Lateral Diffusion of the GABA\textsubscript{B2} Receptor Is Regulated by the GABA\textsubscript{B2} C Terminus*\S

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GABA\textsubscript{B} (\gamma-aminobutyric acid, type B) is a heterodimeric G-protein-coupled receptor. The GABA\textsubscript{B1} subunit, which contains an endoplasmic reticulum retention sequence, is only transported to the cell surface when it is associated with the GABA\textsubscript{B2} subunit. Fluorescence recovery after photobleaching studies in transfected COS-7 cells and hippocampal neurons revealed that GABA\textsubscript{B2} diffuses slowly within the plasma membrane whether expressed alone or with the GABA\textsubscript{B1} subunit. Treatment of cells with brefeldin A revealed that GABA\textsubscript{B2} moves freely within the endoplasmic reticulum, suggesting that slow movement of GABA\textsubscript{B2} is a result of its plasma membrane insertion. Disruption of the cytoskeleton did not affect the mobility of GABA\textsubscript{B2}, indicating that its restricted diffusion is not due to direct interactions with actin or tubulin. To determine whether the C terminus of GABA\textsubscript{B2} regulates its diffusion, this region of the subunit was attached to the lymphocyte membrane protein, CD2, which then exhibited a slower rate of lateral diffusion. Furthermore, co-expression of a cytoplasmically expressed soluble form of the GABA\textsubscript{B2} C terminus increased movement of the GABA\textsubscript{B2} subunit. We constructed forms of GABA\textsubscript{B2} with various C-terminal truncations. Truncation of GABA\textsubscript{B2} after residue 862, but not residue 886, caused a dramatic increase in its mobility, suggesting that the region between these two residues is critical for restricting GABA\textsubscript{B2} diffusion. Finally, we investigated whether activation of GABA\textsubscript{B} might modulate its movement. Treatment of COS-7 cells with the GABA\textsubscript{B} receptor agonist baclofen significantly increased its mobile fraction. These data show that the restricted movement of GABA\textsubscript{B} at the cell surface is regulated by a region within its C terminus.

GABA\textsubscript{B} receptors are metabotropic receptors for the inhibitory neurotransmitter \(\gamma\)-aminobutyric acid (GABA).\textsuperscript{2} Pre- and post-synaptic GABA\textsubscript{B} receptors are coupled to inhibitory G-proteins and can regulate neurotransmission via several mechanisms, including modulation of adenyl cyclase (1), inhibition of voltage-gated Ca\textsuperscript{2+} channels (2), and modulation of K\textsuperscript{+} channels (3, 4). Formation of a functional receptor requires the heterodimerization of two subunits, GABA\textsubscript{B1} and GABA\textsubscript{B2} (5). Previous work has demonstrated that the stable assembly of these subunits occurs, to some extent, via association of coiled-coil domains within their C termini (6). The subunits appear to serve different functions within the fully formed receptor. GABA\textsubscript{B1} contains the agonist binding site on its large extracellular N terminus, and the affinity of this site for agonists is increased following heterodimerization with GABA\textsubscript{B2} (7). The GABA\textsubscript{B2} subunit contains intracellular loops that couple the receptor to the G-protein (8–10).

Heterodimerization of the GABA\textsubscript{B} subunits is important not only for proper receptor function but also for forward trafficking of the receptor to the cell surface (5, 7). In the absence of GABA\textsubscript{B2}, the GABA\textsubscript{B} subunit is retained within the endoplasmic reticulum due to the presence of a C-terminal RSRR retention motif on its C terminus. The interaction of GABA\textsubscript{B2} with GABA\textsubscript{B1} apparently masks this motif, allowing the fully formed receptor to traffic to the cell surface, where it may be targeted to the synapse.

The number of neurotransmitter receptors within post-synaptic membranes is dependent not only on insertion of new receptors but also on lateral diffusion of extrasynaptic receptors into the synaptic compartment (11). Regulation of receptor movement within the plasma membrane is therefore likely to be important for plasticity at individual synapses. At excitatory synapses, lateral diffusion of \(\alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors has been suggested to regulate synaptic strength following either long-term potentiation or long-term depression (12–14). Furthermore, \(N\)-methyl-D-aspartate receptors readily exchange between synapt and extrasynaptic compartments via movement within the plasma membrane (15). However, the behavior of inhibitory neurotransmitter receptors is less well understood. The dynamics of the glycine receptor (16) and the ionotropic GABA\textsubscript{A} receptor (17) have recently been examined. Extrasynaptically, both receptors diffuse freely, but within the synaptic compartment interactions with the synaptic scaffolding protein gephyrin significantly slow their movement. To date, however, membrane dynamics of the GABA\textsubscript{B} receptor have yet to be investigated.

