Review Article

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From Bound Cells Comes a Sound Mind: The Role of Neuronal Growth Regulator 1 in Psychiatric Disorders

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Cell-to-cell adhesion is important for maintenance of brain structure and function. Abnormal neuronal cell adhesion and loss of its connectivity are considered a main cause of psychiatric disorders such as major depressive disorder (MDD). Various cell adhesion molecules (CAMs) are involved in neuronal cell adhesions and thereby affect brain functions such as learning and memory, cognitive functions, and psychiatric functions. Compared with other CAMs, neuronal growth regulator 1 (Negr1) has a distinct functioning mechanism in terms of its cross-talk with cytokine receptor signaling. Negr1 is a member of the immunoglobulin LON (IgLON) family of proteins and is involved in neuronal outgrowth, dendritic arborization, and synapse formation. In humans, Negr1 is a risk gene for obesity based on a genome-wide association study. More recently, accumulating evidence supports that it also plays a critical role in psychiatric disorders. In this review, we discuss the recent findings on the role of Negr1 in MDD, focusing on its regulatory mechanism. We also provide evidence of putative involvement of Negr1 in other psychiatric disorders based on the novel behavioral phenotypes of Negr1 knockout mice.

Key words: Cell adhesion molecule (CAM), Neuronal growth regulator 1 (Negr1), Immunoglobulin LON family (IgLON), Psychiatric disorders, Major depressive disorder (MDD)

INTRODUCTION

Tight connections between presynaptic bouton and postsynaptic density by cell adhesion molecules (CAMs) are critical to form stable and functional synapses [1-6]. Two types of CAMs, Ca^{2+}-dependent and Ca^{2+}-independent, work as synaptic adhesion molecules in the central nervous system (CNS). Cadherin and selectin are dependent on Ca^{2+} ion for binding, while members of the immunoglobulin superfamily (IgSF) contribute to establishment and remodeling of neuronal synapses [7-9] in a Ca^{2+}-independent manner. Among IgSF, immunoglobulin LON (IgLON) family proteins function as synaptic adhesion molecules that play important roles in axonal extension, dendritic arborization, and synaptogenesis during brain development [10-13]. Molecules of the IgLON family including LAMP, OBCAM, neurotrimin, GP55, and AvGP50 have three Ig-like domains and localize to the membrane raft of the Triton-insoluble low-density fraction via a glycosylphosphatidylinositol (GPI)-anchor [14-18]. Among the IgLONs, neuronal growth regulator 1 (Negr1), also named Kilon (a kindred of IgLON), is the most recently characterized IgLON subgroup member [19, 20].

