Jin, P., Zarnescu, D.C., Zhang, F., Pearson, C.E., Lucchesi, J.C., Moses, K., and Warren, S.T. (2003). Neuron *39*, 739–747.

Khateb, S., Weisman-Shomer, P., Hershco-Shani, I., Ludwig, A.L., and Fry, M. (2007). Nucleic Acids Res. *35*, 5775–5788.

López Castel, A., Cleary, J.D., and Pearson, C.E. (2010). Nat. Rev. Mol. Cell Biol. *11*, 165–170.

Lu, C., Lin, L., Tan, H., Wu, H., Sherman, S.L., Gao, F., Jin, P., and Chen, D. (2012). Hum. Mol. Genet. *21*, 5039–5047.

Mori, K., Weng, S.M., Arzberger, T., May, S., Rentzsch, K., Kremmer, E., Schmid, B., Kretzschmar, H.A., Cruts, M., Van Broeckhoven, C., et al. (2013). Science *339*, 1335–1338.

Napierala, M., Michalowski, D., de Mezer, M., and Krzyzosiak, W.J. (2005). Nucleic Acids Res. *33*, 451–463.

Oostra, B.A., and Willemsen, R. (2009). Biochim. Biophys. Acta *1790*, 467–477.

Pearson, C.E. (2011). PLoS Genet. *7*, e1002018.

Pearson, C.E., Nichol Edamura, K., and Cleary, J.D. (2005). Nat. Rev. Genet. *6*, 729–742.

Reddy, K., Zamiri, B., Stanley, S.Y., Macgregor, R.B., Jr., and Pearson, C.E. (2013). J. Biol. Chem. *288*, 9860–9866.

Todd, P.K., Oh, S.Y., Krans, A., He, F., Sellier, C., Frazer, M., Renoux, A.J., Chen, K.C., Scaglione, K.M., Basrur, V., et al. (2013). Neuron *78*, this issue, 440–455.

Zu, T., Gibbens, B., Doty, N.S., Gomes-Pereira, M., Huguet, A., Stone, M.D., Margolis, J., Peterson, M., Markowski, T.W., Ingram, M.A., et al. (2011). Proc. Natl. Acad. Sci. USA *108*, 260–265.

Evidence for a Common Endocannabinoid-Related Pathomechanism in Autism Spectrum Disorders

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In this issue of *Neuron*, Földy et al. (2013) report that endocannabinoid-mediated signaling at inhibitory synapses is dysregulated in mouse models of autism-associated Neuroligin-3 mutations. These findings carry implications regarding the pathophysiology of autism spectrum disorders and the development of treatment strategies.

The correct wiring of the brain during development is an extremely complex biological process, during which a staggering number of synapses with often very diverse characteristics have to be formed and maintained in a precise and delicate balance. Not surprisingly, therefore, numerous neurodevelopmental and psychiatric diseases appear to be disorders of aberrant synaptogenesis and synapse function, or "synaptopathies." Particularly in the context of autism spectrum disorders (ASDs), an ever-growing number of mutations in genes encoding synaptic proteins have been identified in affected individuals (Murdoch and State, 2013), and major research efforts are currently focusing on strategies to transform this knowledge base into viable treatment strategies.

However, the corresponding challenges are substantial. For example, very little is known about the role of ASD-related synaptic proteins in vivo, e.g., in neuronal circuits that control autism-relevant behavior. Second, many known ASD-related proteins are structural proteins with adhesion or scaffold functions and therefore poor targets for pharmacological intervention with small molecule drugs. Third, many ASD-related mutations lead to a loss of the corresponding protein so that no target for pharmacological intervention remains. Finally, each individual ASD-related mutation is rare, with the vast majority accounting for less than 1% of affected individuals each. In view of these difficulties, the focus in the field of ASD biology has shifted toward the identification of cellular protein-protein interactions or signaling pathways that are common to the various ASD-related proteins and therefore expected to be perturbed by a wide range of ASD-related mutations—with the hope that such pathways may represent more promising treatment targets than the ASD-linked proteins discovered so far.