In the present study we explored the movement of the GABA\textsubscript{B} receptor within the plasma membrane. We found lateral diffusion of GABA\textsubscript{B} at the cell surface to be slow, due to restricted mobility of the GABA\textsubscript{B2} subunit. Disruption of the cytoskeleton did not affect GABA\textsubscript{B2} diffusion; however, truncation of the GABA\textsubscript{B2} C-terminal region allowed GABA\textsubscript{B2} to diffuse more rapidly. We show, therefore, that GABA\textsubscript{B} exhibits
**Diffusion of GABA<sub>B</sub> Receptor**

**A.** GABA<sub>B2</sub>-YFP and CD2-YFP

**B.**

![frap-graph](image)

**C.**

![frap-graph](image)

**FIGURE 1.** GABA<sub>B</sub> diffuses slowly at the cell surface. **A,** COS-7 cells were transfected with either GABA<sub>B2</sub>-YFP (top) or CD2-YFP (bottom). A circular region (arrow) was bleached with a high intensity laser, and recovery of the YFP signal was imaged over time. **B,** diffusion of GABA<sub>B2</sub>-YFP into the bleached region is very slow, whether expressed alone or together with the GABA<sub>B1b</sub> subunit. **C,** COS-7 cells expressing GABA<sub>B2</sub>-YFP were treated with brefeldin A, which causes retention of proteins in the endoplasmic reticulum. Within the endoplasmic reticulum GABA<sub>B2</sub>-YFP diffused rapidly, indicating that GABA<sub>B2</sub> only exhibits restricted movement within the plasma membrane.

distinct cell surface dynamics that can be regulated by a region within the C terminus of the GABA<sub>B2</sub> subunit.

**MATERIALS AND METHODS**

**Cell Culture and Transfection**—COS-7 cells were maintained in minimal essential medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin and incubated at 37 °C, 5% CO<sub>2</sub>. For experiments, cells were seeded onto glass coverslips and transfected 24 h later using JetPEI (Autogen Bio-chem, Calne, UK) according to the manufacturer's instructions. Cells were incubated for 48 h post-transfection before FRAP (fluorescence recovery after photobleaching) analysis. Primary neuron cultures were prepared from E18 Sprague-Dawley rat embryos. Briefly, dissected hippocampi were mechanically dissociated in Hanks' balanced salt solution lacking calcium and magnesium, supplemented with 1 mM pyruvate and 10 mM HEPE. Cells were plated immediately onto glass coverslips coated with poly-D-lysine (5 μg/ml) and cultured in Neurobasal medium containing 2% B-27, 0.5 mM l-glutamine, 25 μM glutamate, 0.05% gentamicin. To restrict the proliferation of non-neuronal cells, after 4 days the medium was changed to Neurobasal medium supplemented with 2% B27, 0.5 mM l-glutamine, 3 μM cytosine arabinofuranoside, 0.05% gentamicin. Neurons were transfected with Lipofectamine (Invitrogen) 5 days in vitro according to the manufacturer's instructions and imaged 48 h later. Approximately 1–3% of cells were transfected per coverslip; 8–10 dendrites from distinct cells were analyzed in each independent experiment.

**Plasmids**—HA-tagged GABA<sub>B2</sub> and Myc-tagged GABA<sub>B1b</sub> were obtained from GlaxoSmithKline, as were the GABA<sub>B1b</sub>-YFP and GABA<sub>B2</sub>-YFP and their CFP derivatives. All GABA<sub>B2</sub> truncations and chimeras were generated by PCR from the HA-tagged construct in pCDNA3.1(-). The truncated forms of GABA<sub>B2</sub> were produced by excising the EcoRV/HindIII fragment from the HA-tagged GABA<sub>B2</sub> in pCDNA3.1(-). The different regions of interest were obtained using polymerase chain reaction (PCR) to amplify them using the common forward primer 5'-GCA GGA CGG GAT ATC TCC ATC CGC CCT TCT CTC C-3' that covers the EcoRV site positioned in the third extracellular loop of GABA<sub>B2</sub>. The reverse primers covered the C terminus from amino acids 920, 886, 862, or 841 and contained codons at these positions that permitted ligation in-frame into eYFP-N1 (Clontech).