Negr1 is a GPI-anchored membrane protein of 46 kDa with three immunoglobulin-like domains and six putative glycosylation sites (Fig. 1A) [19, 20]. Initial studies during mouse development show that Negr1 expression gradually increases during postnatal brain development and reaches a constant level in adulthood [20-22]. Both immunohistochemistry and western blot analysis confirm that the Negr1 protein is expressed in the cerebral cortex and hippocampus of the adult rat [19]. *In situ* hybridization studies revealed that Negr1 mRNA is expressed in the cerebral cortex,
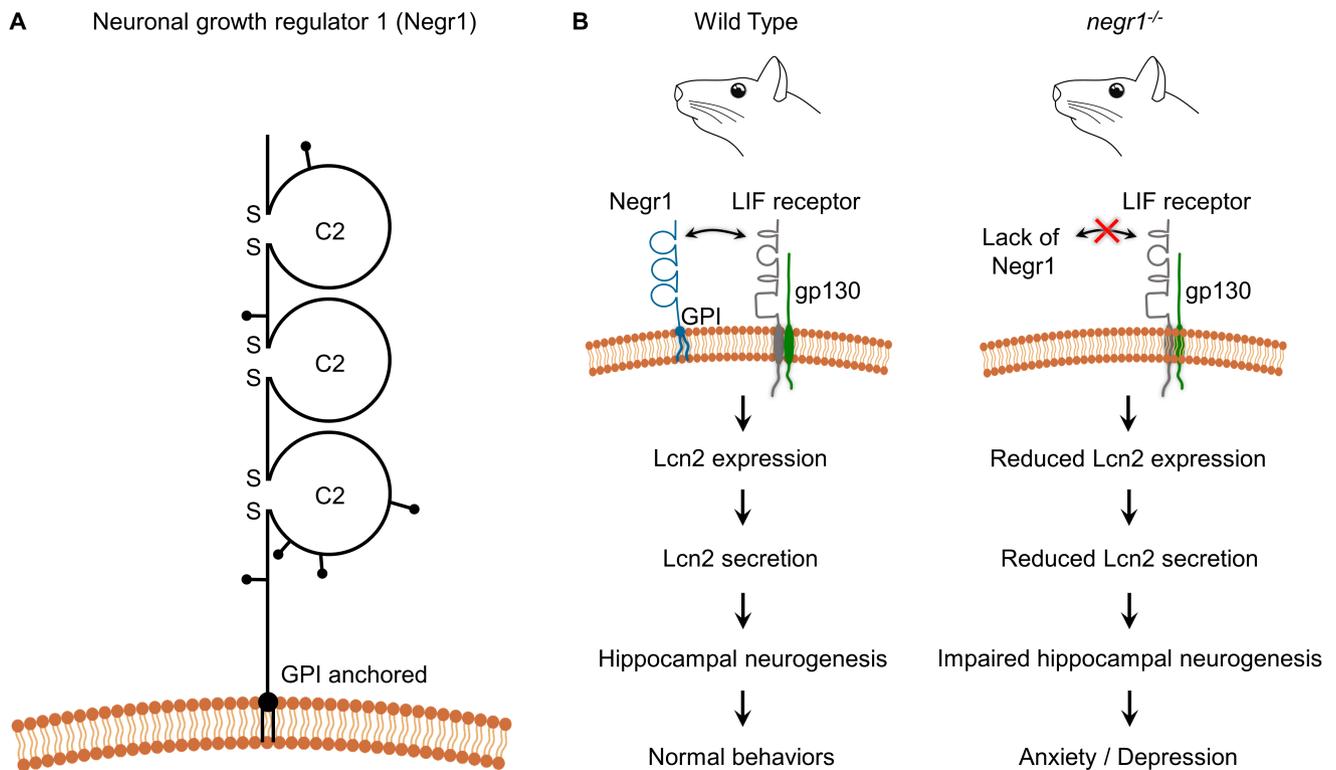
Fig. 1. (A) Structure of Negr1. Loops represent immunoglobulin-like domains. Lines ending with dots are putative *N*-linked glycosylation sites. (B) Schematic illustration of the molecular mechanism underlying the regulatory role of Negr1 in anxiety- and depressive-like behaviors. Negr1 interacts with LIF receptor and potentiates Lcn2 expression. Lcn2, in turn, induces hippocampal neurogenesis in adult mice. In *negr1*-/- mice, impaired adult hippocampal neurogenesis due to reduced Lcn2 expression results in anxiety- and depression-like behaviors.

hippocampus, amygdala, hypothalamus, and olfactory bulb [23]. Moreover, electron microscopic analysis showed specific localization of Negr1 at postsynaptic regions of dendritic synapses in the cerebral cortex and hippocampus of the adult brain [22]. In another study, Negr1 expression was detected in reactive astrocytes, which contribute to neurite outgrowth of hippocampal neurons [21]. These data suggest that Negr1 has distinct functions depending on its cellular and subcellular localization in the brain.

ROLE OF NEGR1 IN NEURONAL SYNAPTOGENESIS AND NEURITE OUTGROWTH

The role of Negr1 in synaptogenesis and neurite outgrowth has been reported mainly in *in vitro* studies. Comparing cultured cortical neurons from wild type and Negr1 knockout (*negr1*-/-) mice, *negr1*-/- cortical neurons showed significantly reduced neurite numbers, length, and branches [23]. It was suggested that Negr1 is shed from the cortical neuronal membrane by metalloproteinase ADAM10 [10], and the soluble form of Negr1 can promote synaptogenesis and neurite outgrowth [24]. Pischedda and Piccoli [24] showed that membrane-released soluble Negr1 promoted

