Chapter III

ANALYSIS OF β-CATENIN DYNAMICS IN NEURONS

As discussed above, I collaborated with Dr. Sachiko Murase, a postdoctoral scholar in the Schuman laboratory to help analyze the dynamics of β-catenin in neurons in response to stimulation (Murase, Mosser et al. 2002). My role in this collaboration was the processing and analysis of the images acquired by Dr. Murase. I was blind to the experimental conditions of each image to ensure a lack of bias in the results of the analysis.

Dr. Murase demonstrated that KCl induced depolarization caused the movement of β-catenin into spines and increased β-catenin/cadherin interactions, as measured by coimmunoprecipitation. Application of tyrosine kinase inhibitor mimicked these effects, while application of a tyrosine phosphatase inhibitor had the opposite effects, indicating that this redistribution of β-catenin was phosphorylation mediated.

The β-catenin phosphorylation mutations (Y654E and Y654F) were sufficient to cause redistribution in a manner consistent with this; the phophorylation-mimic (Y654E) accumulates in the dendritic shaft, while the dephophorylation-mimic (Y654F) accumulates in spines.

As noted above, β-catenins interaction with cadherin is required for cadherin-mediated adhesion. The phosphorylation-mediated movement of β-catenin into spines may promote stabilization and strengthening of cadherin-cadherin interactions, which may be a part of the local expansion of synaptic contacts. To address this issue, we examined the morphological properties of neuronal synapses expressing one of three EGFP-β-catenin fusions; wild-type β-catenin, and two mutants: Y654E, and Y654F. Y654E has tyrosine-654 of β-catenin mutated to glutamic acid; the negative charge of the

glutamate mimics the phosphorylated state of wild-type tyrosine-654. Y654F has its tyrosine-654 mutated to phenylalanine, which has the same aromatic group as tyrosine, but cannot be phosphorylated. This mimics the unphosphorylated state of wild-type tyrosine-654.

To determine whether changes in spine β-catenin concentration result in changes in the size of the postsynaptic density, Dr. Murase fixed the transfected neurons and immunostained them for PSD-95, a prominent component of the postsynaptic density in forebrain neurons (Cho, 1992).

She then captured images, which I was given. I analyzed the images of EGFP signal by selecting the spines, and overlaying the PSD-95 immunostaining images, then identifying the immunostained puncta that overlapped with EGFP labeled spines. I then analyzed the area and intensity of these puncta to determine the effect of mutating tyrosin-654 on PSD clustering.

In Y654F-expressing neurons, the size of the PSD-95 puncta was significantly larger than those of either the Y654E- or wild-type β-catenin-expressing cells, which did not differ significantly from each another (figure 2, B and C). In addition, the intensity of the PSD-95-containing puncta was substantially lower in the Y654E-expressing neurons (figure 2, B and C) and slightly, but significantly, lower in the Y654F-expressing neurons. Thus, the Y654F mutants possessed larger PSD-95 puncta, while the Y654E mutants possessed PSD-95 puncta of the same size, but of lesser intensity.

This suggests that increasing or decreasing the concentration of β-catenin in spines via the point mutation is sufficient to produce changes in the size and "density" of the PSD.