These were as follows: GABA<sub>B2</sub>-Δ920, 5'-GCT AAG CCT GAC GCA GGG GCT GAC ACA GCT GGC-3'; GABA<sub>B2</sub>-Δ886, 5'-GCT AAG CCT TGG GAG AGT TTA TAT CTT CTA TAC G-3'; GABA<sub>B2</sub>-Δ862, 5'-TGT GTT CCA CTG AAG CTT GGG ATT TTG ATC GAG-3'; GABA<sub>B2</sub>-Δ841, 5'-GCA AGC TTT CCC AGG TTG AGG ATG TCA TTG AGC-3'. The PCR products were then ligated into the EcoRV/HindIII-digested HA-tagged GABA<sub>B2</sub> to produce the protein of interest. For production of the YFP-tagged truncated forms of GABA<sub>B2</sub> the plasmids containing the ligated PCR products were excised from pCDNA3.1 using Nhel and HindIII, and this fragment was then ligated into Nhel/HindIII-digested eYFP-N1. The plasmid containing CD2 fused to the C-terminal tail of mGluR1α previously described (18) was digested with BamHI and NotI to remove the mGluR1α sequence. YFP (Clontech) was amplified by PCR with the primers 5'-CGC TGG AGG ATG TTA TAT CTT CTA TAC G-3' and 5'-GCA GGA CGG GAT ATC TCC ATC CGC CCT TCT CTC C-3' that covers the EcoRV site positioned in the third extracellular loop of GABA<sub>B2</sub>. The reverse primers covered the C terminus from amino acids 920, 886, 862, or 841 and contained codons at these positions that permitted ligation in-frame into eYFP-N1 (Clontech).
CTC GAG ACC CTG AGA ACA AAC-3’ and 5’-CCA CGG ATC CAG GCC CGA GAC CAT GAC TCG-3’, after digestion with the same enzymes, to give CD2-R2-YFP. This contained the N-terminal and transmembrane domain of CD2 followed by the C terminus of the subunit that could be detected in cells, the C terminus of the subunit was amplified by PCR using the primers 5’-CTC ATC ACC CTG AGA TCT AAC CCA GAT GCA GC-3’ and 5’-CGT ATC TAG ATT ACA GGC CCG AGA CCA TGA CTC G-3’. The product was digested with BglII and XbaI and the product inserted into sim-ilarly digested pECFP-C1 (Clontech). All PCR reactions were carried out using the proofreading KOD polymerase (Invitrogen) and DNA alter-

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before being bleached by 15 cycles at 90% maximal laser power. The fluo-

crescence intensity of the whole cell was captured for 2 min at 1% laser power; recovery is presented as percentage of original fluorescence, cor-

rected for any bleaching due to repet-

tive scanning. To examine the effects of brefeldin A (5 μg/ml; Sigma-Al-

drich), baclofen (100 μM; Sigma), lact-

runculin (5 μM; Sigma), or colchicine (5 M; Sigma), the cells were incu-

bated with the appropriate com-

pound at 37 °C for 1 h prior to FRAP analysis. For antagonist treatments, cells were incubated with the GABA<sub>B</sub> antagonist CGP-54626 (4.2 μM; To-

cris Cookson, Ellisville, MO) for 10 min and then co-treated with antago-

nist (4.2 μM) and baclofen (100 μM) for 1 h prior to FRAP analysis. For treat-

ment with antagonist only, cells were incubated with 4.2 μM CGP-54626 for 1 h.

For diffusion analysis of HA-tagged proteins, we incubated COS-7 cells expressing these constructs with an anti-HA antibody (kindly provided by GlaxoSmithKline) conjugated with the fluorophore Alexa 488 (Alexa Fluor 488 Protein Labeling kit; Molecular Probes, Eugene, OR) for 20 min at 37 °C. The cells were washed twice with Hanks’ balanced

saline solution and then imaged as described above, except using an Argon 488 laser instead of the 514 laser.