neurite outgrowth by modulating ERK1/2 activation and the fibroblast growth factor receptor 2 (FGFR2) signaling pathway as an underlying mechanism. In cultured hippocampal neurons, Negr1 was detected mainly in the presynaptic axon terminals in the early culture stage (DIV 3-14); however, at late culture stage (DIV21-28), it was detected mainly in the dendritic postsynaptic spine of mature neurons [25]. This suggests that Negr1 expression and subcellular localization are differentially regulated depending on developmental stage of hippocampal neurons. Moreover, heterologous overexpression of *negr1* in hippocampal neurons decreased dendritic synapse number at early culture stages (DIV 3-14), whereas it increased dendritic synapses in late culture stages (DIV 21-28) [25]. Together, these studies argue that Negr1 differentially modulates neural outgrowth depending on developmental stage [25]. The morphology of neuronal dendritic processes is also affected by Negr1 expression [26]. When Negr1 expression was knocked-down by miRNA in cultured cortical neurons at DIV 1, it altered the distribution of neurites in each branching order at a late culture stage (DIV 16) but not at an early culture stage (DIV 6) [26]. Taken together, these results indicate that Negr1 is an important regulator of not only synapse number, but also of maturation

of dendritic processes; moreover, its regulatory effects are dependent on the developing stages of neurons. Direct control by Negr1 of synapse number and neurite outgrowth was also demonstrated in an *in utero* study [27]. When *negr1* expression was silenced in mouse embryonic brain at E15.5 by *in utero* electroporation, dendritic length and number of neurite processes of layer II/III cortical neurons at P7 decreased significantly compared with their controls [27]. These results confirm that Negr1 deficiency prevents normal morphological development of pyramidal neurons *in vivo*.

ROLE OF NEGR1 IN OBESITY

Since the cloning of negr1, the function of this putative synaptic adhesion molecule has been investigated mainly in synaptogenesis and neurite outgrowth. Therefore, it was unexpected when human genetics studies showed Negr1 as a major risk factor for human obesity by genome-wide association studies (GWAS) [28-31]. These studies have been replicated in human subjects of various ethnic groups including Europeans [32-37], Asians [38-40], and African Americans [41, 42], suggesting that common genetic variants of *negr1* increase the risk of obesity in diverse ethnic backgrounds. Moreover, one animal study demonstrated that increased fat mass, enlarged adipose cells, and decreased muscle mass were observed in *negr1*-deficient mice [43], suggesting that Negr1 may serve as a potential drug target that could be exploited for treatment of human obesity. However, there also were some studies that failed to pinpoint Negr1 as an obesity-associated risk factor [44-46]. An obesity risk locus that was identified in obese adults was not related to weight gain of overweight children [44]. Moreover, SNPs of *negr1* identified in a European population were not related to and had no significant effects in a Chinese population [45, 46]. Thus, although most reports indicate Negr1 as an obesity risk factor, further precise investigation is still needed.

Currently, the molecular mechanisms regarding how Negr1 serve as a risk factor for human obesity are not clear. Although an *in vitro* study showed a putative role of Negr1 in lipid metabolism [43], studies using animal models failed to show any mechanism but rather showed conflicting data. For example, one study showed significantly reduced body mass, food intake, and physical activity in *negr1*$^{-/-}$ mice while energy expenditure remained unchanged [47]. In contrast, another study that targeted Negr1 expression in a specific brain region showed an opposite phenotype; blocking Negr1 expression in the periventricular hypothalamus area *in vivo* led to increase in body weight and food intake and reduced locomotion activity [48]. Therefore, it can be speculated that Negr1 expression in the hypothalamus may play a different role in body mass increase compared with that in other brain/body regions.

Therefore, the brain region-specific effects of Negr1 polymorphisms on the pathogenesis of obesity need to be addressed.

