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Altered trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptors (AMPARs) in the striatum leads to behavioral changes in emotional responses

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HIGHLIGHTS

- Transient expression of G2CT increases surface expression of AMPARs in the striatum.
- G2CT in the dorsomedial striatum reduces mobility in high-anxiety circumstances.
- G1CT in the dorsomedial striatum transiently alters anxiety-related behavior.

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ABSTRACT

The striatum receives and integrates multiple inputs from diverse areas in the brain and plays a critical role in the regulation of motor activity. However, whether the striatum is involved in the alteration of behavior in the presence of emotional challenges is unknown. Here, we examined whether alterations in the surface expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptors (AMPARs) in the dorsal striatum would affect anxiety-related behaviors. We found that the transient expression of G1CT or G2CT, AMPAR-derived peptides, in the dorsomedial striatum led to decreased mobility in high-anxiety circumstances; however, the expression of these peptides in the dorsolateral striatum did not affect anxiety-related behavior. These data suggest that excitatory connections within the dorsomedial striatum play important roles in the control of motor actions in the presence of emotional challenges.

1. Introduction

Glutamatergic synapses are the major excitatory connections in the brain. α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors (AMPARs) at the glutamatergic synapses are particularly important for fast synaptic transmission. The regulation of AMPARs is one of the key mechanisms of synaptic plasticity, which is believed to be the major cellular mechanism that mediates learning and memory. We have previously used AMPAR-derived peptides, termed G1CT and G2CT, to interfere with AMPAR trafficking in vivo [10,22]. G2CT was designed to disrupt the interaction between AMPARs and the AP2 clathrin adaptor complex in mice [13]. The transient expression of G2CT has been shown to block NMDA-stimulated AMPAR endocytosis in dissociated cortical neurons and long-term depression (LTD) in layer 4 of the primary visual cortex [22]. In contrast, G1CT has been demonstrated to interfere with the insertion of GluA1-containing AMPARs into synaptic surface [16], thereby restricting experience-dependent cortical long-term potentiation (LTP) [6,22]. These data indicated that G1CT and G2CT could be effective tools for modulating the plasticity of the excitatory synapses.

Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptors; DMS, dorsomedial striatum; DLS, dorsolateral striatum; EPM, elevated plus maze; FST, forced swim test; HSV, herpes simplex virus; NAc, nucleus accumbens; OFT, open field test.

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The striatum, which integrates multiple inputs from many areas of the brain, is the main input nucleus of the basal ganglia. As the basal ganglia provide outputs to brain systems that are involved in the generation of behavior, such as the thalamus, the superior colliculus, the pedunculopontine nucleus, and the hypothalamic systems, the basal ganglia have been suggested to play important roles in behavioral controls. Furthermore, many research findings suggest that the basal ganglia play roles in action selection and decision-making [2,18]. That is, the basal ganglia determine which of the numerous inputs that contain competing action plans should survive, presumably by integrating sensorimotor, cognitive, motivational, and emotional information [9]. While motivational regulation of the basal ganglia function has been extensively studied [5,8,12], how emotional challenges affect the function of the striatum has attracted little attention.

Here, we examined whether G1CT or G2CT expression within the striatum affected behavioral responses in mouse models of anxiety. We expressed G1CT or G2CT in the striatum via herpes simplex viral vector (HSV)-mediated gene transfer and examined the resulting behavioral alterations. Our data suggest that glutamatergic neurotransmissions in the striatum, the dorsomedial striatum (DMS) rather than the dorsolateral striatum (DLS), play a critical role in the regulation of behavioral responses to emotional challenges and that the aberrant alterations of striatal synapses may be responsible for certain aspects of mood disorders.

2. Materials and methods

2.1. Animals

Male C57BL/6N mice were purchased from Orient Bio (Seoul, Korea) and housed (4 mice/cage) in the Korea University animal facility under standard conditions (12:12 h light–dark cycles, 23 ± 1 °C. The animals were approximately 9–10 weeks old (20–22 g) at the beginning of the experiments. The experimental protocols used in this study were approved by the Laboratory Animal Care and Use Committee of Korea University.

2.2. Behavioral analyses

All experiments were performed under dim lighting conditions (10 lx). All test sessions were video recorded and analyzed using video tracking software (Ethovision XT 7.1, Noldus).

