant changes following the reversal learning task. This may not be the case in older mice, but this remains to be tested. This effect of ArcKR expression on LTP magnitude may at first appear contradictory to the results of (Kyrke-Smith *et al.* , 2021), who showed that deletion of Arc had no effect on LTP. However, it may be that WT levels of Arc have no effect on LTP, but if Arc expression is enhanced or Arc can accumulate at dendritic spines, it may then play a role in LTP.

mGluR dependent priming is abolished in ArcKR mice.

One major difference in synaptic plasticity in hippocampal slices from ArcKR mice compared to slices from WT mice was the prevention of GI-mGluR-dependent facilitation of LTP. The behavioural role for the enhancement of LTP induction by low level mGluR activation is currently unclear, but its loss does not seem to affect the acquisition of spatial memories in young animals (Wall *et al.* , 2018). Corroborating this observation is the finding that although LTP and NMDA receptor-mediated LTD are intact at hippocampal SC-CA1 synapses in MAPK-activated protein kinase 2 (MK2) KO mice when compared to WT littermate mice, the mGluR-mediated priming of LTP is abolished. Similar to the cognitive dysfunction observed in ArcKR mice (Wall *et al.* , 2018), MK2 KO mice are able to learn a hippocampal-dependent spatial task (the Barnes maze), but showed marked deficits in performing the reversal version of the previous learned task (Privitera*et al.* , 2019). These findings suggest that mGluR-mediated facilitation of LTP may be involved in the process of re-learning a previously learned task, and not in the initial learning of the task. However, this is difficult to test, as there are currently no methods to selectively block the priming of LTP *in vivo* without effecting other forms of plasticity.

It is currently unclear how the mGluR-dependent priming of LTP is abolished by the expression of ArcKR, which is relatively resistant to degradation (Wall *et al.* , 2018). Increased Arc protein expression facilitates AMPA receptor endocytosis, in particular the GluA1 subunit, following the induction of its expression with the mGluR-agonist DHPG (Waung *et al.* , 2008; Wall *et al.* , 2018). Endocytosis of AMPA-lacking GluA2 subunits, which are calcium permeable, would reduce synaptic strength and the probability of inducing potentiation. This hypothesis would support our observation that there is prolonged inhibition of basal transmission following the application of 20 μM DHPG in some slices obtained from ArcKR mice but not from WT mice (Figure 4D). Although this is consistent with our previous studies, where we found that a lower concentration of DHPG (50 μM) was sufficient to induce LTD in ArcKR mice (Wall *et al.* , 2018), we could not measure any significant differences in Arc protein expression levels induced by exposure to 20 μM DHPG (this may be because any increases are below the limits of detection).

Environmental enrichment reveals deficits in LTP in ArcKR mice.

There is considerable evidence demonstrating that exposure to enriched or complex environments positively influences neuronal structure and (Rosenzweig & Bennett, 1996; Nithianantharajah & Hannan, 2006; Correa *et al.* , 2012; Privitera *et al.* , 2020; Cooper & Frenguelli, 2021). ((Privitera *et al.* , 2020) showed that LTP magnitude at SC-CA1 synapses was enhanced by enriched conditions compared to standard house wildtype mice, with no enrichment-induced increase in basal synaptic transmission. In these mice the basal expression of Arc was significantly reduced after prolonged enrichment (Privitera *et al.* , 2020). We also found that there were no significant changes in basal synaptic transmission in WT mice, comparing standard house to environmentally-enriched (compare Fig 1A and 4D). In contrast there does appear to be a reduction in synaptic strength for ArcKR mice upon enrichment which may reflect the persistence of Arc and the resultant AMPA receptor internalisation. When we examined LTP (measured after 1 hour) and STP (short term

