Chronic Treatment of C6 Glioma Cells with Antidepressant Drugs Results in a Redistribution of Gsα

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ABSTRACT

Previous studies have demonstrated that chronic treatment of C6 glioma cells with the antidepressants desipramine and fluoxetine increases the Triton X-100 solubility of the G protein Gsα (Toki et al., 1999). The antidepressants also caused a 50% decrease in the amount of Gsα localized to caveolae-enriched membrane domains. In this study, laser scanning confocal microscopy reveals that Gsα is localized to the plasma membrane as well as the cytosol in both treated and control cells. However, striking differences are seen in the distribution of Gsα in the long cellular processes after chronic treatment with these antidepressant drugs. Control cells display Gsα along the entire process with an especially high concentration of that G protein at the distal ends. Desipramine- or fluoxetine-treated cells show a more centralized clustering of Gsα in the Golgi region of the cell and a drastic reduction of Gsα in the cellular processes. There is no change in the distribution of Goα after desipramine treatment and the antipsychotic drug chlorpromazine does not alter Gsα. These results suggest that antidepressant-induced changes in the association of Gsα with the plasma membrane may translate into altered cellular localization of this signal transducing protein. Thus, modification of the coupling between Gs-coupled receptors and adenylyl cyclase may underlie both antidepressant therapy and depressive illnesses. This report also suggests that modification of the membrane domain occupied by Gsα might represent a mechanism for chronic antidepressant effects.

Over the past 4 decades, electroconvulsive therapy and antidepressant drugs have been used for the treatment of clinical depression and other psychiatric disorders. Several distinct pharmacological compounds show therapeutic efficacy. These include monoamine oxidase inhibitors, tricyclic compounds, selective serotonin and norepinephrine reuptake inhibitors, as well as some atypical drugs. The possibility that these diverse agents converge on a single postsynaptic target has been an area of great research interest. Menkes et al. (1983) first reported that long-term administration of various antidepressants enhanced guanylyl-5’-imidodiphosphate- and fluoride-stimulated adenylyl cyclase activity in rat cortex and hypothalamus membranes. This suggested that the stimulatory α-subunit of the Gs protein was a target of antidepressant action and that antidepressant treatment facilitated the activation of adenylyl cyclase by Gs. These initial findings involving the stimulation of adenylyl cyclase via Gso after antidepressant treatment have been substantiated by later studies (Ozawa and Rasenick, 1989, 1991; De Montis et al., 1990; Kamada et al., 1999). Increased cAMP activity has been demonstrated in rat cerebral cortex in response to antidepressant treatment (Perez et al., 1989, 1991). Consistent with these findings, it has been reported that chronic antidepressant treatment increases the expression and activity of cAMP response element binding protein in the rat brain (Nibuya et al., 1996; Duman et al., 1997; Takahashi et al., 1999; Thome et al., 2000). Furthermore, similar antidepressant-induced increases in guanylyl-5’-imidodiphosphate-stimulated adenylyl cyclase activity have been observed in vitro using C6 glioma cells (Chen and Rasenick, 1995a).

There has been much recent interest in the organization of G protein signaling complexes at the plasma membrane (Huang et al., 1997). G proteins interact with several other membrane-associated proteins and are unlikely to diffuse freely through the plasma membrane (Neubig, 1994). The localization of G proteins to specific membrane domains such as caveolae (Li et al., 1995) and rafts has generated interest in these cholesterol- and sphingolipid-rich, detergent-resistant membrane domains and how they effect G protein targeting and function (Brown and London, 2000; Moffett et al., 2000). Bayewitch et al. (2000) have shown that chronic exposure to agonists of Gα-coupled receptors leads to a decrease in the cholate solubility of these G protein subunits and a “superactivation” of adenylyl cyclase. These studies

ABBREVIATIONS: PBS, phosphate-buffered saline; CRE, cAMP response element.
indicate that the lipid environment of the G protein may play an important role in its function.

Previous studies demonstrated that Gsα from C6 rat glioma cells migrates from a Triton X-100 (TTX-100) insoluble membrane domain to a TTX-100 soluble membrane domain in response to chronic antidepressant treatment (Toki et al., 1999). In this same study, it was also reported that there was a comigration of adenyl cyclase with Gsα into the more TTX-100 soluble membrane fractions. Interestingly, there was no comparable shift in the localization of Gαo to a more TTX-100 soluble membrane domain after antidepressant treatment, suggesting that the antidepressant effect on G protein membrane localization is Gsα specific.