Data analyses were performed using Igor Pro 5.05 software

(Wavometrics, Lake Oswego, OR) with FRAP plug-in written by K. Miura (EMBL Heidelberg, Germany). Data are presented as means ± S.E., representing at least three independent exper-

iments/group, each group containing a minimum of 10 cells. For the region of interest of each cell, the fluorescence recovery curve was best fitted with a double exponential function with Phair normalization (20). The mobile fraction and time to half-

maximal recovery (t<sub>1/2</sub> maximal) was calculated from each curve.

Immunocytochemistry—Following the transfection period, COS-7 cells were treated for 1 h at 37 °C with either lactruncu-

lin, to block actin polymerization, or colchicine, to block tubu-

lin polymerization. After treatment, cells were washed once with PBS, fixed for 5 min at in 4% paraformaldehyde at room

temperature, and then washed twice with PBS and twice with Tris-saline. To detect intracellular proteins, cells were perme-

abilized with 0.2% Triton X-100 for 5 min. Cells were incubated for 1 h at room temperature in blocking solution containing 1% bovine serum albumin and 1% normal goat serum. To visualize actin, cells were incubated in PBS containing fluorescein iso-
thiocyanate-labeled phalloidin (Molecular Probes) for 1 h, washed with PBS, and mounted on slides. For tubulin detection,
the cells were incubated for 2 h at room temperature in PBS containing mouse anti-β-tubulin (1:1000; Sigma), washed three times with PBS, and incubated for 1 h with anti-mouse ALEXA 546 (Molecular Probes). After three washes with PBS, the coverslips were mounted and viewed with a Zeiss LSM510 inverted confocal microscope with a ×40 oil objective.

RESULTS

GABA<sub>B</sub> Diffuses Slowly within the Plasma Membrane, as Measured by FRAP Analysis—To determine the diffusional mobility of GABA<sub>B<sub>2</sub> at the cell surface, we performed FRAP experiments on COS-7 cells transiently transfected with a YFP-tagged GABA<sub>B<sub>2</sub> construct. We monitored subunit movement within the plasma membrane by focusing the laser excitation at the cell surface. Fluorescent proteins within a defined region of the cell were photobleached by high intensity laser, and the diffusion of unbleached proteins into the bleached region was monitored for 2 min. GABA<sub>B<sub>2</sub> diffused very slowly at the cell surface compared with the diffusion rate of YFP-tagged CD2, a membrane-targeted protein known to freely move within the plasma membrane (Fig. 1A). To examine the mobility of the GABA<sub>B</sub> receptor, cells were co-transfected with GABA<sub>B<sub>1b</sub>-CFP and GABA<sub>B<sub>2</sub>-YFP. In the absence of GABA<sub>B<sub>2</sub>, GABA<sub>B<sub>1b</sub> did not reach the cell surface (data not shown). Movement of the GABA<sub>B<sub>1b</sub>-GABA<sub>B<sub>2</sub> heterodimer was similar to the diffusion rate of GABA<sub>B<sub>2</sub> (Fig. 1B), suggesting that the restricted movement of GABA<sub>B</sub> is limited by the diffusion of the GABA<sub>B<sub>2</sub> subunit. Slow diffusion of GABA<sub>B<sub>2</sub> is specific to the plasma membrane, because retention of GABA<sub>B<sub>2</sub> in the endoplasmic reticulum following brefeldin A treatment increased its diffusion rate (Fig. 1C). To confirm that the YFP tag was not affecting subunit diffusion, we also analyzed movement of HA-GABA<sub>B<sub>2</sub> by live labeling of surface receptors with fluorescein isothiocyanate-labeled antibodies directed against HA (supplemental Fig. S1). No difference in mobility was detected between YFP-tagged GABA<sub>B<sub>2</sub> and HA-tagged GABA<sub>B<sub>2</sub>. Diffusion of GABA<sub>B<sub>2</sub> was also restricted in plasma membrane of cultured rat hippocampal neurons. As expected, transfection of primary neurons using Lipofectamine yielded transfection rates of ~1–3%. FRAP analysis of neurons transiently transfected with either GABA<sub>B<sub>2</sub>-YFP or CD2-YFP revealed that movement of GABA<sub>B<sub>2</sub> was constrained within dendrites relative to movement of CD2-YFP (Figs. 2, A and B). The mobility of GABA<sub>B<sub>2</sub>-YFP was not affected by co-expression with GABA<sub>B<sub>1</sub> (Fig. 2C).

