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Contribution of mGluR5 to hippocampal pathophysiology in a mouse model of human chromosome 16p11.2 microdeletion

Di Tian[1,2,3,6], **Laura J. Stoppel**[1,2,3], **Arnold J. Heynen**[1,2,3], **Lothar Lindemann**[4], **Georg Jaeschke**[4], **Alea A. Mills**[5], and **Mark F. Bear**[1,2,3,7]

[1]Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge MA, 02139, USA [2]The Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge MA, 02139, USA [3]Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge MA, 02139, USA [4]Pharmaceuticals Division, F. Hoffmann-La Roche, CH-4070 Basel, Switzerland [5]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11724, USA [6]The C.S. Kubik Laboratory for Neuropathology, Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston MA 02114. Current address: Department of Pathology and Laboratory Medicine, Developmental Neuroscience Program, The Saban Research Institute, Children's Hospital Los Angeles, Los Angeles CA, 90027, USA

Abstract

Human chromosome 16p11.2 microdeletion is the most common gene copy number variation in autism, but the synaptic pathophysiology caused by this mutation is largely unknown. Here we show using a mouse with the same genetic deficiency that metabotropic glutamate receptor 5- (mGluR5-) dependent synaptic plasticity and protein synthesis is altered in the hippocampus, and that hippocampus-dependent memory is impaired. Remarkably, chronic treatment with a negative allosteric modulator of mGluR5 reverses the cognitive deficit.

Introduction

Autism spectrum disorder (ASD) is characterized by behavioral, cognitive and language impairment. Over the past decade, studies on monogenetic syndromes with high prevalence of ASD, such as fragile X (FX) and tuberous sclerosis (TS), have provided insights into the pathophysiology of diseases that can cause autism[1]. For example, it has been shown that altered signaling downstream of metabotropic glutamate receptor 5 (mGluR5) plays a pivotal role in the pathogenesis of FX, and that genetic and pharmacological modulation of mGluR5 can ameliorate numerous impairments in FX animal models[2].

[7]Corresponding author: mbear@mit.edu.

Competing interests: M.F.B. holds patents on the use of mGluR5 inhibitors for treatment of fragile X and autism; M.F.B. and D.T. have patents pending on use of mGluR5 inhibitors for treatment of 16p11.2 microdeletion; L.L., G.J. are employees of Roche Pharmaceuticals.

Chromosomal copy number variations (CNVs) have been associated with 5–10% of patients with ASD[3, 4]. Variation at human chromosome 16p11.2 is the most common of these and accounts for approximately 0.5–1% of all ASD cases[4]. The affected region harbors ~27 annotated protein-coding genes, many of which are expressed in the brain[5, 6]. The common clinical presentations in individuals carrying chr16p11.2 microdeletion are language impairment, intellectual disability (ID), ASD, anxiety, attention deficit hyperactive disorder (ADHD), and epilepsy[7]. Recent studies on animal models of human chr16p11.2 microdeletion have demonstrated morphological, behavioral and electrophysiological deficits[8–10], however the synaptic pathophysiology remains largely unexplored.

Using electrophysiology, biochemistry and behavioral tests we characterized hippocampal function of a mouse model for human chr16p11.2 microdeletion[8]. We uncovered alterations in mGluR5-mediated synaptic plasticity, Arc expression, basal protein synthesis, and hippocampus-dependent learning that are reminiscent of previous observations in mouse models of syndromic autism and ID[1]. Importantly, we were able to ameliorate the behavioral abnormalities using an mGluR5 negative allosteric modulator (NAM), 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1*H*-imidazol-4-yl)ethynyl)pyridine (CTEP), suggesting a pathophysiology shared with FX.

Results and Discussion

Mutant mice (termed 16p11.2 *df/+*), engineered to be heterozygous null at the region of chromosome 7qF3 that is syntenic to human chromosome 16p11.2[8], were back-crossed for 5–10 generations to C57BL/6N mice (Charles River) to allow a comparison of synaptic physiology with previous studies[1, 2]. As noted previously[8], loss of the genes in this region can compromise survival, and we found that this effect was amplified in successive generations on the C57BL/6N background (Supplementary Fig. 1).

