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RESEARCH ARTICLE

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Neur1 and *Neur2* are required for hippocampus-dependent spatial memory and synaptic plasticity

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Abstract

Neur1 and *Neur2*, mouse homologs of the *Drosophila neur* gene, consist of two neuralized homology repeat domains and a RING domain. Both *Neur1* and *Neur2* are expressed in the whole adult brain and encode E3 ubiquitin ligases, which play a crucial role in the Notch signaling pathways. A previous study reported that overexpression of *Neur1* enhances hippocampus-dependent memory, whereas the role of *Neur2* remains largely unknown. Here, we aimed to elucidate the respective roles of *Neur1* and *Neur2* in hippocampus-dependent memory using three lines of genetically modified mice: *Neur1* knock-out, *Neur2* knock-out, and *Neur1* and *Neur2* double knock-out (D-KO). Our results showed that spatial memory was impaired when both *Neur1* and *Neur2* were deleted, but not in the individual knock-out of either *Neur1* or *Neur2*. In addition, basal synaptic properties estimated by input–output relationships and paired-pulse facilitation did not change, but a form of long-term potentiation that requires protein synthesis was specifically impaired in the D-KO mice. These results collectively suggest that *Neur1* and *Neur2* are crucially involved in hippocampus-dependent spatial memory and synaptic plasticity.

KEYWORDS

E3 ligase, hippocampus-dependent learning and memory, long-term potentiation, *Neur1*, *Neur2*, spatial memory, synaptic plasticity, ubiquitination

1 | INTRODUCTION

Neuralized encodes ubiquitin E3 ligase (Yeh et al., 2001) and is a highly conserved gene in various species from flies to humans (Nakamura et al., 1998; Price, Chang, Smith, Bockheim, & Laughon, 1993). In mammals, there are five different neuralized homology repeat (NHR) domain-containing proteins, namely *Neuralized-1* (or *Neurl 1a*, *Neur1*), *Neuralized-2* (or *Neurl 1b*, *Neur2*), *Ozz-E3* (or *Neurl 2*), *LINCR* (or *Neurl 3*), and *KIAA1787* (or *Neurl 4*) (Liu & Boulianne, 2017). Of interest in this study, *Neur1* and *Neur2* are the mouse homologs of the Drosophila *neur* gene and are each composed of two copies of the neuralized homology repeat (NHR) domain and a C-terminal C3HC4 RING Zn-finger (RING) domain (Pavlopoulos et al., 2002; Song et al., 2006).

Previous studies suggested that both *Neur1* and *Neur2* are expressed in all parts of the adult mouse brain. The mRNA expression of *Neur1* was found to be highly localized in the dendrites of major

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excitatory neurons in the hippocampus (Timmusk, Palm, Belluardo, Mudo, & Neuman, 2002), whereas *Neur2* was preferentially found in the cerebral cortex, thalamus, striatum, and olfactory bulb (Rullinkov et al., 2009).

Both *Neur1* and *Neur2* are involved in the Notch signaling pathways. For instance, *Neur1* represses Notch signaling by downregulating the expression of a Notch ligand, Jagged1 (Koutelou et al., 2008), whereas *Neur2* regulates endocytosis of the Notch ligand Delta in association with Mind Bomb-1 (Song et al., 2006). These studies suggest that both *Neur1* and *Neur2* participate in Notch signaling, but with distinct mechanisms. Although the Notch signaling pathway is involved in crucial developmental processes (Andersson, Sandberg, & Lendahl, 2011; Bolos, Grego-Bessa, & de la Pompa, 2007), *Neur1* knock-out (*N1*-KO) and *Neur2* knock-out (*N2*-KO) mice have been found to be developmentally intact (Koo et al., 2007).

Previous reports suggest ubiquitin E3 ligases have diverse roles in the learning and memory of mature animals (Chakraborty et al., 2015; Sun et al., 2015; Zhang et al., 2013). In Drosophila, *Neur* mutant flies showed impairment of long-term memory, which was rescued by overexpression of *Neur* in the peripheral neurons of the α/β lobes of the mushroom bodies (Pavlopoulos, Anezaki, & Skoulakis, 2008). Moreover, overexpression of *Neur1* in the mouse hippocampus improved long-term potentiation (LTP) and hippocampus-dependent memory, and these effects were found to be mediated by the monoubiquitinated form of cytoplasmic polyadenylation element-binding protein 3 (CPEB3), which increases the number of the AMPAR subunits GluA1 and GluA2 (Bear et al., 2018; Pavlopoulos et al., 2011). In contrast, the role of *Neur2* in learning and memory is hitherto largely unknown. Also, it remains unclear whether interaction between these genes plays a role in regulation of learning and memory.