2.2.1. Elevated plus maze (EPM) test

Each arm of our maze was 67 cm long and 7 cm wide, with 15 cm-high walls around the closed arms. The maze was elevated 55 cm above the ground. The mice were habituated in a soundproof experimental chamber for 1 h prior to the test. The mice were placed in the center area (i.e., the intersection between the open and closed arms) with their heads facing an open arm and were allowed to explore the maze for 5 min.

2.2.2. Open field test (OFT)

The animals were habituated in a soundproof experimental chamber for 20 min before they were placed in the center of an open acrylic box (30 cm × 30 cm × 30 cm). The mice were allowed to move freely while being video recorded for 1 h. The central square area of the box (16% of the entire area of the box) was defined as the center zone.

2.2.3. Mobility analysis

The maximum cutoff for a score of immobility was 0.9%, and the minimum cutoff for a high mobility score was 15%; thus, if fewer than 0.9% (or more than 15%) of the pixels changed between consecutive video frames, an immobility (or high mobility) event was recorded. Our sampling rate was 3 frames/s.

2.3. Stereotaxic virus injections

Stereotaxic virus injections were performed as previously described [10]. The mice were anesthetized with a ketamine (400 mg/kg, Yuhan Corporation)/xylazine (80 mg/kg, BAYER) solution. The virus was injected through glass micropipettes using the Nanoliter 2000 (World Precision Instrument). The following stereotaxic coordinates were used: anteroposterior = 1.1, mediolateral = ±1.1 (for DMS), ±1.8 (for DLS), dorsoventral = −3.0, −2.5, −2.0. The total volume of each viral injection was 230 nl, and the virus was injected at a rate of 23.0 nl/s (23.0 nl per each of 10 injections with a 20-s inter-injection interval).

2.4. Slice biotinylation assay

Slice biotinylation assay was conducted as described with minor modifications [22]. Briefly, the brain of mice that had been injected with viral vectors 3 days before was rapidly removed and transferred to ice-cold dissection buffer supplemented with 1 μM Latrunculin A (Enzo Life Science), constantly carbogenated (5% CO_2 and 95% O_2). Coronal slices (300-μm-thick) were prepared and incubated for recovery in carbogenated artificial cerebrospinal fluid (ACSF) supplemented with 1 μM Latrunculin A at 30 °C for 2 h. The brain slices were then incubated with 1 mg/ml sulfo-NHS-LC-biotin (Thermo Scientific) in ACSF for 20 min on ice and then washed 3 times (1× with TBS, 2× with cold ACSF). The infected areas were microdissected under a fluorescence microscope and homogenized in RIPA buffer supplemented with a protease inhibitor cocktail (Calbiochem). The homogenate was centrifuged (14,000 × *g*) for 15 min at 4 °C, and the supernatant was collected. After the protein concentration of the supernatant was determined, 60 μg of the protein sample was mixed with Neutravidin (60 μl; Thermo Scientific) and incubated at 4 °C overnight. The following day, the biotinylated protein–avidin complex was washed (3× with RIPA), resuspended in SDS sample buffer, and boiled for 10 min. The total protein (avidin-unbound) and pull-down protein (avidin-bound) samples were used for immunoblotting assay (GluA1, Millipore, AB1504; GluA2, Chemicon, MAB397; NCAM, Millipore, AB5032). The surface levels of AMPARs were normalized to the level of the neural cell adhesion molecule (NCAM).

2.5. Quantification of HSV infection

We quantified the overall fluorescence intensity from the infected area to compare relative infection rate in each mouse. A region that includes the whole striatum was set and used to measure total fluorescence intensity from each slice. In order to compensate for slide-to-slide variation, fluorescence intensity from non-injected area was used for normalization. Quantification was performed using ImageJ v.1.46.

2.6. Statistical analyses

All statistical analyses were performed using the SPSS 12.0.1 software (IBM). We used Wilcoxon signed-rank tests for the statistical comparisons of the immunoblot assays, and one-way ANOVAs with LSD post hoc tests and Mann–Whitney test for the other behavioral tests. $p < 0.05$ was considered statistically significant. The data are expressed as the mean ± the SEM.