ROLE OF NEGR1 IN MAJOR DEPRESSIVE DISORDER (MDD)

Dennis et al. [49] recently reported that Negr1 has effects on brain structure independent of its effects on obesity. By comparing white matter integrity and SNPs of Negr1 in healthy young adults, they concluded that *negr1* was closely associated with low white matter integrity. Considering that precise temporal and spatial expression of CAMs is crucial for development, construction, and maintenance of functional neural connectivity [5, 50, 51], it is anticipated that Negr1 may impact the pathogenesis of brain disorders in addition to obesity [52-54], and recent studies suggest that Negr1 is implicated in major depressive disorder (MDD).

Maccarrone et. al. [55] screened for a disease-specific protein bio-signature in the cerebrospinal fluid (CSF) of MDD patients and found a significant elevation of Negr1 in these patients compared to healthy individuals. The involvement of Negr1 in MDD was also supported by a study screening for putative genetic traits associated with treatment response of selective serotonin reuptake inhibitors (SSRIs) [56] in MDD patients. In this recent cross-trait meta-analysis study, *negr1* was uncovered as a key genetic locus associated with SSRI responsiveness in MDD patients and was implicated in both obesity and SSRI treatment response [56]. This implies that the genetic variant of obesity is closely linked to SSRI treatment efficacy in MDD. In line with this, aberrant expression of synaptic adhesion molecules was proposed as an etiologic factor of depressive disorder [57, 58], and a strong association between SNPs of the limbic system-associated membrane protein (LSAMP) gene and human MDD has been reported [59]. These prior studies suggest that *negr1* may also be involved in psychiatric disorders such as MDD.

To understand the relationship between Negr1 and psychiatric disorders, we used *negr1*$^{-/-}$ mice to test whether Negr1 influences affective behaviors such as anxiety and depression [60]. We found that *negr1*$^{-/-}$ mice exhibit anxiety- and depressive-like behaviors caused by impairment of synaptic transmission of granule cells and neurogenesis in the hippocampal dentate gyrus (DG) [60]. Interestingly, we found that expression of Lipocalin-2 (Lcn2), a 24-kD secretory cytokine [61, 62], is severely compromised in *negr1*$^{-/-}$ mice, and this Lcn2-decrease is responsible for the cellular and behavioral abnormalities observed in *negr1*$^{-/-}$ mice [60]. Specifically, heterologous Lcn2 expression in the DG region of *negr1*$^{-/-}$ mice rescued the abnormal electrophysiological properties of granule cells, impaired hippocampal neurogenesis, and anxiety- and depressive-like behaviors in *negr1*$^{-/-}$ mice [60]. Therefore, our data indicate that the

abnormal affective behaviors observed in *negr1*^{-/-} mice are due to lack of expression of Lcn2, an immune mediator. In our effort to elucidate the molecular mechanisms underlying the functions of Negr1, we discovered that Negr1 directly interacts with leukemia inhibitory factor receptor (LIFR), a co-receptor for LIF [63-65], and thereby potentiates Lcn2 expression [60]. Taken together, our data suggest that Negr1 potentiates LIFR-induced Lcn2 expression and thereby affects hippocampal neurogenesis and affective behaviors (Fig. 1B). In previous reports, reduced hippocampal neurogenesis was observed in Lcn2-deficient mice [66], and these mice showed both anxiety and depressive behaviors [61, 62], which is in line with our findings. Thus far, both cytokines and synaptic adhesion molecules have been shown to play pivotal roles in MDD and anxiety [67, 68]; however, the cross-talk between these two depression/anxiety-mediating pathways has not been reported. Consequently, Negr1's function *via* regulation of the expression of immune mediators is unique compared to other synaptic adhesion molecule functions.

## INVOLVEMENT OF NEGR1 IN OTHER PSYCHIATRIC DISORDERS AND COGNITIVE FUNCTIONS

In addition to MDD, Negr1 has been implicated in other psychiatric disorders and neurological diseases. Several studies have provided evidence that Negr1 is involved in Alzheimer disease (AD) [69, 70], autism spectrum disorder (ASD) [27], and schizophrenia (SCZ) [71]. A GWAS study using patients with comorbid MDD and AD identified *negr1* as a genetic risk factor that affects AD development [70]. These results suggest a possibility that *negr1* serves as a common risk factor for both MDD and AD pathologies. Furthermore, downregulation of Negr1 in mice resulted in impaired behaviors that are similar to those of individuals with ASD [27]. In that study, the authors proposed a putative mechanism by which Negr1-deficiency leads to ASD behavior. According to their proposed mechanism, Negr1 directly interacts with FGFR2 and cooperatively regulates cortical development. Thus, Negr1-deficiency led to impaired cortical development resulting in the ASD phenotype. Moreover, a clinical study with schizophrenic patients showed increased expression levels of Negr1 transcript in the dorsolateral prefrontal cortex of these patients [71]. Taken together, these studies strongly support that the altered expression of Negr1 may be associated with abnormal behaviors of various psychiatric disorders (Table 1).