One of the synaptic proteins associated with ASDs is Neuroligin-3 (NLGN3), a member of the Neuroligin family of postsynaptic cell adhesion molecules that interact with presynaptic Neurexins to control synapse development and function. Two distinct mutations in *NLGN3* have been linked to ASDs, a point mutation resulting in an R451C substitution in the Neurexin-binding domain (Jamain et al., 2003) and a deletion of the *NLGN3* gene (Sanders et al., 2011). Studies on the respective mouse models, a $Nlgn3^{R451C}$ knockin (KI) and a Nlgn3 knockout (KO), showed that both mutations cause ASD-related behavioral phenotypes (Radyushkin et al., 2009; Tabuchi et al., 2007) but have strikingly different effects on synapse and network function, with the $Nlgn3^{R451C}$ mutation resulting in a gain-of-function phenotype that is

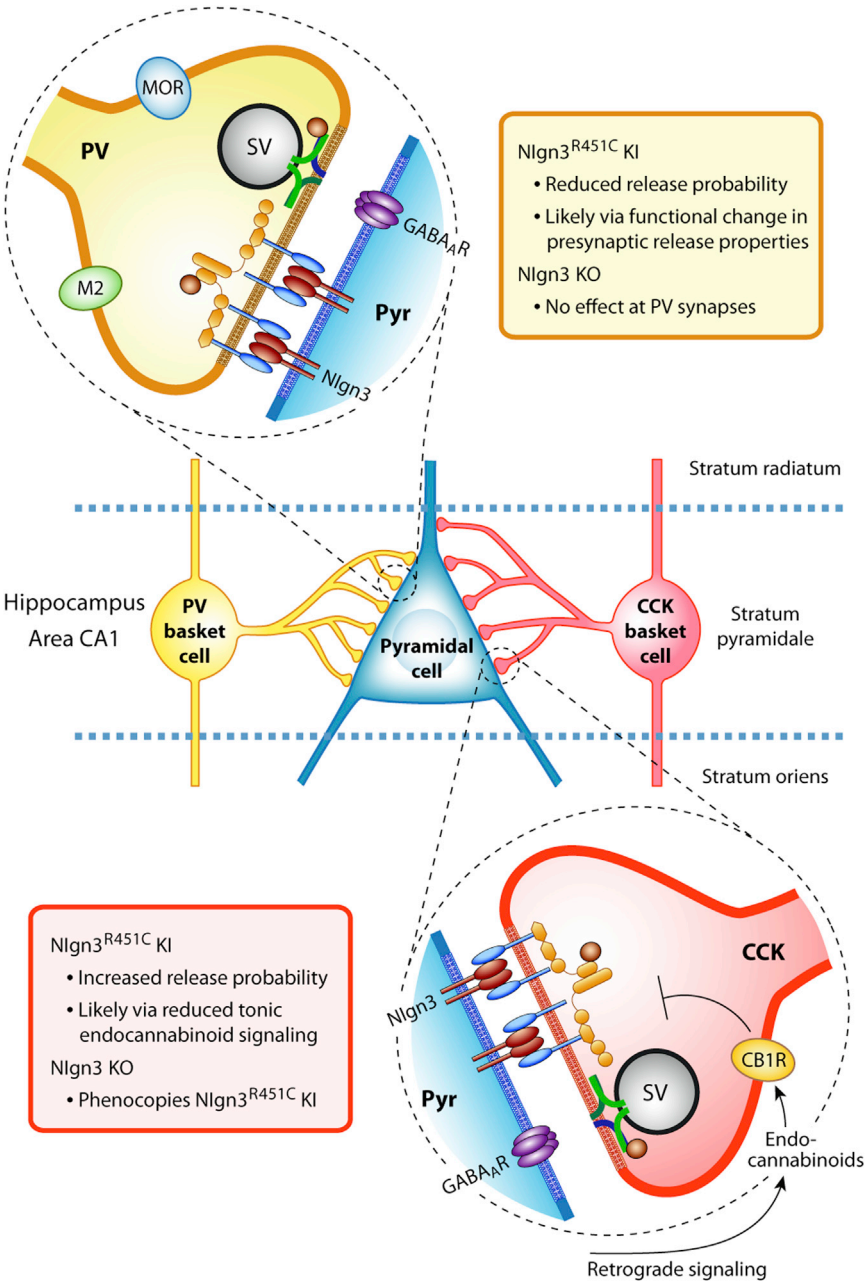

Figure 1. Potential Role of Nlgn3 at Perisomatic Inhibitory Synapses on CA1 Pyramidal Neurons

PV and CCK basket cells form perisomatic inhibitory synapses onto pyramidal neurons. Boxed inserts summarize the data reported by Földy et al. (2013). Expanded views show a model of how Nlgn3 may affect PV basket cell synapses (upper left) and CCK basket cell synapses (lower right) based on these findings. It should be noted that the presence of Nlgn3 at these synapses has not been investigated and is inferred from the data. Abbreviations: Nlgn3, Neuroligin-3; PV, parvalbumin; CCK, cholecystokinin; SV, synaptic vesicle; MOR, μ-opioid receptor; M2, M2 muscarinic acetylcholine receptor; CB1R, cannabinoid receptor type 1.

unrelated to the loss of function caused by the Nlgn3 deletion (Baudouin et al., 2012; Etherton et al., 2011; Tabuchi et al., 2007).

