Nanoscale distribution of the dopamine transporter assessed by super-resolution microscopy: regulation by membrane potential

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The dopamine transporter (DAT), a membrane protein present on dopaminergic neurons and responsible for the uptake of dopamine from the extracellular space, resides in discrete nanodomains on the plasma membrane of neuronal projections. Through direct stochastic optical reconstruction microscopy (dSTORM) on primary dopaminergic cultures and mouse brain slices, changes in the clustered, nanoscale distribution of DAT were observed upon manipulations affecting the neuronal membrane potential. The dynamic character of the the clustered architecture was supported by dispersing of clusters following both NMDA receptor activation and nicotinic acetylcholine receptor activation. However, nanoclusters were not affected by the presence of the DAT inhibitor cocaine, nor was the NMDA induced declustering influenced by the inhibition of nitric oxide production. The declustering of DAT following NMDA receptor activation was muted through the sequesting of calcium by BAPTA-AM, and it was blocked by inhibition of voltage gated calcium channels, indicating the change in clustering is dependent on calcium influx. Interestingly, the changes in clustering architecture were also induced following viral transduction of ion channels into the dopaminergic neurons that caused the neuronal membrane to either hyperpolarize or depolarize. The data suggest that the dynamic changes in DAT clustering is a generalized phenomenon and is a response to membrane potential or neuronal activity in dopaminergic neurons. We also assessed the context for dopaminergic clusters by dual color dSTORM imaging of DAT with other presynaptic proteins, revealing concomitant declustering of some but increased clustering of other proteins. In order to observe if our observed effect take place also in the developed brain, live mouse brain slices were treated with NMDA, and the changes in DAT clustering were observed following dSTORM imaging of DAT in the striatum. In summary, DAT is organized into nanoscale clusters that are dynamic, and these clusters change in response to stimuli that influence membrane potential, indicating that the clustered distribution of DAT serves a functional role in dopaminergic neurotransmission.