To address the breadth of Negr1 influence in psychiatric disorders, we subjected our *negr1*^{-/-} mice to a series of cognitive tasks assessing spatial memory, recognition memory, and context fear memory. In the spatial version task of Morris water maze for measuring spatial memory, our *negr1*^{-/-} mice performed worse than wild-type mice in locating the hidden platform (Fig. 2A) while

Table 1. Studies showing putative involvement of Negr1 in psychiatric disorders

Literature	Disease targeted	Subject	Main findings
Singh et al. 2019 [23]	General psychiatric disorders	*negr1*^{-/-} mouse	- Enlarged ventricle. - Decreased number of parvalbumin-positive inhibitory interneurons in hippocampus. - Hyperactivity in social interaction. - Impaired social dominance behavior.
Maccarrone et al. 2013 [55]	Major depressive disorder (MDD), Bipolar disorder, Schizophrenia (SCZ)	Human	- Identification of association of Negr1 as a MDD-specific protein biosignature in cerebrospinal fluid of MDD patient.
Szczurkowska et al. 2018 [27]	Autism spectrum disorder (ASD)	*negr1*^{-/-} mouse	- Impaired ultrasonic vocalization. - Increased latency to respond to thermal stimuli. - Less sniffing, More grooming.
Ni et al. 2018 [70]	Major depressive disorder (MDD), Alzheimer's disease (AD)	Human, mouse	- Identification of Negr1 as a common variants in the MDD GWAS loci with AD. - Reduced mRNA expression in the entorhinal cortex and temporal cortex in human AD patient. - Significant correlation of Negr1 mRNA expression level with both amyloid-β (Aβ) and tau (Tau) burden in AD mouse model.
Karis et al. 2018 [71]	Schizophrenia (SCZ)	Human	- Increased Negr1 transcript level in dorsolateral prefrontal cortex in SCZ patient.
Noh et al. 2019 [60]	Major depressive disorder (MDD)	*negr1*^{-/-} mouse	- Increased anxiety- and depressive-like behavior. - Decreased hippocampal neurogenesis. - Reduced Lipocalin-2 (Lcn2) expression in hippocampus. - Impaired LTP and mEPSC in hippocampal dentate gyrus.
Amare et al. 2018 [56]	Major depressive disorder (MDD)	Human	- Identification of association of Negr1 in SSRI treatment response.
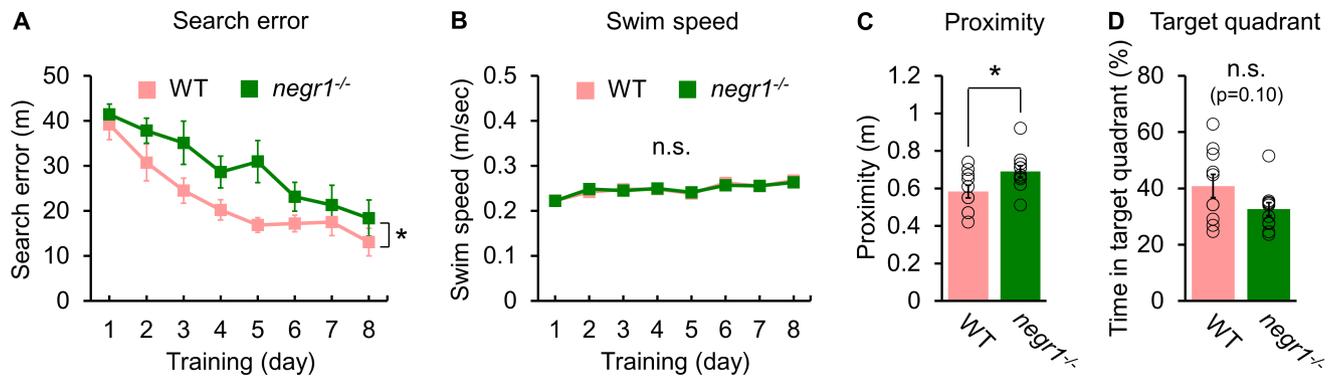
Fig. 2. Spatial memory impairments of *negr1*−/− mice. To assess the spatial memory of the *negr1*−/− mice, the Morris water maze task was conducted. (A) The *negr1*−/− mice were slower in finding the hidden platform (ANOVA with a repeated measure; Group, $F_{(1,19)}$=5.13, p<0.05; Training = $F_{(7,133)}$=17.94, p<0.001, Group x Training, $F_{(7,133)}$=0.96, p=0.46). (B) No differences between the two groups were found in swimming speed during the place training. (C, D) The *negr1*−/− mice exhibited less spatial bias than the wild-type mice on the probe trial (independent *t*-test; proximity, $t_{(19)}$=-2.36, p<0.05; Target quadrant, $t_{(19)}$=1.78, p=0.10). *p<0.05; n.s., not significant. Data are presented as mean±s.e.m.