Immunofluorescence laser scanning confocal microscopy was used to investigate the effect of chronic antidepressant treatment on the distribution of Gsα in C6-2B cells. This study reports that chronic antidepressant treatment results in the redistribution of Gsα from the cell processes and process tips to the cell body. This may be caused in part by an alteration of the lipid environment in which Gsα normally resides, allowing the protein to be more mobile and thus able to interact with downstream effectors. On the other hand, antidepressant-induced increased mobility of Gsα may be caused by a disruption of the interactions between Gsα and other membrane-bound proteins or cytoskeletal elements.

**Materials and Methods**

**Cell Culture.** C6-2B cells (between passages 30 and 50) were plated onto coverslips and allowed to attach overnight in Dulbecco’s modified Eagle’s medium, 4.5 g/l glucose, 10% bovine serum, and 100 μg/ml penicillin and streptomycin at 37°C in a humidified 10% CO₂ atmosphere. As reported previously, desipramine treatment regimens of 3 μM for 5 days and 10 μM for 3 days yielded similar biochemical results (Chen and Rasenick, 1995b). Therefore, the latter treatment paradigm was used in these experiments because it was easier to maintain the cell cultures for 3 days. In some instances, 10 μM fluoxetine was used. The culture media and drug were changed daily. Neither desipramine nor fluoxetine treatment altered cell growth (as determined by the confluence of the cell monolayer and total protein estimation) or cell viability (as determined by 4, 6-diamidino-2-phenylindole staining and visualization under a fluorescence microscope with UV light). During the treatment duration, no morphological changes were observed in the cells. After the treatment duration, the cells were incubated in drug-free media for 45 to 60 min before fixation.

**Indirect Immunofluorescence Laser Scanning Confocal Microscopy.** After treatment, cells were washed once with phosphate-buffered saline (PBS; 136 mM NaCl, 2.6 mM KCl, 5.4 mM Na₂HPO₄, 7H₂O, pH 7.4) and fixed with ice-cold methanol for 10 min. Cells were then washed three times with PBS followed by 2 h of blocking in 5% normal goat serum/0.2% fish skin gelatin in PBS. Primary antibody was added for 1.5 h, Gsα/RM1 (PerkinElmer Life Sciences, Boston, MA) 1:50 and Gαo (Santa Cruz Biotechnology, Santa Cruz, CA) 2 μg/ml, followed by three washes with PBS. Oregon Green-labeled secondary antibody (Molecular Probes, Eugene, OR) was added at a concentration of 8 μg/ml for 1 h followed by three PBS washes. The coverslips were mounted onto slides with Vectashield (Vector Laboratories, Burlingame, CA) containing diamidino-2-phenylindole as a mounting medium. Images were acquired using a Zeiss LSM510 laser-scanning confocal microscope (Carl Zeiss Inc., Thornwood, NY). A single 488-nm beam from an argon/krypton laser was used for excitation of the Oregon Green. Differential interference contrast images were also acquired. Five experiments were performed and coverslips were examined. Approximately 2100 cells from control and desipramine-treated coverslips were counted by two investigators blind to the experimental conditions over the course of the five experiments.

**Fluorescence Quantification.** The cellular distribution of Gsα was quantified in confocal imaged C6-2B cells using NIH-Image software (http://rsbinfonihgov/nih-image) as described previously (Southwell et al., 1998a,b; Jenkinson et al., 1999). Images of 9 × 1 μm optical, planar sections taken from four randomly selected control and four randomly selected desipramine-treated cells were captured and the middle five sections from each cell were quantified. Total cellular Gsα fluorescence was measured by counting the number of pixels with intensity above threshold (determined by minimum intensity above background, in this case 50 pixels). The areas of intensity were numbered and divided visually into those localized to the cell body and those localized to the processes and process tips. The total from each region was divided by the total pixel intensity and expressed as a percentage of total. This was done for each section of each cell and the sections were averaged per cell to give an average percentage total per cell.