potentiation, 5 minutes after induction) we observed no significant difference in their amplitudes between environmentally-enriched ArcKR and WT mice. We did however observe a clear difference in the pattern of EPSP potentiation, with a difference in the decay of potentiation after LTP induction. In standard-housed conditions, the slope of fEPSPs remained enhanced to around the same degree as with short term potentiation (STP, LTP magnitude ˜ 95 % of STP) for both WT and ArcKR mice. Whereas in environmentally-enriched mice, LTP decayed to ˜ 78 % of the STP level after an hour in ArcKR mice. The ratio of LTP to STP was significantly different between the genotypes (unlike the absolute magnitude of LTP, which may not have reached significance due to the larger variation in magnitude between slices). Thus, environment enrichment reveals an effect of ArcKR expression on potentiation. Recent studies (reviewed in (France *et al.* , 2022) have investigated the processes underlying STP and LTP and have shown that it is possible to differentially regulate STP and LTP using NMDA subunit-specific positive allosteric modulators and antagonists. Thus, different NMDA receptors appear to contribute to these two processes. It is unclear why expression of ArcKR has no effect on STP and LTP in standard housed conditions, but it does in environment enriched conditions. It is possible that changes in NMDA receptor expression in environment enriched mice could be involved (Rampon *et al.* , 2000) but this needs to be further tested.

Conclusion

We have shown that the magnitude of LTP is diminished in hippocampal slices from ArcKR mice compared to WT mice and that induction of LTP facilitated by priming of GI-mGluR is impaired in ArcKR mice perhaps suggesting that below threshold activation of mGluR is sufficient to induce Arc protein expression triggering endocytosis of AMPA receptors. Previous studies have shown that when Arc expression is induced, the level of expression is enhanced, and the expression remains elevated for longer in ArcKR mice. Such enhancement could also occur when there is marked increases in neural activity during pathologies (such as during epileptic seizures) and could occur when there is Arc misexpression such as in Fragile X Syndrome (Niere *et al.* , 2012) and in neurodegenerative diseases. Our experiments using ArcKR expression, suggest that under these conditions it is possible that increased Arc expression could negatively modulate LTP and contribute to cognitive impairment.

Author Contributions

Experiments were designed by MJW, BGF, SALC. Electrophysiology and biochemistry experiments were carried out by MH, JB, MF, VTA, SALC and MJW. Data was analysed by MH, JB, MF AMM, VTA, SALC and MJW. The manuscript was written by MJW and SALC.

Declaration of interest

The author(s) confirm that this article content has no conflicts of interest.

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Figure Legends

Figure 1. Expression of a degradation-resistant form of Arc ($Arc_{(K268A;K269A)}$; ArcKR), does not affect basal synaptic transmission or basal Arc protein expression levels but significantly enhances PP-LFS-induced long-term depression.

A, Graph plotting mean fEPSP slope against stimulus strength for WT (n =17 slices, 10 mice) and ArcKR mice (n = 17 slices, 9 mice). Inset, superimposed fEPSP waveforms at increasing stimulus strengths (0.5 to 5 V) from a WT and ArcKR slice. B, Graph plotting mean paired-pulse ratio against paired-pulse interval for WT (n =13 slices, 6 mice) and ArcKR mice (n =15 slices, 6 mice). C, Bar graph showing no difference between Arc protein levels in hippocampal lysate obtained from WT (n = 4) and ArcKR (n = 4) mice (p = 0.944). GAPDH was used as loading control. Inset, example western blots for Arc and GAPDH. Statistical comparisons were performed with one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons. D, Graph plotting mean normalised (to the baseline) fEPSP slope against time. Long-term depression was induced with low frequency paired-pulse stimulation (LFS-PP; 900 paired-pulse at 1 Hz, interval between pulses 50 ms). Inset, example waveforms from WT and ArcKR mice. E, Bar chart summarising mean percentage depression 55-60 minutes after LFS-PP stimulation (WT n =10 slices 4 mice; ArcKR n = 11 slices, 6 mice). The amplitude of depression was significantly (p = 0.0358, U = 28, Z = -2.07 Mann Whitney) enhanced in slices from ArcKR mice (mean depression in WT 21.1 ± 4.3 compared to 35.8 ± 4.3 % in ArcKR mice). Data are represented as mean ± SEM. Data points are from individual experiments.