In the present study, we investigated the roles of *Neur1* and *Neur2* in various aspects of hippocampus-dependent learning and memory via deletion of either *N1*-KO or *N2*-KO; these mice were compared with double knock-out (D-KO) and wild-type (WT) littermates. Our results show that spatial memory was impaired only when both *Neur1* and *Neur2* were absent. Additionally, we revealed that basal synaptic properties were unchanged, but protein synthesis-dependent long-term synaptic plasticity was selectively impaired in the D-KO mice.

2 | MATERIALS AND METHODS

2.1 | Animals

The genetic background and generation of the *N1*-KO, *N2*-KO, and *Neur1* and *Neur2* D-KO mouse have been previously described (Koo et al., 2007; Ruan et al., 2001). Briefly, genomic regions encoding amino acids from 218 to 574 of the Neur1 protein, and amino acids from 115 to 319 of Neur2 protein were ablated. In both cases, the genomic deletion led to a truncation of the carboxy-terminal region of the proteins, including the RING zinc finger domain essential for ubiquitylation. The established mouse lines are whole body knock-out mice derived from the C57BL/6J strain. Both male and female genotypes were used, and there were no gender-based differences in their behavior. The animal facility was on a 12-hr light, 12-hr dark cycle (lights switched on at 9:00 a.m., lights switched off at 21:00 p.m.). Under temperature regulated (approximately 24°C) conditions, all animals cohabited, with food and water provided ad libitum. This research was performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Seoul National University.

2.2 | Behavioral tests

2.2.1 | Object location memory test

The object location memory (OLM) test was performed as previously described (Cho et al., 2015). Briefly, mouse handling was performed for 5 min for five consecutive days. In the next 2 days, the subjects were habituated to a chamber (Plexiglas, 33 cm × 33 cm × 30 cm), where one side of the wall had a visual cue and the opposite side was transparent, for 15 min daily. The entire experimental process was performed under a dim light. On each training day, two identical objects were positioned in the chamber, and the mice were allowed to explore and learn the location of the objects for 10 min. On the test day, one of the objects was relocated to the opposite side, and mice were permitted to explore for 5 min. Between each trial, the chamber and the objects were washed with distilled water and 70% ethanol (EtOH). The interaction time with objects was manually measured by experimenters, and the discrimination index was determined by the relative time spent near the relocated object.

2.2.2 | Morris water maze test

The Morris water maze (MWM) test was performed as previously described (Lim et al., 2017). Briefly, a round-shaped tank (140 cm in diameter, 100 cm in height) was placed in a room with spatial cues and filled with white opaque water. The entire experiment was performed under a dim light and the water temperature was maintained in the range of 21–23°C. The tank was split into four virtual quadrants, and a platform (10 cm in diameter) was located at the center of one of the quadrants, named as the target quadrant (TQ). Mice were put into the water tank and, for training, were allowed to reach the platform in the TQ, and each trial lasted no more than 60 s. For five consecutive days, each trial was performed four times per day, with 1 min intertrial intervals. This was followed by the probe test, which was performed under the same conditions, but without a platform.

2.2.3 | Contextual fear conditioning test

The contextual fear conditioning (CFC) test was performed as previously described (Choi et al., 2018). Briefly, mice were handled for

3 min each day in the 3 days prior to the training day. On the conditioning day, mice were permitted 3 min to explore freely in the conditioning chamber, (Coulbourn Instruments) followed by a foot shock (2 s duration, 0.4 mA intensity) delivered through the floor grid. At the end of the task, mice were immediately returned to their home cages. Twenty-four hours later, mice were reexposed to the same chamber, and freezing behavior was automatically quantified by Freeze Frame software (Coulbourn Instruments).

2.3 | Electrophysiology

Mice were anesthetized with isoflurane and sacrificed by decapitation in accordance with the regulations of the Institutional Animal Care and Use Committee at Seoul National University. Transverse hippocampal slices (350 μm) were prepared using a vibratome (Leica, VT1200S) in an ice-chilled slicing solution that contained (in mM): 210 sucrose, 3 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 5 $MgSO_4$, 10 D-glucose, 3 sodium ascorbate, and 0.5 $CaCl_2$, and was saturated with 95% O_2 and 5% CO_2. The slices were transferred to an incubation chamber that contained the recording solution (artificial cerebrospinal fluid; mM): 124 NaCl, 3 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $MgSO_4$, 10 D-glucose, and 2 $CaCl_2$ (carbonated with 95% O_2 and 5% CO_2). Slices were allowed to recover at 32–34°C for 30 min and then maintained at 26–28°C for a minimum of 1 hr before recordings were made.