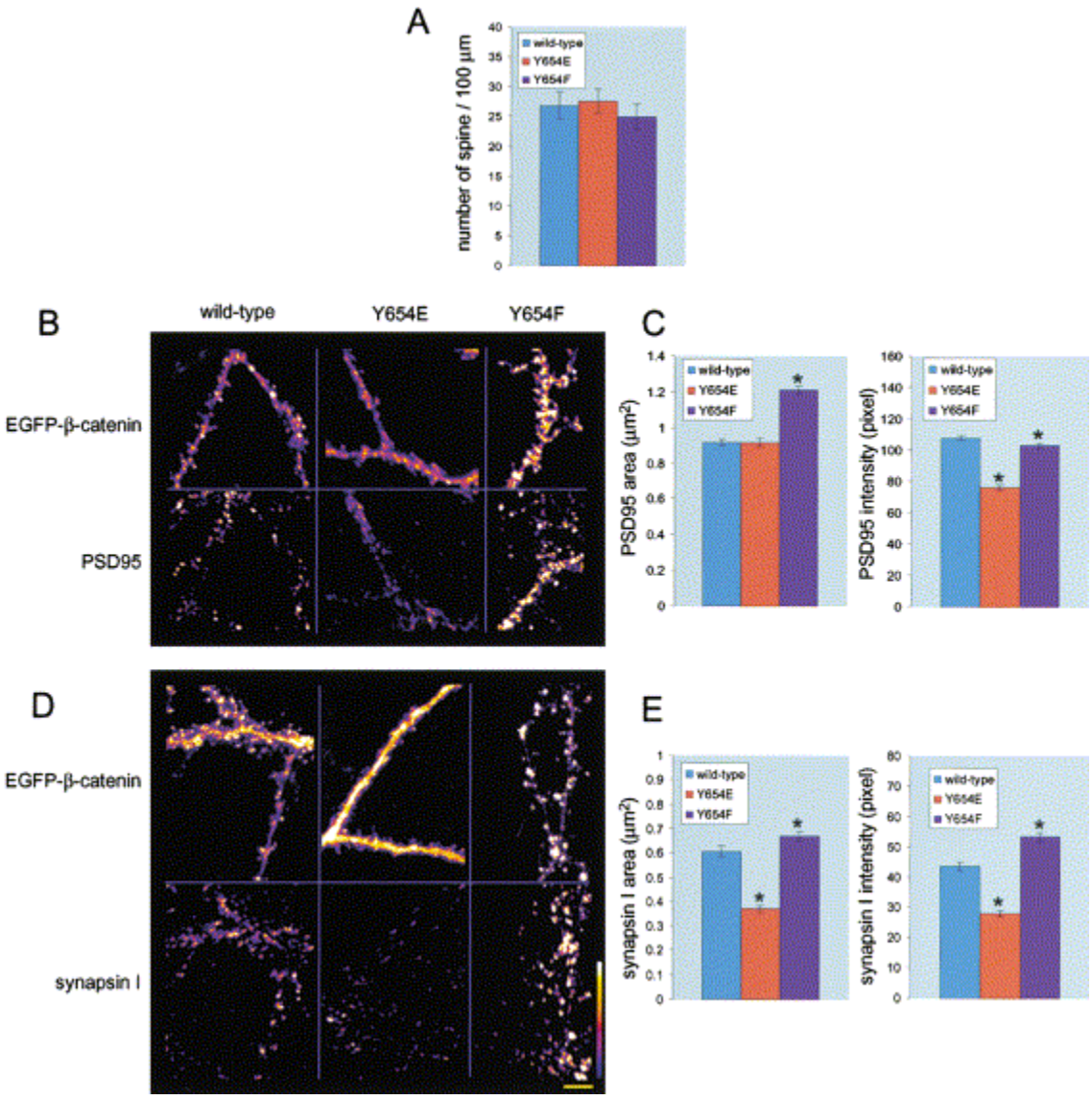

Figure 2. Mutation of a β-catenin tyrosine residue affects the size and intensity of presynaptic and postsynaptic protein clusters.
A. The mean number of spines did not differ between the groups, with N = 22, N = 22, and N = 22, respectively. B. EGFP-β-catenin with anti-PSD95 immunostaining. C. Summary data (n = 12 for each group). D. EGFP-β-catenin and anti-synapsin I immunostaining. E. Summary data (n = 10 for each group). Scale bars: 20 µm (Murase, Mosser et al. 2002).

Because cadherins span the synapse through their *trans* interactions, we reasoned that a postsynaptically driven increase in the interaction of β-catenin with cadherin might also cause changes across the synapse in presynaptic structures. Two other studies have observed increases in immunolabeling for presynaptic proteins following synaptic plasticity (Antonova, Arancio et al. 2001 and Bozdagi, Shan et al. 2000). We thus conducted a similar analysis of a presynaptic protein, synapsin-I, to determine whether the size or intensity of synapsin-I-positive puncta were altered by the expression of either β-catenin mutant. Dr. Murase immunostained transfected cells for synapsin-I and as above, I analyzed only the synapsin-I-positive puncta that were associated with spines from a β-catenin-EGFP-expressing neuron.

Both the size and intensity of synapsin-I puncta from Y654F mutants were significantly enhanced, relative to wild-type or Y654E-expressing cells (figure 2, D and E). In contrast, the synapsin-I clusters in Y654E-expressing neurons were significantly smaller and less intense than those observed in wild-type or Y654F-expressing cells. These data show that increases in spine β-catenin are associated with increases in the size and intensity of synapsin-I clusters, whereas decreases in spine β-catenin are associated with smaller and less intense synapsin-I clusters.

These data suggest that the local concentration of β-catenin in spines can drive changes in presynaptic protein clustering across the synaptic junction, which may reflect the transsynaptic action of cadherins (summarized in figure 3).