We first characterized basal synaptic transmission at the Schaffer collateral-CA1 synapse using hippocampal slices from 4–5 week old mice and found no difference from wild type (WT) in input-output or paired-pulse facilitation (PPF) (Supplementary Fig. 2). To investigate NMDA receptor-dependent synaptic plasticity, we induced long-term potentiation (LTP) with theta-burst stimulation (TBS), and long-term depression (LTD) with low-frequency (1 Hz) stimulation (LFS). Again, there was no difference from WT (Fig. 1a–b), suggesting basic excitatory synaptic transmission and plasticity mechanisms are intact in the mutant mice.

We next assayed mGluR5 mediated long-term depression (mGluR-LTD). mGluR-LTD was induced either by brief application of the mGluR1/5 agonist S-3,5-dihydroxyphenylglycine (DHPG) or by applying a series of paired pulses at 50 ms interval (PP-LFS)[1]. We again found no difference between WT and the 16p11.2 *df/+* mutant with either induction protocol (Fig. 1c–f).

A distinctive property of mGluR-LTD in WT animals is a requirement for mRNA translation at the time of induction[11]. In slices from *Fmr1*$^{-/y}$ mice, however, mGluR-LTD is unaffected by translation inhibitors[12] because basal synaptic synthesis of LTD-regulatory

proteins such as Arc[13–15] is elevated downstream of constitutive mGluR5 activity due to loss of the translational repressor FMRP[16]. We were therefore compelled to investigate the protein synthesis-dependence of mGluR LTD in the 16p11.2 *df/+* mice, and discovered a striking difference from WT. Like FX model mice, mGluR-LTD in the 16p11.2 *df/+* mice was unaffected by cycloheximide (CHX) (Fig. 1d, f).

As reported previously in WT animals, DHPG induces LTD via two mechanisms: a postsynaptic reduction in AMPA receptors and a presynaptic reduction in glutamate release probability. Only the postsynaptic mechanism is CHX sensitive[1]. To test whether the different sensitivity of LTD to CHX in the mutant was due to a qualitatively different expression mechanism, we analyzed PPF at the beginning and end of each DHPG-LTD experiment. No difference was observed between the WT and mutant slices (Supplementary Fig. 3). These findings point to a deficiency in postsynaptic regulation of protein synthesis in the 16p11.2 *df/+* mice.

We next tested the mutant mice in two hippocampus-dependent behavioral assays, contextual fear conditioning (CFC) and inhibitory avoidance (IA), which have been shown in previous studies to reveal cognitive impairments in *Fmr1*$^{+/y}$ mice[1, 2]. CFC requires intact mGluR5 signaling and new protein synthesis at the time of conditioning[17]. In this assay, mice are exposed to a distinctive environmental context in which a foot-shock is delivered, and 24 h later the mice are returned to either the same (familiar) or a different (novel) context (Fig. 2a). WT mice expressed fear memory by freezing in the familiar context, and demonstrated an ability to discriminate different contexts by freezing less in the novel context. In contrast, the mutant mice showed significantly less freezing in the familiar context, and were unable to distinguish between the familiar and novel context (Fig. 2b). Mutant and WT mice exhibited comparable sensitivity to foot-shock (Fig. 2c), suggesting the difference in freezing at 24 h was due to an impairment in memory formation in the mutant mice.

In the IA assay, mice received a foot shock upon entry into the dark side of a two-chamber box (Fig. 2d). Memory strength and extinction were measured as the latency to enter the dark side when given the opportunity at 6, 24, and 48 h intervals[2, 18]. The 16p11.2 *df/+* mice showed impaired acquisition and extinction of IA memory. Similar IA deficits in *Fmr1*$^{-/y}$ mice have been ameliorated by chronic post-adolescent treatment with the mGluR5 NAM CTEP[2]. Therefore we repeated the IA assay on WT and mutant mice that were treated every second day with CTEP (2 mg/kg *p.o.*) or vehicle for 4 weeks[2]. Although treatment had no effect in the WT mice, it corrected the deficits in the mutants both in terms of acquisition and extinction (Fig. 2e).