Figure 2. The amplitude of long-term potentiation (LTP) induced by theta burst stimulation is significantly reduced in ArcKR compared to WT mice.

A**,** Graph plotting mean normalised (to the baseline) fEPSP slope against time for WT (n = 13 slices, 11 mice) and ArcKR mice (n = 13 slices, 8 mice). After a 20-minute baseline, LTP was induced with theta burst stimulation (TBS, 4 trains separated by 20 s, with each train consisting of 10 bursts of 4 pulses at 100 Hz, separated by 200 ms). Inset, example fEPSP waveforms before and after LTP induction (average of waveforms at 115-120 minutes) from WT and ArcKR mice. B, Bar chart plotting the mean potentiation of fEPSP slope 5 minutes post LTP induction (short term potentiation; STP). There was no significant difference between the STP produced in slices from ArcKR and from WT mice (mean STP: WT 117 ± 20 %; ArcKR 69.7 ± 7.3 %, P = 0.054, Z = -1.923, U = 47, Mann Whitney). C, Bar chart plotting the mean potentiation of fEPSP slope 115-120 minutes post TBS (LTP). The magnitude of LTP was significantly reduced in slices from ArcKR vs WT mice (mean LTP: WT 113.4 ± 28.8 %; ArcKR 48.7 ± 6.6 %, P = 0.013, Z = -2.487, U = 36, Mann Whitney). Data are represented as mean ± SEM. The data points in B and C are from single slices.

Figure 3. ArcKR expression prevents GI-mGluR-mediated priming of long-term potentiation (LTP)

A**,** Graph plotting mean normalised (to the baseline) fEPSP slope against time for WT (n = 10 slices, 5 mice) and ArcKR mice (n = 12 slices, 6 mice). After a 20-minute baseline, LTP was induced with high frequency stimulation (1s, 100 Hz). Inset, example fEPSP waveforms before and after LTP induction from WT and ArcKR mice. B, Bar chart plotting the mean potentiation of fEPSP slope (between 55-60 minutes post LTP induction). There was no significant difference (p = 0.3791, Z =-0.89, U = 46, Mann Whitney) in the amplitude of long-term potentiation between WT (mean potentiation 60.2 ± 17.9 %) and ArcKR slices (74.0 ± 14.9 %). C, Graph plotting mean normalised (to the baseline) fEPSP slope against time for WT (n = 7 slices, 4 mice) and ArcKR mice (n = 7 slices, 5 mice). Following a 20-minute baseline, a low concentration of DHPG (20 μM) was applied for 10 minutes to activate GI-mGluRs (mean peak inhibition in WT 41.9 ± 4.8 %; ArcKR 42.8 ± 4.7 %). Following a 20-minute wash, LTP was induced by high frequency stimulation (1s, 100 Hz). Inset, example fEPSP waveforms before and after LTP induction (average of waveforms at 55-60 minutes) from WT and ArcKR mice. D, Bar chart plotting mean potentiation (55-60 minutes post LTP

induction). There was a significant difference (p = 0.022, U =37, Z = 2.2) in the amplitude of LTD following GI-mGluR priming in WT (mean potentiation 76.6 ± 8.6 %) compared to ArcKR slices (mean potentiation 36.6 ± 8.3 %). Data points are the mean potentiation from individual experiments. E, Graph plotting mean normalised (to the baseline) fEPSP slope against time for WT (n = 7 slices, 4mice) and ArcKR mice (n = 7 slices, mice) illustrating the actions of 20 μM DHPG. F, Bar chart plotting the mean inhibition after 20 minutes following DHPG wash. There was no significant difference in the amount of inhibition in WT compared to ArcKR mice (P = 0.069, Z = -1.78, U =14.5, Mann Whitney). The points (B, D and F) are the means from individual experiments. G, Western blot showing Arc protein expression in hippocampal lysates obtained from WT (n = 4) and ArcKR (n = 4) mice. Slices were incubated in control (vehicle), 20 μM DHPG and 100 μM DHPG for 10 min. H, Bar chart analysis show a significant increase in Arc expression after DHPG (100 μM) exposure compared to control in WT (*p=0.012) and ArcKR (**p=0.008), but not after 20 μM DHPG exposure (WT: p=0.26 and ArcKR: p=0.75). GAPDH was used as loading control. The data points are the Arc/GAPDH ratios from individual experiments. Error bars indicate ± S.E.M. Statistical comparisons were performed with one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons.