In a separate investigation, seven sets of 300 cells each from control group and desipramine-treated cells from five experiments were counted to determine the primary localization (processes and process tips or cell body) of Gsα within these cells. The majority of the cells stained positively for Gsα throughout the entire cell, but there was usually an enhancement in one of these regions. Overly flattened and fragmented cells were omitted from counting, as were cells that did not display processes. The counts are displayed as the ratio of process and process tip localization/cell body localization.

**Data Analysis.** Images were evaluated by two investigators blinded to the treatment condition. Student’s t test was performed for statistical analysis. Values of p < 0.05 were taken to indicate significance.

**Results**

**Chronic Antidepressant Treatment Leads to a Shift in the Cellular Localization of Gsα.** Studies have shown that chronic antidepressant treatment of C6-2B glioma cells alters the detergent solubility of Gsα (Toki et al., 1999). C6-2B cells were treated with the tricyclic antidepressant desipramine (10 μM) for 3 days and were then examined by laser scanning confocal microscopy to visualize these changes in membrane localization. Examination of 300 to 500 control and desipramine-treated cells by three independent researchers revealed that desipramine treatment did not alter the overall structure of C6-2B cells (Fig. 1), but drastically reduced the presence of Gsα in the process tips (Fig. 2, arrowheads and Fig. 3). In addition, there was an increase in the presence of Gsα within the cell body of many of the desipramine-treated cells (Fig. 2, arrows), as well as a decrease within the cell processes themselves (Fig. 2, asterisks). In some instances, there was an intense clustering of Gsα staining in the cell body (Fig. 2C, arrows), but the majority of the cells did not exhibit such a focused increase in Gsα staining.

Twenty-one hundred cells from each group (control versus desipramine-treated) over a series of five experiments were examined to quantify the extent of the antidepressant effect. The cells were grouped into two categories: those that displayed intense staining at the process tips as well as overall staining in the processes and cell body (category A) versus those that displayed intense staining in the cell body region and decreased process and process tip staining (category B). Abnormal cells or those not displaying processes were not included in the cell count. Cells (300–450) were counted per
experiment and the ratio of category A cells to category B cells for each group is shown in Fig. 4. Twice as many control cells (64%) displayed Gs\(\alpha\) staining at the process tips and throughout the entire cell than those treated with desipramine (32%). This demonstrates that Gs\(\alpha\) relocalization is not an all-or-none response to antidepressant treatment and that some cells may be more responsive to treatment than others.

To determine quantitative differences between the groups, five 1 \(\mu\)m optical, planar sections through each of four cells in each group were examined by confocal microscopy and the digital images were captured. These images were then analyzed using the program NIH Image according to methods published previously (Southwell et al., 1998a,b; Jenkinson et al., 1999). This was done to account for changes in Gs\(\alpha\) localization at different focal planes of the cell. The percentages of Gs\(\alpha\) localized to the cellular processes and process tips of control versus treated cells were compared by dividing the pixel density above threshold in these regions by the total cellular pixel density (Table 1). There was a 3-fold decrease in Gs\(\alpha\) localization in the processes and process tips between control cells and desipramine-treated cells as 12% of the total cellular Gs\(\alpha\) was located in the process tips of control cells versus 4% present in the tips after desipramine treatment.

**Antidepressant Induced G Protein \(\alpha\) Subunit Cellular Relocalization Is Specific to Gs.**

To determine whether antidepressant-induced mobility is specific to Gs\(\alpha\), Go\(\alpha\) distribution was examined in approximately 500 cells

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**Fig. 1.** Chronic desipramine treatment of C6-2B glioma cells does not alter the overall shape of the cell. Differential interference microscopy images of control and desipramine treated C6-2B cells are shown to demonstrate similarities in overall cell shape between the two groups. Cells were treated and processed for microscopy as described under Materials and Methods. A representative image of five independent experiments is shown. Bar, 10 \(\mu\)m.

**Fig. 2.** Chronic desipramine treatment results in an enhancement of Gs\(\alpha\) immunofluorescence in the cell body and a decrease in the cell processes and process tips. Untreated C6-2B glioma cells (A and B) display ubiquitous staining of Gs\(\alpha\) with an enhancement at the process tips (arrowheads) and cell processes (asterisks). Desipramine treated cells (C and D) show a decrease in Gs\(\alpha\) staining at the process tips (arrowheads) and cell processes (asterisks) and simultaneously display an increase in cell body staining (arrows). Cells were treated and prepared for microscopy as described previously. Bar = 10 \(\mu\)m.