We were motivated to investigate the possibility that FX and 16p11.2 microdeletion have shared pathophysiology for several reasons, including the fact that four deleted genes are targets of FMRP (*MAZ*, *SEZ62L*, *TAOK2*, *ALDOA*)[19] and disruption of several genes in this region are predicted to affect mGluR5 signaling (*MVP*, *CDIPT*, *MAPK3*) or protein turnover (*KCTD13*)[9]. The most straightforward prediction from our results is that synaptic protein synthesis downstream of mGluR5 is exaggerated by the 16p11.2 microdeletion, and this gives rise to cognitive impairment. In the *Fmr1*$^{-/y}$ mouse, bulk protein synthesis in

hippocampal slices is elevated and corrected by manipulations of mGluR5[16, 18]. However, we found that basal protein synthesis in hippocampal slices in the 16p11.2 *df/+* mice was reduced, possibly explained by the decrease in ERK pathway activity (Fig. 3). Nevertheless, immunoblots for Arc protein showed a significant increase. The mGluR5-dependent synthesis of Arc protein normally gates LTD and synapse elimination[13–15]. Therefore, constitutive elevation of Arc could render mGluR-LTD insensitive to acute inhibition of protein synthesis and contribute to cognitive impairment.

These findings support two important conclusions. First, the data suggest that some cognitive and neuropsychiatric symptoms of 16p11.2 microdeletion disorder arise from altered synaptic signaling that is amenable to targeted drug therapy. Second, the data strengthen the hypothesis that multiple causes of ASD and ID converge on common pathophysiological processes, and one of these is the synaptic regulation of protein synthesis[20].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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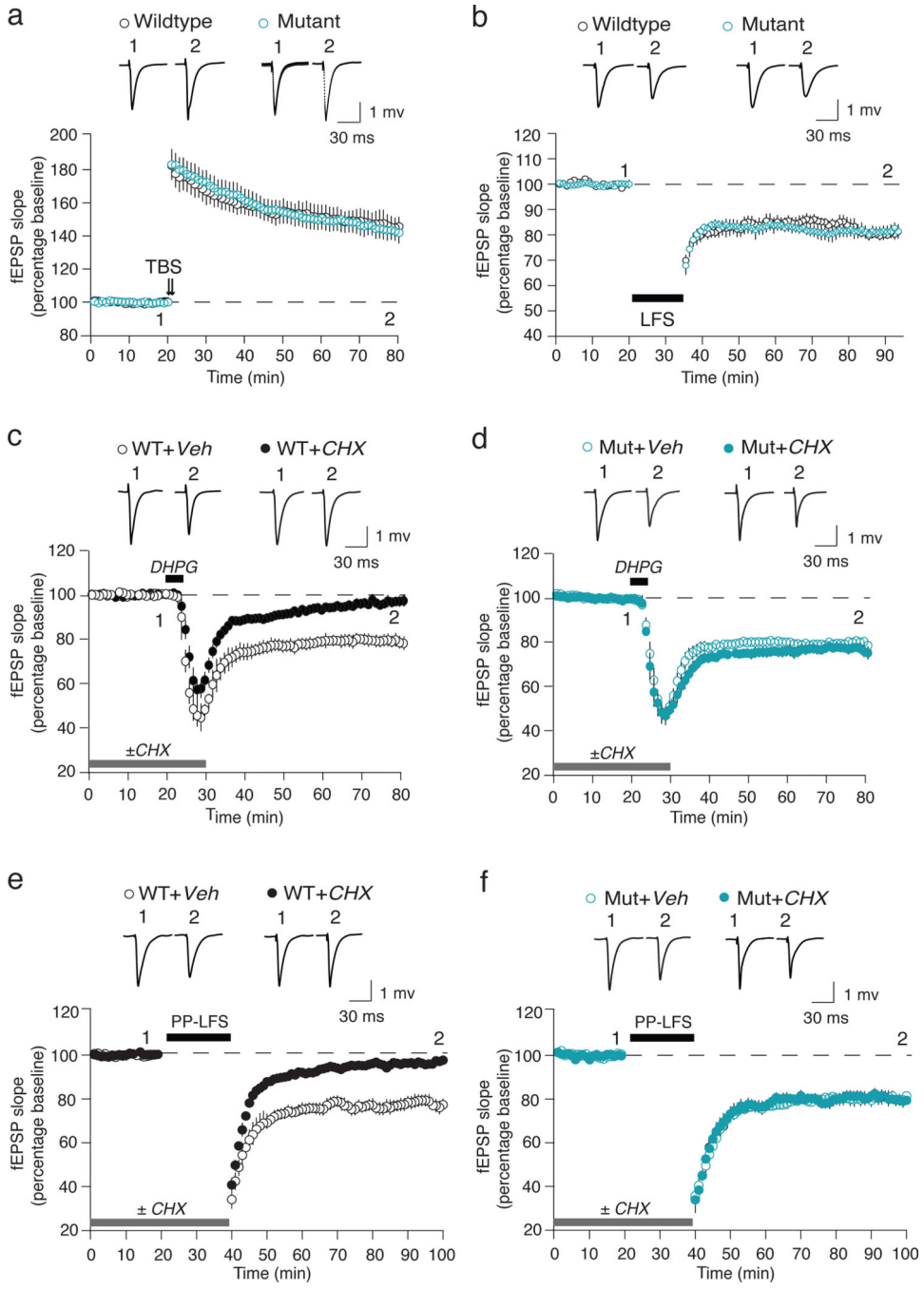