Standard extracellular recordings were performed in the CA1 region of the hippocampal slices maintained at 32°C, as described previously (Park et al., 2016), to measure the slope of the evoked field EPSPs. Responses were obtained using a Multiclamp 700B amplifier (Molecular Devices) and digitized with a Digidata 1322A A/D board at a sampling rate of 20 kHz (low-pass filtered at 10 kHz). Recordings were monitored and analyzed using WinLTP (Anderson & Collingridge, 2007). Two independent Schaffer collateral-commissural pathways were stimulated alternately, each at a frequency of 0.1 Hz. After a stable baseline for at least 20 min, LTP was induced using theta-burst stimulation (TBS) delivered at the basal stimulus intensity. An episode of TBS comprised five bursts at 5 Hz, with each burst composed of five pulses at 100 Hz. Either a single episode of TBS or a train of three TBS episodes with an inter-episode interval of 10 min was applied. A representative sample trace was the average of four consecutive responses collected from typical experiments (stimulus artifacts were blanked for clarity). The input–output (I–O) relationships were estimated by varying the stimulus intensity from 1.5 to 15 V. Interstimulus intervals from 20 to 400 ms at the basal stimulus intensity were used for paired-pulse facilitation (PPF).

2.4 | Statistics

Statistical significance was assessed using one-way analysis of variance (ANOVA) with Bonferroni's post hoc correction or Student's *t* test as appropriate. The level of significance was denoted as follows: $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, and $^{****}p < .0001$. The GraphPad Prism 8 program was used for data plots and statistics. All graphs have been presented as means ± *SEM*. All tests were conducted in blind with respect to the information on genotypes.

3 | RESULTS

3.1 | D-KO mice displayed impaired hippocampus-dependent spatial memory

We initially tested the roles of *Neur1* and *Neur2* in hippocampus-dependent spatial memory by employing the OLM and the MWM tests.

In the OLM test, mice were allowed to learn the location of two identical objects and then tested on whether they could recognize the position of one object when it was relocated the next day (Figure 1a). We calculated the discrimination index as the ratio between the exploration time with the relocated object and the total exploration time; therefore, a low discrimination index indicates that the subjects spent significantly less time near the relocated object than near the original object. We found a significantly lower discrimination index in D-KO mice compared with the WT littermates ($^{*}p < .05$, one-way ANOVA with Bonferroni's post hoc test; $n = 15$ and 10 for WT and D-KO) (Figure 1b).

In the MWM test, mice were trained for five consecutive days in order for them to learn the location of a platform in a round tank with opaque white water (Figure 2a). Compared to WT littermates, D-KO mice showed delayed learning during the training sessions (Figure 2b) and also showed significantly retarded escape latency on training Day 5 ($^{***}p < .001$, one-way ANOVA with Bonferroni's post hoc test; $n = 13$ and 8 for WT and D-KO) (Figure 2c) and longer swimming distance on training Day 5 ($^{***}p < .0001$, one-way ANOVA with Bonferroni's post hoc test) (Supplementary Figures S1 and S2). We performed the probe test to examine whether the mice remembered the location of the platform. The tracked paths illustrate the movement of mice of each genotype in the water maze apparatus. *N1*-KO, *N2*-KO, and WT mice swam near the location of the platform, whilst D-KO mice revolved around the water tank (Figure 2d). This tendency was likewise present with other indices. For instance, D-KO mice spent significantly less time in the TQ (Figure 2e,f), showed a decreased number of platform crossings (Figure 2g), and, on average, stayed significantly farther from the location of the platform compared to WT littermates (Figure 2h). We compared the swimming speed of all mouse groups on five training days and at the time of the probe test. There was no statistically significant difference across all genotypes (Supplementary Figure S3). We observed a strong correlation between the escape latency and the swimming distance (Supplementary Figure S2). These results suggest that the poor memory performance in MWM of D-KO mice was not due to poor swimming ability. Taken together, these results reveal that, under the expression of either *Neur1* or *Neur2*, hippocampus-dependent spatial memory is unharmed, but this might not be the case when both genes are deleted.

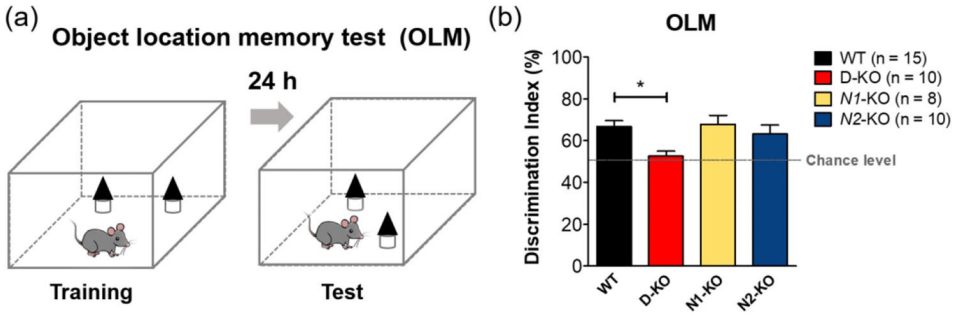

FIGURE 1 Double knock-out (D-KO) mice showed impaired hippocampus-dependent memory in the object location memory (OLM) test. (a) Mimetic drawings of the OLM test. (b) The D-KO mice showed a low object discrimination index in the OLM test. Wild-type (WT) (black; n = 15, [male = 9, female = 6]), D-KO (red; n = 10, [male = 3, female = 7]), *N1*-KO (yellow; n = 8, [male = 6, female = 2]), *N2*-KO (blue; n = 10 [male = 5, female = 5]) (*p < .05, one-way analysis of variance (ANOVA) with Bonferroni's post hoc test) [Color figure can be viewed at wileyonlinelibrary.com]