**Fig. 3.** Enlarged view of the process tips in control versus desipramine treated C62B cells shown in Fig. 2. The process tips from the control cell in Fig. 2A were enlarged to show the intense Gs\(\alpha\) staining (A and B) and the corresponding process tips of the desipramine treated cell in Fig. 2C are shown to demonstrate the reduction of Gs\(\alpha\) staining after antidepressant treatment (C and D).
under the same treatment conditions. Figure 5 demonstrates that there was little if any change in the distribution of Goα after desipramine treatment. Goα appears throughout the cell without specific regions displaying an increased staining intensity in control or treated cells. Some of the control cells (Fig. 5, A and B) have a slight increase in staining intensity at the process tips, but this is also seen in the treated cells (Fig. 5, C and D), indicating that antidepressant treatment does not effect Goα localization within the cell.

Fluoxetine Treatment Also Promotes Gsα Migration. If the redistribution of Gsα is truly an antidepressant effect, then other classes of antidepressant drug should have a similar effect. Desipramine and fluoxetine have both been shown to evoke a similar biochemical redistribution of Gsα (Toki et al., 1999). Confocal microscopic images of C6-2B cells treated with 10 μM fluoxetine for three days show a similar Gsα staining pattern compared with desipramine-treated cells (Fig. 6 A). The most striking similarity of desipramine and fluoxetine effects on Gsα localization is the loss of staining in the processes and process tips (compare Fig. 2, C and D, and Fig. 6A with Fig. 2, A and B). Approximately 100 cells were examined for qualitative differences as described above for Fig. 4. Of the fluoxetine-treated cells, 45% displayed intense staining in the process tips compared with the 64% of control and 32% of desipramine-treated cells mentioned previously.

Chlorpromazine Treatment Does Not Alter the Distribution of Gsα. The antipsychotic drug chlorpromazine

![Fig. 4](image)

**TABLE 1** Percentage of Gsα Immunofluorescence Distribution in the Cell

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cell Body</th>
<th>Processes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>83.9</td>
<td>16.1</td>
</tr>
<tr>
<td>C1</td>
<td>89.0</td>
<td>11.0</td>
</tr>
<tr>
<td>C2</td>
<td>83.5</td>
<td>16.5</td>
</tr>
<tr>
<td>C3</td>
<td>95.6</td>
<td>4.4</td>
</tr>
<tr>
<td>C4</td>
<td>95.6</td>
<td>4.4</td>
</tr>
<tr>
<td>Average</td>
<td>88.0 ± 5.7</td>
<td>12.0 ± 5.7</td>
</tr>
<tr>
<td>Desipramine</td>
<td>91.4</td>
<td>8.6</td>
</tr>
<tr>
<td>D1</td>
<td>92.2</td>
<td>7.8</td>
</tr>
<tr>
<td>D2</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>D3</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>D4</td>
<td>95.9 ± 4.7</td>
<td>4.1 ± 4.7</td>
</tr>
<tr>
<td>Average</td>
<td>95.9 ± 4.7</td>
<td>4.1 ± 4.7</td>
</tr>
</tbody>
</table>

![Fig. 5](image)

Goα does not undergo antidepressant induced realocation. Untreated (A and B) and desipramine-treated (C and D) cells show similar Gsα immunofluorescence profiles. There is staining throughout the cell body and processes of both sets of cells. The figure is typical of approximately 500 cells that were examined. Bar, 10 μm.
was used as a control for antidepressant effects. When cells were treated with 10 µM chlorpromazine for 3 days, Gsα staining was evident throughout the cell body (Fig. 6 B). There is Gsα immunostaining throughout the cell body, cell process, and process tip. This pattern of Gsα distribution was similar to other control cells; 68% of approximately 100 cells demonstrated distinct staining in the cell processes and process tips.