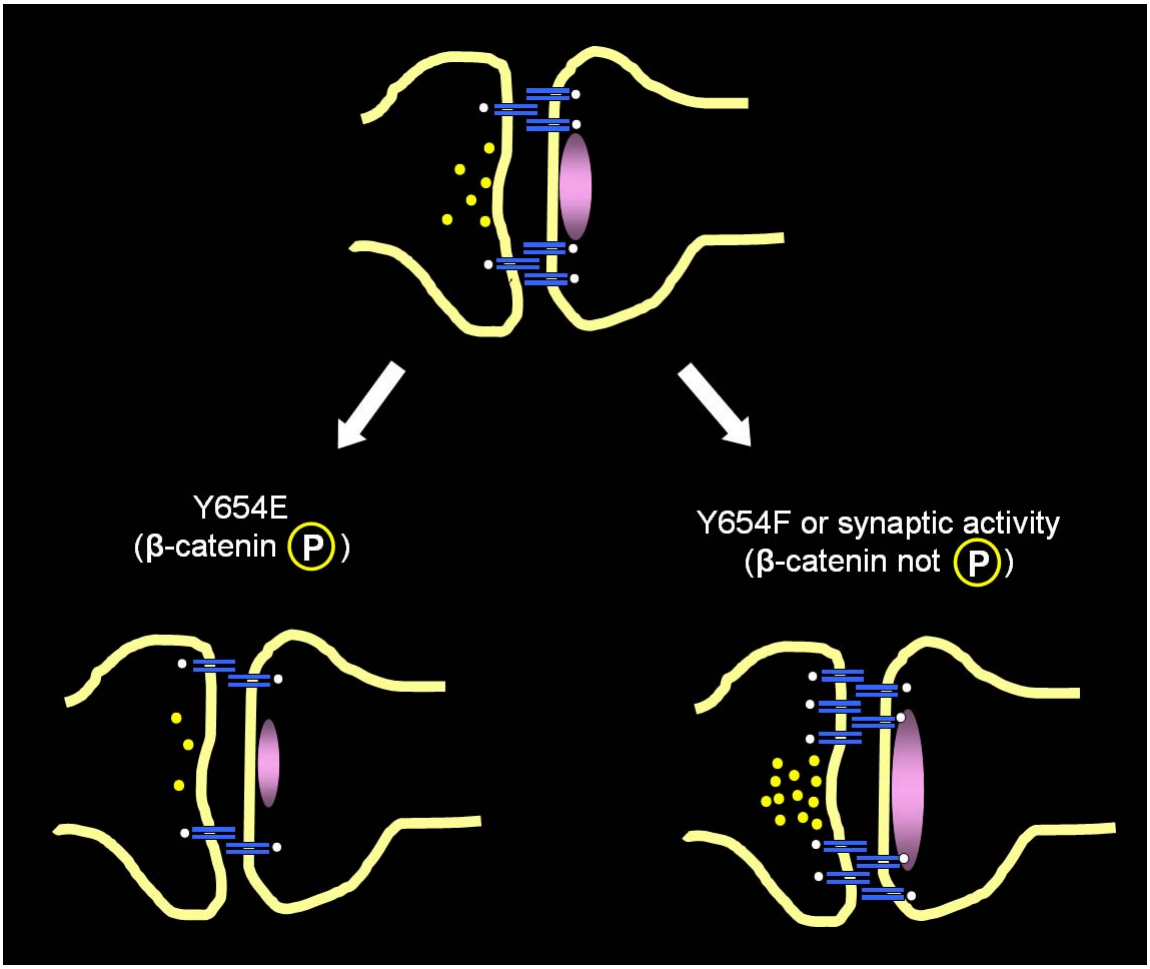

Figure 3. Scheme of β-catenin's redistribution following synaptic activity or mutation of tyrosine 654.
The symbols in the diagram represent the following: cadherin dimers, blue bars; β-catenin, white circles; the PSD, pink oval; synaptic vesicles, yellow spheres. Conditions that lead to the phosphorylation of β-catenin or mimic the phosphorylation (the Y654E mutant) may result in decreased β-catenin cadherin interactions, decreased adhesion, and decreases in the PSD and the synaptic vesicle pool size of associated presynaptic terminals. Conversely, synaptic activity or conditions that favor a dephosphorylated state for β-catenin lead to increased association with cadherins, increases in the PSD, and increases in the synaptic vesicle pool size of associated presynaptic terminals (adapted from Murase, Mosser et al. 2002).