Figure 1. mGluR-LTD is protein synthesis independent in 16p11.2 *df*/+ mice

(**a**) TBS-LTP is unchanged in mutant (n = 9 animals, 16 slices) compared with WT (n = 7 animals, 15 slices) mice. (**b**) LFS-LTD is unchanged in mutant (n = 6 animals, 10 slices) compared with WT (n = 6 animals, 10 slices) mice. (**c**, **d**) The magnitude of DHPG-LTD is comparable in hippocampal slices from the WT (n = 17 animals, 18 slices) and mutant (Mut, n = 16 animals, 20 slices) mice in the absence of CHX. However, CHX blocks DHPG-LTD in WT (n = 17 animals, 21 slices) but not mutant slices (n = 16 animals, 20 slices) (Two-way ANOVA, genotype × CHX, p = 0.0074). (**e**, **f**) The magnitude of PP-LFS-LTD is

comparable in hippocampal slices from WT (n = 12 animals, 14 slices) and mutant (n = 8 animals, 10 slices) mice in the absence of CHX. CHX significantly attenuates PP-LFS-LTD in the WT (n = 12 animals, 14 slices) but not mutant slices (n = 8 animals, 9 slices) (Two-way ANOVA, genotype × CHX, p = 0.013). Representative fEPSP traces (average of 10 sweeps) were taken at the times indicated by numerals. All data are plotted as mean ± SEM.