To confirm that spatial memory deficits observed in D-KO mice were not due to changes in the level of anxiety, we employed the open-field (OF) test, the elevated zero maze (EZM) test, and the light–dark (LD) box test (Clement, Calatayud, & Belzung, 2002). The four genotypes did not show any significant differences in the EZM and the LD box tests (Supplementary Figure S4e,g). Nonetheless, a significantly lower level of anxiety was observed for *N2*-KO mice in the OF test (Supplementary Figure S4b). These results suggest that the spatial memory deficit displayed by D-KO mice may not be due to an alteration in mood. Additionally, *N2*-KO mice showed decreased anxiety-like behavior in the OF test, but they were not impaired in hippocampus-dependent long-term memory.

Moreover, we performed the Y-maze task to investigate hippocampus-dependent spatial working memory (Aggleton, Hunt, & Rawlins, 1986). All genotypes showed a similar level of spontaneous arm alterations. Therefore, the impairment of hippocampus-dependent spatial long-term memory was not due to changes in working memory (Supplementary Figure S5).

3.2 | D-KO mice displayed lower, albeit not statistically significant, freezing levels in the CFC test

We conducted the CFC test to examine whether hippocampus-dependent fear memory was changed in *N1*-KO, *N2*-KO, and D-KO mice. Handled mice were conditioned in the chamber with a foot shock, and, on the following day, mice were reexposed to the same chamber to test memory retrieval (Figure 3a). All genotypes presented a significantly higher level of freezing during the retrieval session compared to the conditioning session (Figure 3b), suggesting fear memory formation was intact in all genotypes. However, during the retrieval session, D-KO mice presented a lower, albeit not statistically significant (p = .1155; one-way ANOVA analysis, Bonferroni's multiple comparison test), level of freezing than WT littermates (Figure 3c). We further analyzed the freezing data with consideration for individual variations in the basal freezing level by normalizing the values into the change in freezing (%) (Barnet & Hunt, 2005; Moita, Rosis, Zhou, LeDoux, & Blair, 2004). D-KO mice showed a significantly decreased change in freezing compared to WT littermates (Figure 3d). Taken together, the freezing level of D-KO mice was noticeably lower compared to other groups, although, admittedly, the degree of change was not dramatic.

3.3 | Reduced late LTP in *Neur1*, *2* D-KO mice

To understand the physiological role of *Neur1* and *Neur2* at synapses, we investigated the basal synaptic properties using extracellular field EPSP recordings. I–O relationships and the PPF ratio were measured at the Schaffer collateral-commissural pathway in acute hippocampal slices. All genotypes showed comparable levels of basal synaptic properties (Figure 4a,b), suggesting synaptic functions were intact despite the deletion of one or both *Neuralized* genes.

Next, we performed synaptic plasticity experiments using TBS for LTP induction. A single episode of TBS was given for a weak LTP, whereas three episodes of TBS with a 10 min inter-episode interval were used for a strong and de novo proteinsynthesis-dependent form of LTP (Park et al., 2014; Park et al., 2016; Park et al., 2018). These two forms of LTP are conventionally referred to as early LTP (E-LTP) and late LTP (L-LTP) (Huang & Kandel, 1994). Results showed intact E-LTP in all genotypes (p = .58, $F_{(3,37)}$ = 0.67, one-way ANOVA; Figure 4a,b). The levels of LTP quantified after 1 hr of LTP induction were 133 ± 4% (n = 14 from 12 mice) and 124 ± 7% (n = 11 from nine mice) of baseline for the WT and D-KO groups, respectively. In contrast, a significantly lower level of L-LTP was found in D-KO mice (Figure 4c,d) when this quantified 2 hr after LTP induction; the value was 142 ± 7% of baseline (n = 9 from nine mice) compared to WT littermates with a value of 173 ± 7% of baseline (n = 12 from 12 mice, *p < .05, $F_{(3,31)}$ = 2.95, one-way ANOVA with Bonferroni's post hoc test). These findings suggest that the presence of at least one of these genes is specifically required for L-LTP.