In a new study published in the current issue of *Neuron*, Földy and colleagues searched for common phenotypic changes in Nlgn3^{R451C} KI and Nlgn3 KO mice that might explain the similar ASD-related behavioral changes in these mouse lines and may therefore be particularly relevant to ASDs (Földy et al., 2013). To this end, the authors investigated GABAergic synaptic transmission in the hippocampus of Nlgn3^{R451C} KI and Nlgn3 KO mice, focusing on the synaptic connections between inhibitory basket cells and pyramidal neurons, which are known to play a fundamental role in the generation of the network oscillations that underlie a number of cognitive functions controlled by the hippocampus (Lisman and Buzsáki, 2008). Two types of basket cells are particularly relevant for this process, parvalbumin-containing (PV) and cholecystokinin-containing (CCK) basket cells, and Földy et al. (2013) employed paired whole-cell recordings to monitor perisomatic synapses formed by each of these inhibitory cell types onto postsynaptic pyramidal neurons (Figure 1).

The authors found that synaptic transmission is substantially impaired at PV basket cell synapses in Nlgn3^{R451C} KI mice, with IPSC amplitudes reduced by ~70%. No such alterations were observed in the Nlgn3 KOs, consistent with the previously published notion that the R451C substitution exerts its influence by a gain-of-function mechanism. Unexpectedly in view of the postsynaptic localization of Nlgn3, this decrease in IPSC amplitude appears to be of presynaptic origin and due to a reduction in presynaptic transmitter release probability. In contrast, no evidence for changes in postsynaptic GABA receptor number or composition, in the total number of synapses, in quantal size or the number of release sites, or in the activation of presynaptic receptors that modulate release probability was observed. The authors conclude that the Nlgn3^{R451C} KI affects the presynaptic transmitter release machinery at PV basket cell synapses through gain-of-function alterations in transsynaptic signaling, although the precise mechanism has yet to be elucidated.

While these experiments provided valuable new insights into the mechanisms by which the R451C substitution might affect Nlgn3 function, they failed to uncover common phenotypic features of the two Nlgn3 mutants that might be related to pathways of particular relevance

for ASD pathophysiology. Hence, Földy et al. (2013) next investigated transmission at CCK basket cell synapses. Unexpectedly, the authors found that the Nlgn3^{R451C} KI phenotype at these CCK basket cell synapses was diametrically opposite to the one found at PV basket cell synapses, with IPSC amplitudes substantially increased rather than decreased. As with the PV basket cell synapses, this phenotypic change is again likely the result of an alteration in presynaptic GABA release probability. However, in the case of the CCK basket cell synapses, the change in IPSC amplitude was phenocopied in the Nlgn3 KO mouse, indicating that it represents a loss-of-function effect that is mechanistically distinct from the one observed at PV basket cell synapses of Nlgn3^{R451C} KI mice.

It was shown previously that GABA release at CCK basket cell synapses can be suppressed by tonic endocannabinoid-mediated activation of presynaptic CB1 receptors, most likely via constitutive release of endocannabinoids from the postsynaptic neuron (Katona and Freund, 2012). The authors therefore tested if the increase in GABA release probability observed at CCK basket cell synapses of Nlgn3^{R451C} KI and Nlgn3 KO mice is caused by a deficiency in tonic endocannabinoid signaling. In support of this notion, bath application of a CB1 receptor antagonist resulted in an increase in IPSC amplitudes at wild-type synapses, but failed to further enhance transmission at Nlgn3^{R451C} KI or NL3 KO or R451C KI synapses, indicating that CB1 receptor signaling was already reduced in the two mutants. Interestingly, Nlgn3 loss-of-function impaired tonic endocannabinoid signaling at all CB1-containing GABAergic synapses throughout the hippocampus, but showed no effect on glutamatergic transmission or on phasic endocannabinoid signaling. These data led the authors to conclude that Nlgn3 is required to specifically localize the release machinery for tonic endocannabinoid release to CB1-containing synapses.

There are several interesting lessons to be learned from this study. First, the observation that the same Nlgn3 mutation can have such different effects on two types of presynapses contacting the same postsynaptic neuron highlights the fundamental importance of synaptic context in understanding Neuroligin function. The function of Neuroligins and Neurexins is not only diversified by extensive alternative splicing, but also by alternate transsynaptic binding partners such as LRRTMs or N-cadherin (reviewed recently in Krueger et al., 2012). Accordingly, each synapse type may express its own distinct transsynaptic signaling complex, dependent on the identity of both the presynaptic and the postsynaptic neuron. As Földy et al. (2013) discuss, it is conceivable that the Nlgn3^{R451C} substitution may exert distinct effects on the binding affinity to various transsynaptic partners, thereby differentially shifting the composition of the transsynaptic signaling complex at PV basket cell and CCK basket cell synapses. The consequence of this complexity is that it becomes challenging to predict the relevance of a given mutation for ASD-related phenotypes without directly assessing its effects in a synapse-specific and circuitry-specific manner. The use of genetic strategies to selectively target individual types of synapses, as well as methods to elucidate the molecular identity of transsynaptic signaling complexes in a synapse-specific manner, will be essential to fully eludicate the role of Neuroligins in normal synapse development and in disorders of the synapse.