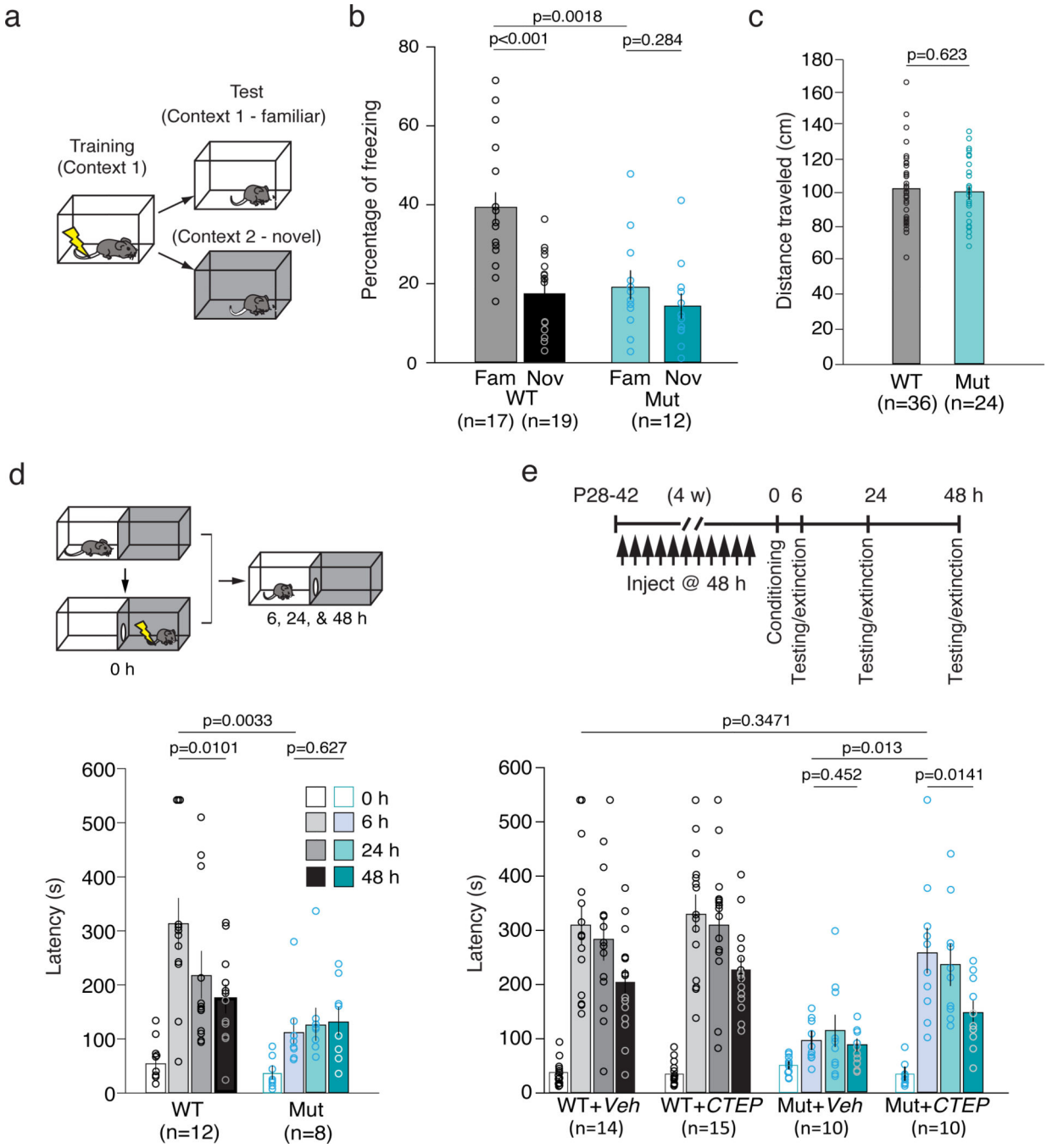

Figure 2. 16p11.2 *df/+* mice exhibit deficits in hippocampal-associated contextual fear conditioning (CFC) and inhibitory avoidance (IA)

(**a**) CFC experimental design. (**b**) Mutant mice show significantly less freezing in the familiar context compared with WT (unpaired t-test, p = 0.0018). While WT mice are able to distinguish a novel from familiar context (unpaired t-test, p < 0.0001), the mutant mice are impaired (unpaired t-test, p = 0.2840). Two-way ANOVA, genotype × context, p = 0.0166. (**c**) Mutant and WT mice have the same running response to foot-shock during the training session (unpaired t-test, p = 0.6234). (**d**) Mutant mice are impaired in IA acquisition

(WT vs Mut, 0 hr vs 6 hr, repeated measures two-way ANOVA, p = 0.0108; WT vs Mut at 6 hr, post-hoc unpaired t-test, p = 0.0033). Unlike WT (6 hr vs 48 hr, post-hoc paired t-test, p = 0.0101), mutant mice show no extinction of fear memory (WT vs Mut, 6 hr vs 48 hr, repeated measures two-way ANOVA, p = 0.0197; Mut, 6 hr vs 48 hr, post-hoc paired t-test, p = 0.6278). (**e**) CTEP treatment ameliorates behavioral deficits in mutant mice in IA. In mutant mice, CTEP treatment enhances acquisition (Mut+Veh vs Mut+CTEP, 0 hr vs 6 hr, repeated measures two-way ANOVA, p = 0.0016; Mut+Veh vs Mut+CTEP at 6 hr, post-hoc unpaired t-test, p = 0.0013) and extinction of fear memory (Mut+Veh vs Mut+CTEP, 6 hr vs 48 hr, repeated measures two-way ANOVA, p = 0.0039; Mut+Veh, 6 hr vs 48 hr, post-hoc paired t-test, p = 0.4281; Mut+CTEP, 6 hr vs 48 hr, post-hoc paired t-test, p = 0.0140). There is no statistically significant difference between WT+Veh and Mut+CTEP at 6 hr (unpaired t-test, p = 0.3471). In WT mice, CTEP has no effect on either acquisition (WT +Veh vs WT+CTEP, 0 hr vs 6 hr, repeated measures two-way ANOVA, p = 0.6564) or extinction of fear memory (WT+Veh vs WT+CTEP, 6 hr vs 48 hr, repeated measures two-way ANOVA, p = 0.9882). All data are plotted as mean ± SEM with individual values superimposed.