Disruption of the Cytoskeleton Does Not Significantly Affect GABA<sub>B<sub>2</sub> Movement—Previous studies investigating receptor movement suggest that receptors may interact directly or indi-
FRAP analysis of COS-7 cells expressing either this construct or CD2-YFP revealed that the mobile fraction of CD2-R2C-YFP was significantly (p < 0.001) smaller compared with the mobile fraction of CD2-YFP (52.82 ± 2.63 versus 80.63% ± 1.43) (Fig. 4, A and B). Conversely, CD2-R2C-YFP took significantly (p < 0.05) more time to reach half-maximal (t½ maximal) recovery than CD2-YFP (9.04 ± 1.42 versus 4.99 s ± 0.41 s) (Fig. 4B). CD2-R2C-YFP also diffused significantly more slowly than CD2-YFP in the dendrites of cultured hippocampal neurons (supplemental Fig. S2). Next, we constructed a soluble version of the GABA_B2 C terminus. Co-expression of COS-7 cells with GABA_B2-YFP and soluble R2C significantly (p < 0.001) increased the mobile fraction of GABA_B2-YFP relative to GABA_B2-YFP expressed alone (72.01 ± 3.14 versus 47.53% ± 3.88) and decreased t½ maximal recovery (24.28 ± 5.01 versus 53.23 s ± 5.96 s) (Fig. 4, C and D). These data suggest that the GABA_B2 C terminus regulates diffusion of GABA_B at the cell surface.

**Lateral Diffusion of GABA_B Is Regulated by a 24-Amino Acid Region within the C Terminus**—To determine which region of the GABA_B2 C terminus might be involved in regulating its lateral diffusion rate, we constructed a series of C-terminal-truncated forms of GABA_B2 with truncations at residues 841, 862, 886, or 920 (Fig. 5A). We then transfected COS-7 cells with one of the following constructs: GABA_B2-YFP, Δ920-YFP, Δ886-YFP, Δ862-YFP, or Δ841-YFP. Although plasma membrane diffusion rates of Δ920-YFP and Δ886-YFP were similar to the diffusion of GABA_B2-YFP, both Δ862-YFP and Δ841-YFP exhibited faster movement as measured by FRAP analysis than GABA_B2-YFP (Fig. 5B). The mobile fraction of Δ862-YFP was significantly (p < 0.01) higher compared with the mobile fractions of either GABA_B2-YFP or Δ886-YFP (67.91 ± 7.74 versus 43.24% ± 3.21 or 43.15% ± 4.39) (Fig. 5C). Correspondingly, the t½ maximal recovery of Δ862-YFP was also significantly reduced relative to recovery of either GABA_B2-YFP or Δ886-YFP (7.80 ± 0.55 versus 47.47 ± 4.69 or 52.89 s ± 4.69 s) (Fig. 5C). Co-expression of GABA_B1-YFP with Δ841, compared with co-expression with GABA_B2 significantly increased its mobile fraction (71.62 ± 3.23 versus 48.83% ± 2.72) and decreased its t½ maximal recovery (15.6 ± 4.32 versus 31.15 s ± 4.26 s) (Fig. 5D). These data suggest that diffusion of GABA_B1 is therefore regulated by the GABA_B2 subunit. Thus, the region of the GABA_B2 C terminus between amino acids 862–886 may be important for regulating mobility of GABA_B at the cell surface.
Agonist Binding Increases Lateral Diffusion of GABA_B2—To determine whether lateral diffusion of GABA_B2 could be dynamically regulated, we treated COS-7 cells co-transfected with GABA_B1b-CFP and GABA_B2-YFP with the GABA_B agonist baclofen. After 1 h of baclofen treatment (100 µM), cells were subjected to FRAP analysis of GABA_B2-YFP. Treatment with baclofen increased lateral diffusion of GABA_B2-YFP (Fig. 6A) and significantly (p < 0.001) increased its mobile fraction relative to untreated controls (63.49 ± 6.92% versus 39.62% ± 2.47) (Fig. 6B). Co-treatment of cells with the GABA_B antagonist CGP-54626 (4.2 µM) prevented the effect of baclofen on the mobile fraction (35.69% ± 4.77; p < 0.01); treatment with the antagonist alone had no effect on the mobile fraction relative to the control group (Fig. 6B). These results suggest that, although GABA_B2 movement is restricted under basal conditions, stimulation of the receptor might alter its membrane dynamics.