A second key implication arising from the present study is that dysregulation of the endocannabinoid system may play an important role in ASD pathophysiology and may therefore represent a target for pharmacological intervention. A similar strategy was previously employed to identify the metabotropic glutamate receptor (mGluR) signaling pathway as a target for drug development in several mouse models of ASD-related disorders, including fragile X syndrome (Bear et al., 2004), tuberous sclerosis complex (Auerbach et al., 2011), and Nlgn3 deletion (Baudouin et al., 2012), and clinical trials based on these findings are underway. Whether targeting the endocannabinoid system in the context of ASDs will prove to be similarly promising remains to be seen, and additional research will be necessary to build upon this notion. Interestingly, however, aberrant activation of the endocannabinoid system was also recently reported in a mouse model of fragile X syndrome (Busquets-Garcia et al., 2013; Jung et al., 2012). Together with these findings, the data presented by Földy et al. indicate that further analyses of the link between endocannabinoid signaling and ASDs may provide valuable insights into the pathophysiology and potential treatment strategies for ASDs.

REFERENCES

Auerbach, B.D., Osterweil, E.K., and Bear, M.F. (2011). Nature *480*, 63–68.

Baudouin, S.J., Gaudias, J., Gerharz, S., Hatstatt, L., Zhou, K., Punnakkal, P., Tanaka, K.F., Spooren, W., Hen, R., De Zeeuw, C.I., et al. (2012). Science *338*, 128–132.

Bear, M.F., Huber, K.M., and Warren, S.T. (2004). Trends Neurosci. *27*, 370–377.

Busquets-Garcia, A., Gomis-Gonzalez, M., Guegan, T., Agustin-Pavon, C., Pastor, A., Mato, S., Perez-Samartin, A., Matute, C., de la Torre, R., and Dierssen, M. (2013). Nat. Med. Published online March 31, 2013. http://dx.doi.org/10.1038/nm.3127.

Etherton, M., Földy, C., Sharma, M., Tabuchi, K., Liu, X., Shamloo, M., Malenka, R.C., and Südhof, T.C. (2011). Proc. Natl. Acad. Sci. USA *108*, 13764–13769.

Földy, C., Malenka, R.C., and Südhof, T.C. (2013). Neuron *78*, this issue, 498–509.

Jamain, S., Quach, H., Betancur, C., Råstam, M., Colineaux, C., Gillberg, I.C., Soderstrom, H., Giros, B., Leboyer, M., Gillberg, C., and Bourgeron, T.; Paris Autism Research International Sibpair Study. (2003). Nat. Genet. *34*, 27–29.

Jung, K.-M., Sepers, M., Henstridge, C.M., Lassalle, O., Neuhofer, D., Martin, H., Ginger, M., Frick, A., DiPatrizio, N.V., and Mackie, K. (2012). Nat. Comm. *3*, 1080.

Katona, I., and Freund, T.F. (2012). Annu. Rev. Neurosci. *35*, 529–558.

Krueger, D.D., Tuffy, L.P., Papadopoulos, T., and Brose, N. (2012). Curr. Opin. Neurobiol. *22*, 412–422.

Lisman, J., and Buzsáki, G. (2008). Schizophr. Bull. *34*, 974–980.

Murdoch, J.D., and State, M.W. (2013). Curr. Opin. Genet. Dev. Published online March 25, 2013. http://dx.doi.org/10.1016/j.gde.2013.02.003.

Radyushkin, K., Hammerschmidt, K., Boretius, S., Varoqueaux, F., El-Kordi, A., Ronnenberg, A., Winter, D., Frahm, J., Fischer, J., Brose, N., and Ehrenreich, H. (2009). Genes Brain Behav. *8*, 416–425.

Sanders, S.J., Ercan-Sencicek, A.G., Hus, V., Luo, R., Murtha, M.T., Moreno-De-Luca, D., Chu, S.H., Moreau, M.P., Gupta, A.R., Thomson, S.A., et al. (2011). Neuron *70*, 863–885.

Tabuchi, K., Blundell, J., Etherton, M.R., Hammer, R.E., Liu, X., Powell, C.M., and Südhof, T.C. (2007). Science *318*, 71–76.