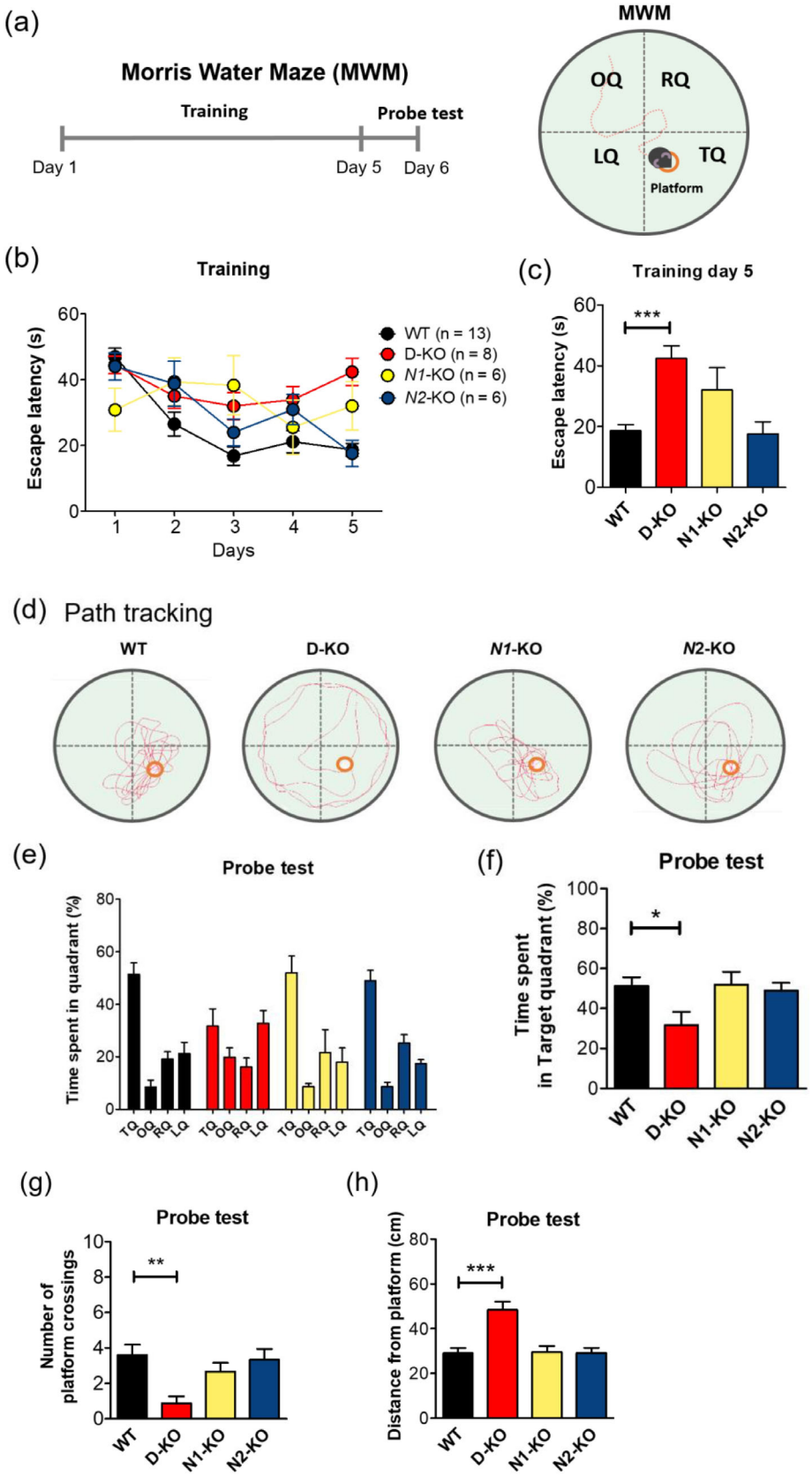

FIGURE 2 Double knock-out (D-KO) mice showed impaired hippocampus-dependent learning and memory in the Morris water maze (MWM) test. (a) Schematic drawings of the MWM test experimental scheme. Wild-type (WT) (black; *n* = 13 [male = 9, female = 4]), D-KO (red; *n* = 8 [male = 6, female = 2]), *N1*-KO (yellow; *n* = 6 [male = 4, female = 2]), *N2*-KO (blue; *n* = 6 [male = 3, female = 3]). (b) Learning curve showing the latency of the mice reaching the platform on five training days. (c) On training Day 5, the D-KO mouse demonstrated delayed escape latency (***$p < .001$, one-way analysis of variance [ANOVA] with Bonferroni's post hoc test; *n* = 13 and 8 for WT and D-KO). (d) The path tracking data representative to each genotype. (e,f) The time spent in each quadrant by each genotype during the 1-min probe test. D-KO mice spent significantly less time in the target quadrant (TQ) during the probe test compared to other genotypes (*$p < .05$, one-way ANOVA of time spent in TQ, Bonferroni's post hoc test; *n* = 13 and 8 for WT and D-KO). (g) During the probe test, the D-KO mice crossed the location of the platform considerably less than other genotypes (**$p < .01$, one-way ANOVA, Bonferroni's post hoc test; *n* = 13 and 8 for WT and D-KO). (h) Throughout the probe test, D-KO mice kept a farther distance from the location of the platform compared to other genotypes (***$p < .001$, one-way ANOVA, Bonferroni's post hoc test; *n* = 13 and 8 for WT and D-KO) [Color figure can be viewed at wileyonlinelibrary.com]