DISCUSSION

In the present study, we examined lateral diffusion of the GABA_B receptor within the plasma membrane using FRAP analysis of fluorescently tagged GABA_B subunits. We found that the GABA_B receptor and the GABA_B2 subunit diffuse slowly at the cell surface in both hippocampal neurons and heterologous cells. Our data suggest that the restricted diffusion of the receptor is regulated by a region within the C terminus of the GABA_B2 subunit.

The diffusion characteristics of GABA_B differ from those reported for other neurotransmitter receptors. We consistently found GABA_B to exhibit a mobile fraction of ~40%. By contrast, AMPA receptors are reported to have a mobile fraction of >80% both in COS-7 cells (27) and in extrasynaptic regions of neurons (13). Similarly, the GABA_A receptor has been shown to diffuse rapidly within the extrasynaptic membrane (28). Rapid diffusion is not restricted to receptor ion channels, as the G-protein-coupled serotonin 5-HT1a (29) and dopamine D1 (30) receptors also move freely in extrasynaptic membrane. Taken together, the data indicate that GABA_B receptors exhibit distinctive diffusion dynamics.

To assess the regulation of GABA_B diffusion, we examined the effect of disrupting the cytoskeleton on receptor movement. We observed that the restricted diffusion of GABA_B2 was not altered following latrunculin or colchicine treatment. In contrast, disruption of microtubules increases the diffusion rate of glycine receptors (28) and disturbance of the actin cytoskeleton by latrunculin has been shown to disrupt AMPA clustering due to interaction between the GluR1 subunit and the actin complex (21–23). Our data indicate that direct interactions between GABA_B2 and either actin or tubulin are unlikely to be responsible for anchoring GABA_B at the cell surface. Similarly, diffusion of the GABA_A receptor appears to be unaffected by latrunculin treatment (31) and diffusion of the R2-YFP as it exhibits a larger mobile fraction and a shorter time to half-maximal recovery. D, GABA_A-YFP displays a significantly higher mobile fraction and significantly lower time to half-maximal recovery when co-expressed with ∆841 than when co-expressed with GABA_B2. N, N terminus; C, C terminus; CCD, coiled-coil domain; TMD, seven-transmembrane domain; R2, GABA_B2.

Values represent means ± S.E.; *, p < 0.05; **, p < 0.01; ***, p < 0.001 versus R2 and ∆886 groups.

**FIGURE 5.** The region of GABA_B2 between residues 862 and 886 regulates mobility of the subunit. A, schematic diagram of the GABA_B2 subunit constructs. Truncations of the subunit were made at the indicated amino acid residues. The YFP tag was placed at the C terminus of each construct. B, ∆862-YFP and ∆841-YFP diffused rapidly within the plasma membrane of COS-7 cells compared with diffusion of R2-YFP, ∆920-YFP, and ∆886-YFP. C, the ∆862-YFP construct is significantly more mobile than either ∆886-YFP or

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We investigated whether stimulation of GABA B might affect receptor movement, as previous studies have demonstrated that movement of other metabotropic receptors, such as the serotonin 5-HT1a receptor, is greater following agonist stimulation (29, 34). We treated cells expressing GABA B1 and GABA B2 with the agonist baclofen and found that baclofen stimulation significantly increased its diffusion rate; the effect of baclofen was prevented by co-treatment with a GABA B antagonist. Agonist binding may induce a conformational change in the receptor that alters its interactions with intracellular scaffolding proteins. Our data suggest a mechanism by which release of neurotransmitter into the synapse might increase diffusion of nearby extrasynaptic receptors, thereby allowing movement into the post-synaptic density.

Regulation of receptor diffusion, and therefore receptor number, is crucial for maintaining synaptic strength. Recently, the dynamics of several neurotransmitter receptors, including AMPA, GABA A, and glycine, have been described (13, 27, 28, 35). In the present study we have demonstrated for the first time that GABA B exhibits restricted movement within the plasma membrane; furthermore, we determined that diffusion of GABA B is regulated by a specific region within the C terminus of the GABA B subunit. These findings provide insight into the regulation of GABA B receptor movement at the cell surface and are consistent with the hypothesis that regulated lateral diffusion is a mechanism by which the cell controls synaptic strength.

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