Other Treatment Paradigms Have a Similar Effect on Gsα. A lower dosage and longer exposure time for desipramine treatment (3 µM for 5 days) was also tested. Control cells have intense staining at the process tips, whereas the desipramine treated cells do not (data not shown). The main difference between the high-dose/3-day and the low-dose/5-day treatment regimens is the cell body localization of Gsα. A majority of C6-2B cells treated with 10 µM desipramine display intense clustering of Gsα in the perinuclear region whereas cells treated with 3 µM desipramine show a more even distribution between intense cell body staining and a more nondescript staining. One-day/10 µM desipramine treatment of C6-2B cells resulted in a Gsα distribution similar to that of cells treated with 3 µM for 5 days (data not shown). Under the acute treatment condition (1 day, 10 µM) the number of cells lacking Gsα in the process tips was not significantly different from the control cell population seen in Table 1 and Fig. 4.

Discussion

Over the past several decades, there has been a great deal of research attempting to determine a common mechanism of antidepressant action. Such a mechanism, if a single one exists, has yet to be clearly established. One of the classic hallmarks of chronic antidepressant treatment is the down-regulation of several types of neurotransmitter receptor in the brain (Sulzer, 1984) and β-adrenergic receptors in rat C6 glioma cells (Fishman and Finberg, 1987). However, the time course between the change in the receptor number and the clinical efficacy of antidepressant treatment cannot be fully explained by these biochemical data (Rasenick et al., 1996).

More recently, much work has focused on postreceptor neuronal cell signaling processes as mechanisms of antidepressant action (Ozawa and Rasenick, 1989; Duman et al., 1997; Takahashi et al., 1999; Toki et al., 1999; Thome et al., 2000). The downstream effects involving cAMP have been the focus of much of this previous work (Perez et al., 1989, 1991; Nibuya et al., 1996; Duman et al., 1997; Takahashi et al., 1999; Thome et al., 2000). Toki et al. (1999) demonstrated that antidepressant treatment results in an alteration in the detergent extractability of Gsα from the plasma membrane of C6 glioma cells and rat cerebral cortex. Altered detergent solubility of Gia and Gβγ has also been demonstrated after chronic activation of Gia-coupled opiate receptors (Bayewitch et al., 2000). This change in detergent solubility corresponds to adenyl cyclase “superactivation”. The current study centers on the visualization of these changes in the detergent solubility of Gsα after antidepressant treatment using laser scanning confocal microscopy. The results of this study suggest that the cellular localization of Gsα is altered after chronic antidepressant treatment.

In this study, it was demonstrated that chronic antidepressant treatment of C6 glioma cells results in a change in the cellular localization of Gsα (Figs. 2–4 and 6). This redistribution of Gsα was observed with two types of antidepressants: desipramine, a tricyclic compound (Figs. 2–4), and fluoxetine, a selective serotonin reuptake inhibitor (Fig. 6A). Chlorpromazine, an antipsychotic agent with chemical similarities to tricyclic antidepressants, did not alter the distribution of Gsα (Fig. 6B). Previous studies have suggested that activated Gsα can be released from the plasma membrane into the cytosol; these results are certainly consistent with those observations (Rasenick et al., 1984; Ransas et al., 1989; Levis and Bourne, 1992). Furthermore, the distribution of Goo was not modified by antidepressant treatment (Fig. 5). The unique antidepressant response of Gso was also seen previously, as Gia solubility in TTX-100/TTX-114 was unchanged by desipramine treatment (Toki et al., 1999). The data reflect a genuine redistribution of Gsα, because the amount of this G protein is not altered by antidepressant treatment (Chen and Rasenick, 1995a; Emamghoreishi et al., 1996; Toki et al., 1999). Thus, these data suggest a reorganization of the extant pool of Gsα rather than an increase in protein synthesis.

The notion that G protein-coupled receptors, G proteins, and effectors are freely mobile in the plasma membrane is becoming less fact and more fiction. Significant limitations on the lateral mobility of plasma membrane proteins (both integral and peripheral) restrict movement much like a “corral” around the protein (Kuo and Sheetz, 1993). It has been suggested that an association with the cytoskeleton (Carlson et al., 1986; Rasenick et al., 1990; Wang et al., 1990) may aid in significantly restricting the lateral mobility of G proteins in the plasma membrane (Neubig, 1994). Furthermore, some G proteins, including Gs, form specific complexes with tubulin, the major microtubule protein (Wang et al., 1990), and this is a bidirectional process, with G proteins participating in the regulation of the cytoskeleton (Roychowdhury and

![Fluoxetine](A) Chlorpromazine (B)