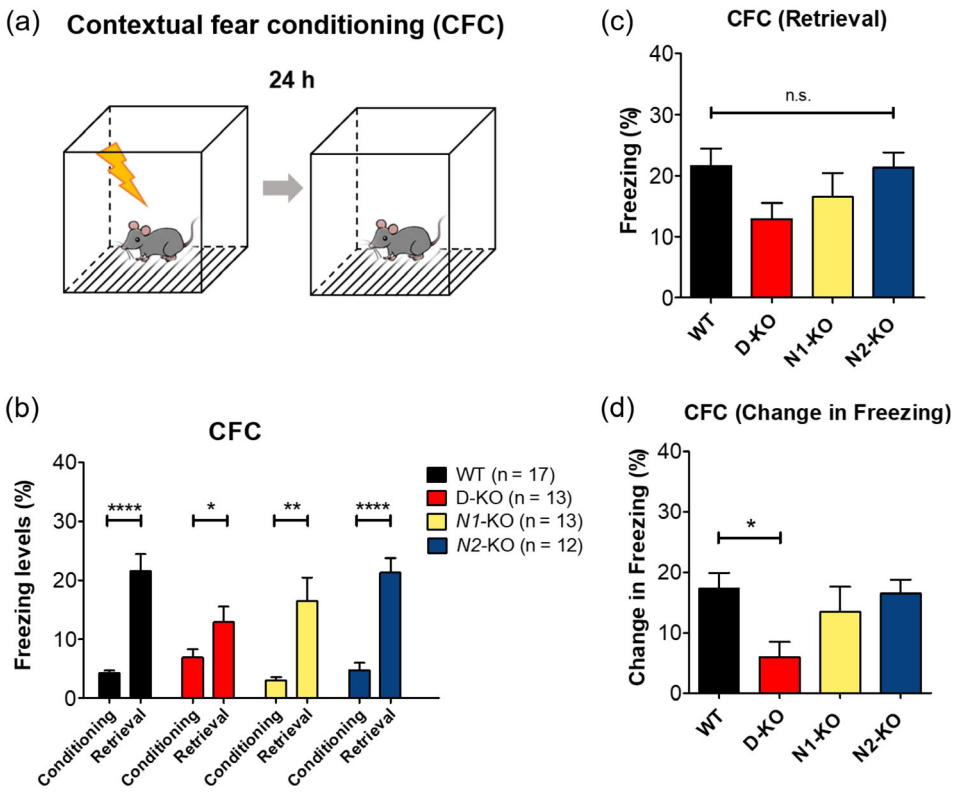

FIGURE 3 Double knock-out (D-KO) mice displayed lower freezing level in the CFC test. (a) Schematic drawings of the CFC test. Wild-type (WT) (black; *n* = 17 [male = 11, female = 6]), D-KO (red; *n* = 13 [male = 5, female = 8]), *N1*-KO (yellow; *n* = 13 [male = 9, female = 4]), *N2*-KO (blue; *n* = 12 [male = 6, female = 6]). (b) All genotypes showed significantly increased levels of freezing behavior in the retrieval session compared to those in the conditioning session (paired *t* test of conditioning and retrieval in WT group ****$p < .0001$, paired *t* test of conditioning and retrieval in the D-KO *$p < .05$, paired *t* test of conditioning and retrieval in the *N1*-KO **$p < .01$, paired *t* test of conditioning and retrieval in *N2*-KO ****$p < .0001$). (c) There was no significant difference in freezing level during the retrieval session (one-way analysis of variance (ANOVA), Bonferroni's post hoc test, $p = .1535$). (d) The results of the analysis of change in freezing. The change in freezing was calculated as follows: ($Freezing_{test} - Freezing_{conditioning}$), (*$p < .05$, one-way ANOVA, Bonferroni's post hoc test; *n* = 17 and 13 for of WT and D-KO) [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