exhibiting no differences in the swimming speed (Fig. 2B). On the probe trial, the *negr1*−/− mice swam much farther from the hidden platform location than the wild-type mice, as measured by proximity (Fig. 2C). These results indicate hippocampal dysfunction of *negr1*−/− mice because the hippocampus is necessarily recruited for performing the hidden platform version of Morris water maze [72]. Meanwhile, in the novel object recognition, the novel object location, and the contextual fear conditioning task, no differences between *negr1*−/− and wild-type mice were observed, suggesting that Negr1 expression is not critical for recognition memory or contextual fear memory (Fig. 3). It is interesting that, although *negr1*−/− mice exhibited impaired spatial learning and memory, they showed no significant differences in the recognition task or contextual fear conditioning task (Fig. 3). One plausible explanation for these distinct behavioral phenotypes is that Negr1 may differentially regulate each neural circuit. Behavioral performance in the Morris water maze depends primarily on the hippocampus region in which Negr1 is highly expressed. However, novel object recognition and location tests are affected by both the cerebral cortex and hippocampus [73], and neural circuits between the cerebral cortex and amygdala areas are critical regions for performance in the contextual fear conditioning test [74-76]. Brain regional differences in expression levels of Negr1 might have caused different cognitive behavioral performances of *negr1*−/− mice (spatial learning vs. fear learning; Table 2). Therefore, future studies using optogenetics or conditional knockout mice dissecting the brain region-specific roles of Negr1 are necessary to elucidate the roles of Negr1 in other psychiatric disorders.

CONCLUSIONS AND FUTURE DIRECTION

In this short review, we summarized and discussed recent studies on the role of Negr1 in psychiatric disorders. Accumulating evidence based on both human and animal studies support critical roles of Negr1 in psychiatric disorders such as MDD, schizophrenia, and ASD. However, the molecular and cellular mechanisms underlying Negr1's role in these psychiatric disorders remain elusive. Considering the diverse brain expression pattern of Negr1 including hippocampus, sensory cortex, and prefrontal cortex region, future studies using brain region/cell type-specific negr1 conditional knockout mice will be instrumental to elucidate the pathophyisological mechanisms of Negr1 in psychiatric disorders. Concerted effort in the fields of genetics, molecular biology, neuroscience, and clinical psychiatric medicine are also needed in the future to dissect the exact pathophysiologic function of this molecule. Although the mechanisms need to be elucidated, studies have proposed Negr1 as a novel target for treatment of psychiatric disorders. Considering the high comorbidity between depression and obesity, Negr1 may act as a central hub bridging the two diseases. If so, Negr1 can be a critical common target for treatment of individuals with MDD comorbid with obesity.