Figure 4. Environment enrichment reveals an LTP phenotype in ArcKR mice.

A, Graph plotting mean normalised (to the baseline) fEPSP slope against time for WT (n = 11 slices, 6 mice) and ArcKR mice (n = 11 slices, 5 mice) in standard housing. After a 20-minute baseline, LTP was induced with theta burst stimulation (TBS, 3 trains separated by 20s, with each train consisting of 10 bursts of 4 pulses at 100 Hz, separated by 200 ms). Inset, example fEPSP waveforms before and after LTP induction (average of waveforms at 55-60 minutes) from WT and ArcKR mice. B, Bar chart plotting the mean potentiation of fEPSP slope 55–60-minute post-LTP induction. There was no significant difference (P = 0.652, Z = 0.459, U = 68, Mann Whitney) in the amplitude of potentiation between WT (122.65 ± 24.6 %) and ArcKR slices (88.5 ± 13 %). C, Bar chart plotting the mean ratio of LTP (55-60-minute post induction) to STP (5 minutes post induction). There is no significant difference in the ratios (mean ratio: WT 0.95 ± 0.1; ArcKR 0.4 ± 0.03 P = 0.623, Z = -0.49, U = 63, Mann Whitney. D, Graph plotting mean fEPSP slope against stimulus strength for WT (n = 31 slices, 11 mice) and ArcKR mice (n = 43 slices, 14 mice) housed in environmentally enriched conditions. There was no significant difference (F(df)1, F=1.673, p=0.208 Two-way repeated measures ANOVA) between WT and ArcKR stimulus input/output relationships. Inset, superimposed fEPSP waveforms at increasing stimulus strengths (0.5 to 5 V) from a WT and ArcKR slice. E, Graph plotting mean paired-pulse ratio against paired pulse interval for WT (n = 18 slices, 7 mice) and ArcKR mice (n = 20 slices, 9 mice) housed in environmentally-enriched conditions. There was no significant difference (F(df)1, F=1.302, p=0.270 Two-way repeated measures ANOVA) in paired-pulse facilitation between WT and ArcKR mice. F, Graph plotting mean normalised (to the baseline) fEPSP slope against time for WT (n = 10 slices, 9 mice) and ArcKR mice (n = 16 slices, 11 mice) housed in environmentally-enriched conditions. After a 20-minute baseline, LTP was induced with theta burst stimulation (TBS, 3 trains separated by 20s, with each train consisting of 10 bursts of 4 pulses at 100 Hz, separated by 200 ms). Inset, example fEPSP waveforms before and after LTP induction (average of waveforms at 55-60 minutes) from WT and ArcKR mice. B, Bar chart plotting the mean potentiation of fEPSP slope 55–60-minute post-LTP induction. There was no significant difference (mean LTP; WT 83.2 ± 16.3; ArcKR 54.2 ± 8.5 %, P = 0.265, Z = 1.11, U = 1.3, Mann Whitney) in the amplitude of potentiation. C, Bar chart plotting the mean ratio of LTP (55-60-minute post induction) to STP (5 minutes post induction). The ratio was significantly smaller in ArcKR mice (mean ratio: WT 0.88 ± 0.04; ArcKR 0.78 ± 0.03 P = 0.0123, Z = 2.5, U = 153, Mann Whitney. Data are represented as mean ± SEM. The data points in (B, C, G and H) show the mean data for individual experiments.