In a previous study, Pavlopoulos et al. showed that *Neur1* inhibition induced impairment of LTP and spatial memory (Pavlopoulos et al., 2011). However, this result is seemingly inconsistent with our results that show hippocampus-dependent spatial memory was impaired when both *Neur1* and *Neur2* were absent, while it was intact when only one *Neuralized* gene was deleted (see also Ruan et al., 2001). We conjecture that these discrepancies were caused by methodological differences. Pavlopoulos et al. used the dominant-negative form of *Neur1* at a specific time point to inhibit the function of normal NEUR1, whereas we and Ruan et al. induced *N1*-KO mice at the embryonic stage. Our results reveal that the relative expression of *Neuralized* transcripts was unchanged in the *N1*-KO and *N2*-KO mice compared to WT littermates (Supplementary Figure S6), suggesting that no compensatory overexpression in the paralog is triggered by the absence of *Neur1* or *Neur2*. Therefore, it can be assumed that *Neur1* and *Neur2* are interchangeable, and do not act collaboratively, in learning and memory. Taken together, these results show that the presence of either *Neur1* or *Neur2* is sufficient for spatial learning and memory, demonstrating the possible functional redundancy of *Neur1* and *Neur2* in hippocampus-dependent spatial memory. However, we cannot rule out the possibility that *Neur1* and *Neur2* may play different roles in other areas of the brain and other types of memory.

In this study, we applied three different memory tasks, the OLM test, the MWM test, and the CFC test, to estimate hippocampus-dependent spatial memory. The memory impairment of D-KO mice was clearer in the OLM and MWM tests. One possibility is that the hippocampus is involved in processing various spatial information in distinct ways. For instance, a goal-directed navigation strategy is required in the MWM test (Cornwell, Johnson, Holroyd, Carver, & Grillon, 2008; Eichenbaum, 2017), while associative learning between context and emotion is crucial during the CFC test (Brasted, Bussey, Murray, & Wise, 2003; Kim & Jung, 2006).

We also found that basal synaptic transmission and E-LTP were unchanged in all genotypes, yet L-LTP was specifically reduced in the D-KO mice. It has been suggested that NEUR1 regulates synaptic plasticity through monoubiquitinated CPEB3, which promotes the production of AMPA receptor subunits GluA1 and GluA2