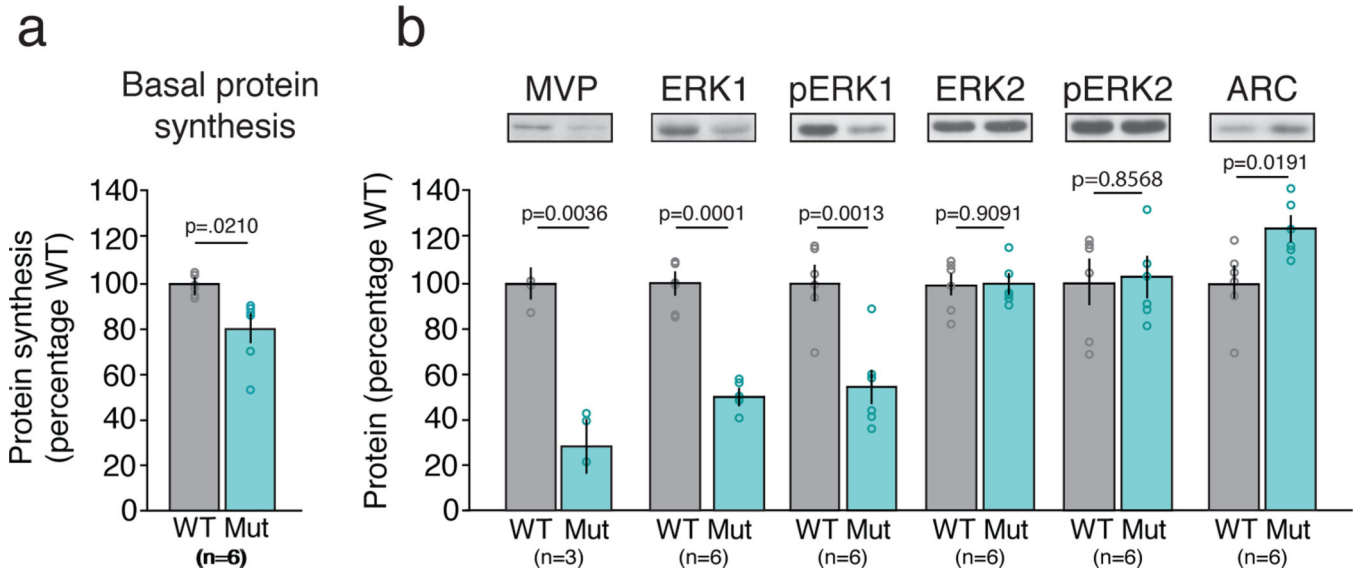

Figure 3. 16p11.2 *df/+* mice exhibit a decrease in basal protein synthesis which is accompanied by an increase in Arc protein levels

(**a**) Metabolic labeling of hippocampal slices reveals a significant reduction of basal protein synthesis in 16p11.2 *df/+* compared to WT mice (unpaired t-test, p = 0.0210). (**b**) MVP, ERK1 and pERK1 are decreased in 16p11.2 *df/+* mice relative to WT mice (unpaired t-test, p = 0.0036; p = 0.0001; p = 0.0013 respectively), whereas ERK2 and pERK2 levels are comparable between 16p11.2 *df/+* and WT mice (unpaired t-test, p = 0.9091; p = 0.8568 respectively). Arc protein levels are significantly increased in 16p11.2 *df/+* mice as compared to WT mice (unpaired t-test, p = 0.0191). All data are plotted as mean ± SEM with individual values superimposed; n indicates number of animals.