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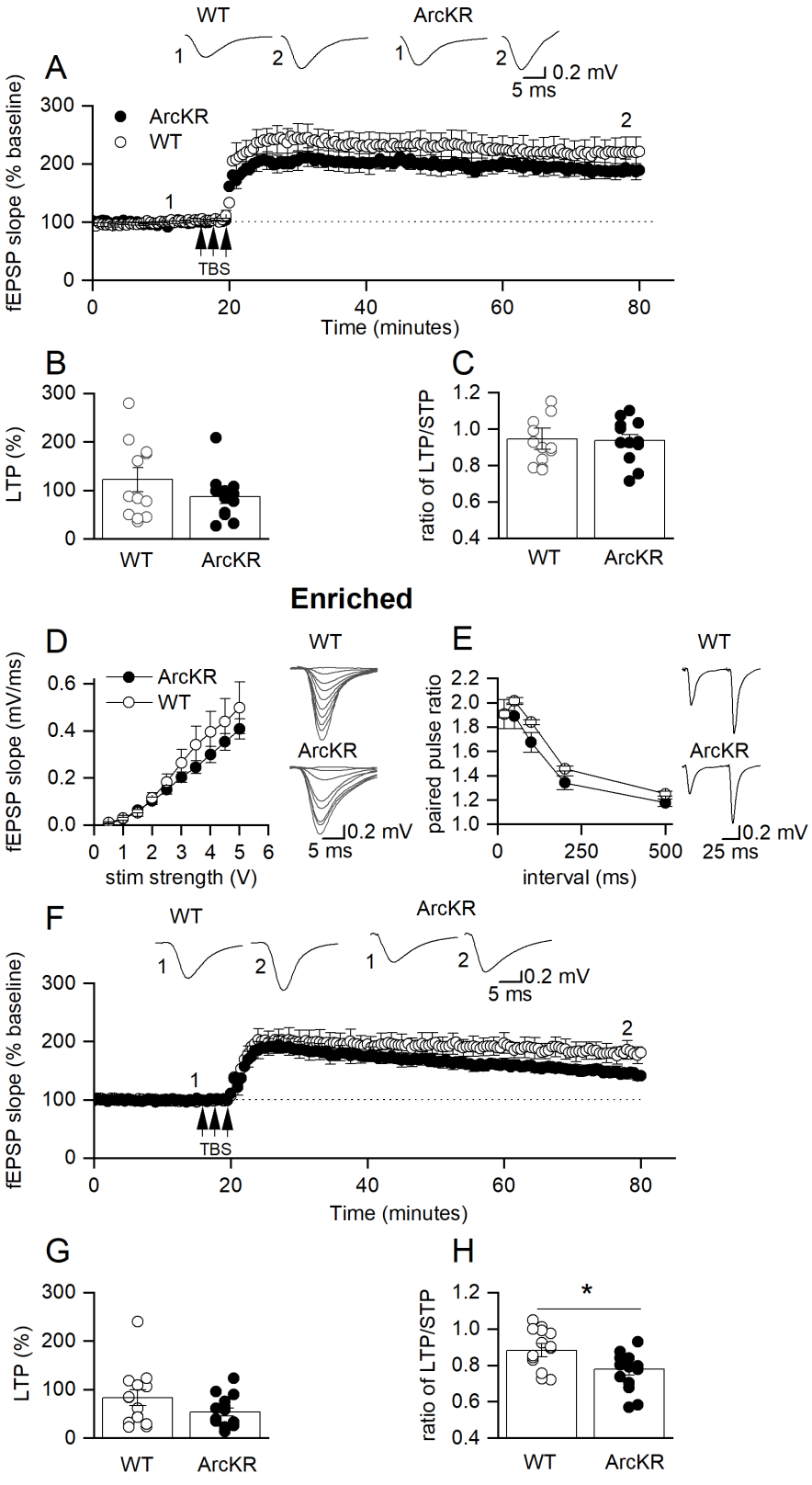
Figure 3.



Hayley et al Figure 4

Figure 4.