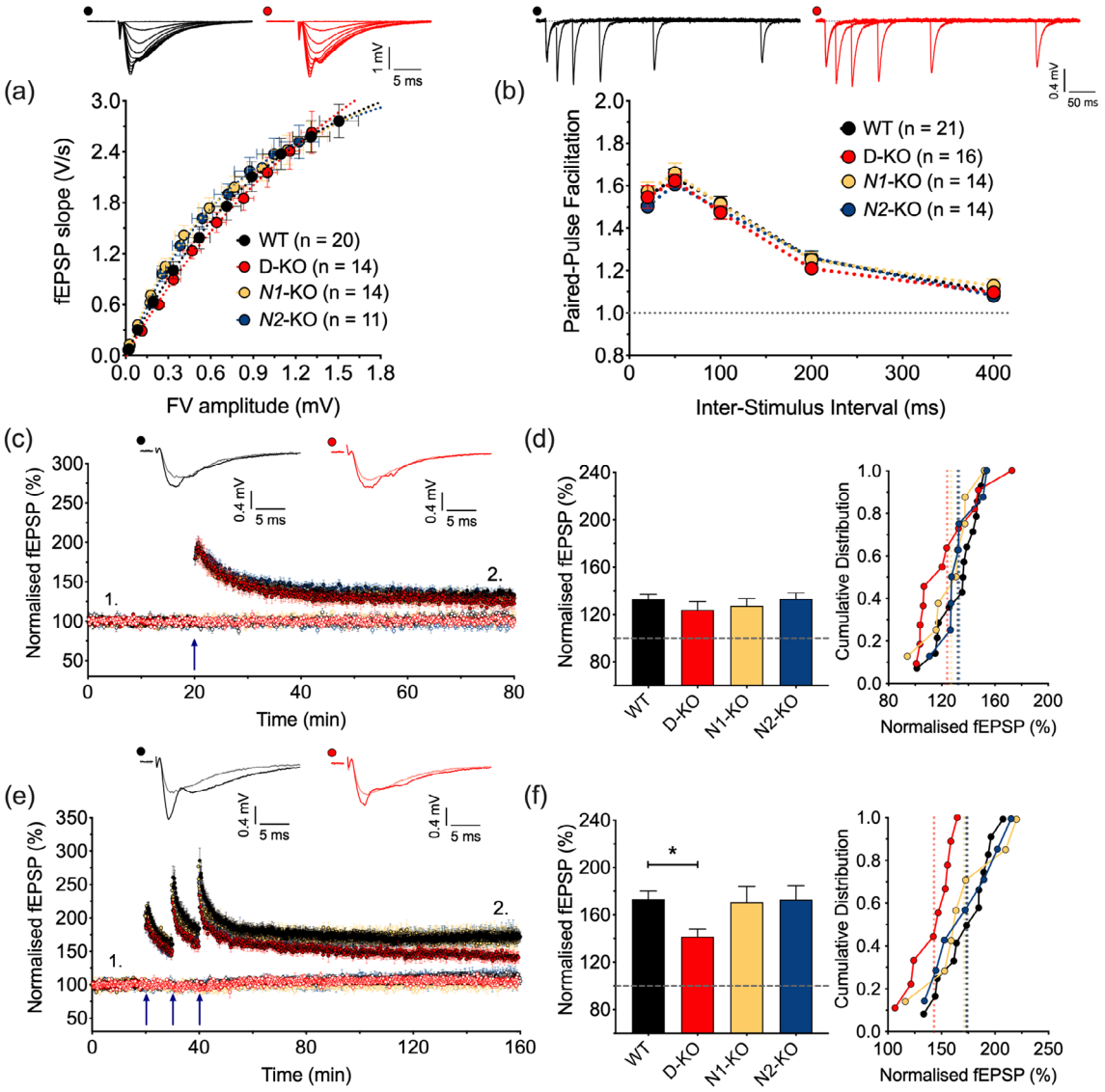

FIGURE 4 Reduced late long-term potentiation (L-LTP) in *Neur1*, *2* double knock-out mice. (a) Input–output relationships of evoked field fEPSP (fEPSP) responses at CA1 synapses for wild-type (WT) (black circles; n = 20 from 12 mice [male = 8; female = 4]), double knock-out (D-KO) (red; *n* = 14 from 9 mice [male = 6; female = 3]), *N1*-KO (yellow; *n* = 14 from 7 mice [male = 4; female = 3]) and *N2*-KO (blue; n = 11 from 7 mice [male = 4; female = 3]). The stimulation intensity was varied by 1.5 V increments, and the insets indicate representative traces (averages of four successive sweeps). (b) Paired-pulse facilitation (PPF) measured at inter-stimulus intervals of 20, 50, 100, 200, and 400 ms for WT (*n* = 21 from 12 mice [male = 8; female = 4]), D-KO (*n* = 18 from nine mice [male = 6; female = 3]), *N1*-KO (*n* = 14 from seven mice [male = 4; female = 3]), and *N2*-KO (*n* = 14 from seven mice [male = 4; female = 3]). (c) Input-specific LTP induced by a single episode of theta-burst stimulation (TBS). (d) Quantification of the levels of LTP and the corresponding cumulative distributions for WT (*n* = 14 from 12 mice [male = 8; female = 4]), D-KO (red; *n* = 11 from nine mice [male = 6; female = 3]), *N1*-KO (yellow; *n* = 8 from seven mice [male = 4; female = 3]), and *N2*-KO (blue; *n* = 8 from seven mice [male = 4; female = 3]), showing no significant difference ($p = .67$; one-way analysis of variance (ANOVA) with Bonferroni's post hoc test). (e) LTP induced by three episodes of TBS with a 10 min inter-episode interval and then monitored for 2 hr following LTP induction. (f) Quantification of the levels of LTP. Significant reduction in the level of LTP observed for double knock-out (D-KO) (*n* = 9 from nine mice [male = 6; female = 3]) compared to WT littermates (*n* = 12 from 12 mice [male = 8; female = 4]) (*$p < .05$, one-way ANOVA with Bonferroni's post hoc test). Sample traces were obtained as indicated by the numbers. Dotted lines in the cumulative distribution indicate the average of each group [Color figure can be viewed at wileyonlinelibrary.com]

(Pavlopoulos et al., 2011). In addition, the deletion of *Neur1* and *Neur2* might have affected other substrates which are known to play a role in learning and memory, such as cGMP-specific phosphodiesterase 9A (Kleiman et al., 2012; Taal et al., 2019) and the Notch signaling pathway ligands (Brai et al., 2015; Tu et al., 2017; Wang et al., 2004). These signaling pathways have critical roles in gene transcription and de novo protein synthesis, which is also consistent with the selective impairment of L-LTP observed in the slice physiology. Further studies are required to elucidate the critical downstream molecules in regulation of learning and memory by *Neuralized* pathways.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Jaehyun Lee, Chaery Lee, Hye-Ryeon Lee, Yeseul Lee, and Eun-Hae Jang conducted the behavioral experiments; Pojeong Park, Dae Hee Han, Hye-Ryeon Lee, and Somi Kim conducted the electrophysiology experiments; Jaehyun Lee, Min Jung Kim, Ji-il Kim, and Hyoung-Gon Ko performed molecular biological works; Jaehyun Lee and Ji-il Kim performed RT-PCR and qRT-PCR experiments. Ki-Jun Yoon and Young-Yun Kong generated KO mouse lines. Jaehyun Lee, Pojeong Park, Young-Yun Kong, and Bong-Kiun Kaang supervised the project and wrote the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

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