**Fig. 6.** Fluoxetine (10 µM) treatment for 3 days has effects similar to those of desipramine on Gsα cellular localization. Cells were treated with fluoxetine (A) or chlorpromazine (B) and processed for confocal microscopy as described under Materials and Methods. Like desipramine, fluoxetine treatment results in a drastic reduction of Gsα immunofluorescence in the cell process (arrow) and process tips (arrowhead), whereas chlorpromazine treatment results in a uniform distribution of Gsα similar to control (compare to Fig. 2, A and B). The differential interference contrast image to the right of the fluorescence image shows that these drugs have no effect on global cell shape. Bar, 10 µm.
Rasenick, 1997; Roychowdhury et al., 1999). G protein-coupled receptors and the kinases that regulate those receptors have been shown to be associated with microtubules as well (Carman et al., 1998; Pitcher et al., 1998; Saunders and Limbird, 2000). Actin and the microfilament cytoskeleton may also interface with G protein signaling (Carlson et al., 1986; Vaiskunaite et al., 2000).

Recently, the lipid environment in which G proteins and its effectors are localized has been under investigation. G proteins seem to be present in caveolin-enriched plasma membrane domains, and caveolin may play a role in G protein-mediated signaling (Li et al., 1995). Ostrom et al. (2000) have recently shown a colocalization of β-adrenergic receptor and adenylyl cyclase type 6 in caveolae of cardiac myocytes. The direct association of G proteins with caveolin has been disputed (Huang et al., 1997); however, these authors conclude that the proteins involved in the hormone-sensitive adenylyl cyclase system are indeed localized to a specialized subdomain of the plasma membrane. In fact, Moffett et al. (2000) have shown that it is the acylation of G protein subunits that targets these signaling molecules to specific cholesterol- and sphingolipid-rich membrane domains called rafts.

Although these data do not show a direct effect on the cytoskeleton or the lipid environment in which Gsα is localized, they demonstrate that Gsα relocates to the cell body of antidepressant-treated cells. This relocalization may reduce the distance between G protein induced cAMP production and the cascade of molecules involved in the up-regulation of cAMP response element (CRE)-mediated gene transcription. In fact, previous data demonstrate an increase in immunoprecipitable Gsα-adenylyl cyclase complexes after treatment of rats with a variety of antidepressants and electroconvulsive shock (Chen and Rasenick, 1995a). The high density of Gsα in the processes and process tips of nontreated cells suggests a “housekeeping” role in which Gsα is involved in maintaining structure and homeostasis in the cell. After chronic antidepressant treatment, Gsα may play a role in stimulating the production of genes involved in cell growth and rearrangement. Levels of brain-derived neurotrophic factor and tyrosine kinase receptor B have been shown to be elevated in the brains of rats chronically treated with antidepressants (Nibuya et al., 1996). The fact that Gsα migrates to the cell body in response to antidepressant treatment suggests that the cAMP signaling machinery leading to increased expression of CRE may be located in close proximity to the nucleus. This relocalization of G proteins followed by increases in CRE-mediated gene expression seem to follow a time frame more consistent with the clinical efficacy of antidepressants than a direct receptor effect.

Although C6 cells have glutamate uptake sites, there is no evidence that they have specific sites for either norepinephrine or serotonin. Nonetheless, these cells have been shown to respond to antidepressants in a manner similar to rat brain (Fishman and Finberg, 1987; Chen and Rasenick, 1995a,b). This is not necessarily problematic; in fact, this may provide an ideal system to detect the existence of a novel target of antidepressant action.

There are probably multiple targets of antidepressant action. Although the data in this study are not sufficient to assign a specific mechanism of action for Gsα in mediating the effects of antidepressants, they do suggest a convergence of different classes of antidepressants that act through a postsynaptic signaling mechanism toward a common end. Further study on Gsα signaling and antidepressant action may illuminate both the biology of depression and the unique heterogeneity of G proteins during the process of cell signaling.

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Ostrom RS, Violin JD, Coleman S and Insel PA (2000) Selective enhancement of CRE-mediated signaling (Li et al., 1995). Ostrom et al. (2000) have shown that it is the acylation of G protein subunits that targets these signaling molecules to specific cholesterol- and sphingolipid-rich membrane domains called rafts.

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