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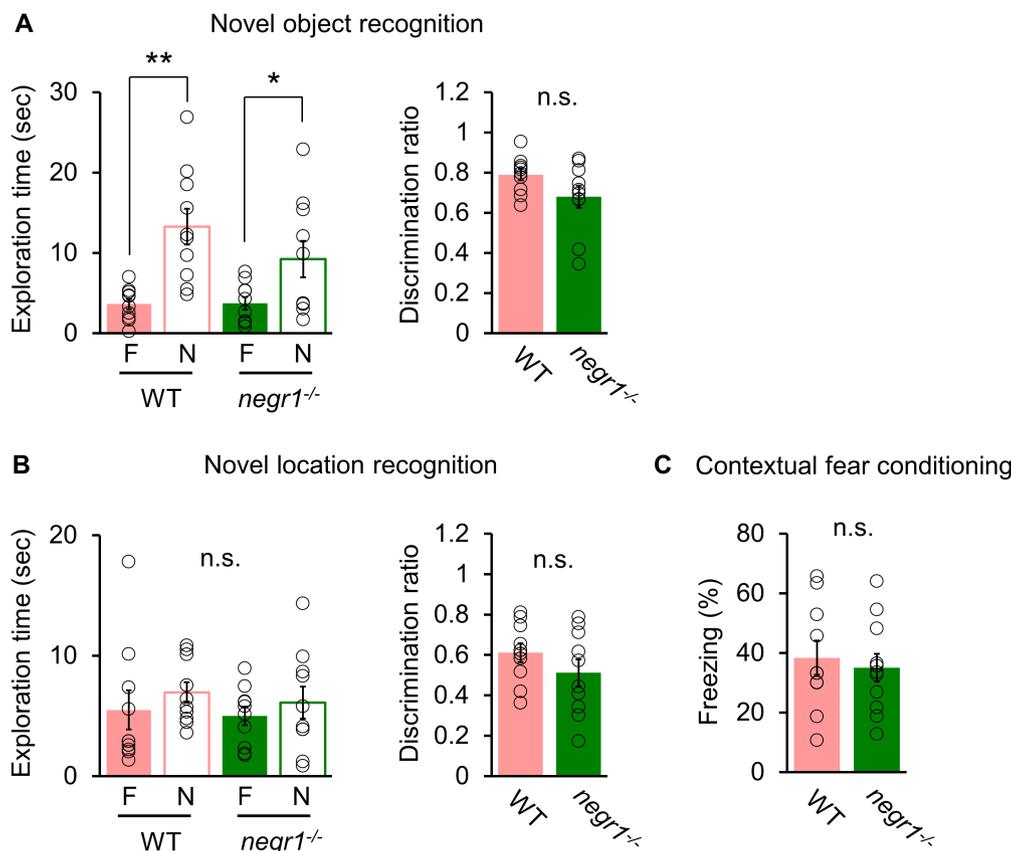
Fig. 3. Performances of the *negr1^-/-* and wild-type mice in the object recognition and the contextual fear conditioning task. Novel object recognition and novel location recognition task were performed to measure the recognition memory of *negr1^-/-* mice. (A, *left*) In the novel object recognition task, both the *negr1^-/-* and wild-type mice preferred the novel object (*N*) to the familiar object (*F*) (paired *t*-test; *negr1^-/-*, $t_{(9)}$=-2.65, p<0.05; wild-type, $t_{(9)}$=-5.06, p<0.05). (A, *right*) No differences between the two groups were found. (B) In the novel location recognition task, no differences between the two groups in the preference of new location and discrimination ratio were observed. (C) In the contextual fear conditioning task, no differences between the two groups in the freezing levels to context were found. *p<0.05; **p<0.01; n.s., not significant. Data are presented as mean±s.e.m.

Table 2. Cognitive phenotypes of *negr1^-/-* mice

Types of memory	Cognitive tasks	Results	Involved brain regions
Spatial memory	Morris water maze	+	Hippocampus
Recognition memory	Novel object recognition	n.s.	Hippocampus, Cerebral cortex
	Novel location recognition	n.s.	
Fear memory	Contextual fear conditioning	n.s.	Cerebral cortex, Amygdala

+, significant behavior; n.s., not significant.

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