more likely. We hope that re-examination of these and other physiological puzzlers will be inspired by the success of Jeworutzki et al. (2012) in uncovering one of only a handful of known auxiliary subunits for the elusive CLC family.

## REFERENCES

Blanz, J., Schweizer, M., Auberson, M., Maier, H., Muenscher, A., Hübner, C.A., and Jentsch, T.J. (2007). J. Neurosci. *27*, 6581–6589.

Brignone, M.S., Lanciotti, A., Macioce, P., Macchia, G., Gaetani, M., Aloisi, F., Petrucci, T.C., and Ambrosini, E. (2011). Hum. Mol. Genet. *20*, 90–103.

Clapham, D. (2001). Neuron *29*, 1–3.

Cuddapah, V.A., and Sontheimer, H. (2010). J. Biol. Chem. *285*, 11188–11196.

Jentsch, T.J. (2008). Crit. Rev. Biochem. Mol. Biol. *43*, 3–36.

Jeworutzki, E., López-Hernández, T., Capdevila-Nortes, X., Sirisi, S., Bengtsson, L., Montolio, M., Zifarelli, G., Arnedo, T., Müller, C.S., Schulte, U., et al. (2012). Neuron *73*, this issue, 951–961.

Leegwater, P.A., Yuan, B.Q., van der Steen, J., Mulders, J., Könst, A.A., Boor, P.K., Mejaski-Bosnjak, V., van der Maarel, S.M., Frants, R.R., Oudejans, C.B., et al. (2001). Am. J. Hum. Genet. *68*, 831–838.

Matsuda, J.J., Filali, M.S., Collins, M.M., Volk, K.A., and Lamb, F.S. (2010). J. Biol. Chem. *285*, 2569–2579.

Menichella, D.M., Goodenough, D.A., Sirkowski, E., Scherer, S.S., and Paul, D.L. (2003). J. Neurosci. *23*, 5963–5973.

Neusch, C., Rozengurt, N., Jacobs, R.E., Lester, H.A., and Kofuji, P. (2001). J. Neurosci. *21*, 5429–5438.

Nighot, P.K., Moeser, A.J., Ryan, K.A., Ghashghaei, T., and Blikslager, A.T. (2009). Exp. Cell Res. *315*, 110–118.

Ratté, S., and Prescott, S.A. (2011). J. Neurosci. *31*, 15838–15843.

Rinke, I., Artmann, J., and Stein, V. (2010). J. Neurosci. *30*, 4776–4786.

Scheper, G.C., van Berkel, C.G., Leisle, L., de Groot, K.E., Errami, A., Jentsch, T.J., and Van der Knaap, M.S. (2010). Genet Test Mol Biomarkers *14*, 255–257.

Wang, X.Q., Deriy, L.V., Foss, S., Huang, P., Lamb, F.S., Kaetzel, M.A., Bindokas, V., Marks, J.D., and Nelson, D.J. (2006). Neuron *52*, 321–333.

Xiong, D., Heyman, N.S., Airey, J., Zhang, M., Singer, C.A., Rawat, S., Ye, L., Evans, R., Burkin, D.J., Tian, H., et al. (2010). J. Mol. Cell. Cardiol. *48*, 211–219.

Yang, L., Ye, D., Ye, W., Jiao, C., Zhu, L., Mao, J., Jacob, T.J., Wang, L., and Chen, L. (2011). J. Cell. Physiol. *226*, 2516–2526.

# How Do Neurons Sense a Spike Burst?

György Buzsáki[1,*]
[1]The Neuroscience Institute, New York University, New York, NY 10016, USA
*Correspondence: buzsaki@andromeda.rutgers.edu
DOI 10.1016/j.neuron.2012.02.013

**In this issue of *Neuron*, Xu et al. (2012) show that knock down of Syt1, a major $Ca^{2+}$ sensor, impairs synaptic transmission similarly in different brain regions but with unexpected, region-specific behavioral outcomes.**

Several decades ago, I used to listen to rock and roll by tuning in to Radio Free Europe with a small headphone, basically a magnetic coil and a metal diaphragm, so that the neighbors could not suspect my illegal activities. That of course was not the same thing as being in a concert hall, enjoying the entire frequency spectrum and perceiving the pitch, melody, harmony, and timbre content of the music but despite the high-pass filtering properties of the low quality earphone the rhythm and other remnant features of the broadcasted music made the experience still enjoyable. As engineers know, high-pass frequency filtering of signals makes communication poorer but not hopeless. Now suppose that we introduce high-pass filters in the communication lines between neurons in the brain. This is exactly what Xu et al., (2012) have accomplished, using molecular biological tools. They find that after such manipulation neuronal transmission becomes sluggish but is not completely abolished. For some structures and tasks, such as the hippocampus-dependent contextual fear learning task, high-pass filtering is tolerated, whereas for a prefrontal cortex-dependent remote memory recall, sluggishness of spike communication leads to a serious behavioral impairment.

Let's examine first how communication between neurons was achieved. Neurons communicate electrochemically. The upstream neuron generates a spike, which is broadcasted to all or most of its presynaptic terminals. Here, electricity is converted to chemically mediated synaptic transmission. This conversion process can be perturbed in multiple ways. For example, tetanus toxin (TetTox) can block transmitter release and thus completely eliminate synaptic communication. Other interventions can produce a more subtle interference. Synaptotagmin-1 (Syt1), together with other vesicle proteins, is essential for the docking and/or fusion of synaptic vesicles with the presynaptic plasma membrane following depolarization and $Ca^{2+}$ influx in presynaptic bouton. Eliminating or interfering with Syt1 also impairs synaptic transmission to single, isolated spikes yet when high enough amount of $Ca^{2+}$ enters the terminal in response to high-frequency spike activity chemical transmission is resumed, although it remains sluggish due to the asynchronous release of the transmitter (Maximov and Südhof, 2005). Put simply, interfering with Syt1 amounts to the introduction of a high-pass frequency filter: no or poor

transmission at low rates of spiking but gradual restoration of the transmitter release at increasing spike frequencies. What are the physiological and, ultimately, behavioral consequences of such frequency-selective mechanisms? To explore this question, Xu and colleagues (2012) used a virus-targeted approach to knock down Syt1 in the brain of mice.

After demonstrating the proof of principle in cultured cortical neurons, the authors generated recombinant adeno-associated viruses (AAV-DJ) to express only enhanced green fluorescent protein (EGFP, which served as a control), or only TetTox, or to express both EGFP and the Syt1-coding shRNA. With such convenient tools in hand, Xu and colleagues (2012) infected neurons in the dorsal hippocampus, the entorhinal cortex, and prefrontal cortex. As expected, electrical stimulation of TetTox expressing CA1 pyramidal cells failed to excite their subicular targets. The situation was similar in Syt1-infected mice when the stimulation frequency was low, but synaptic transmission increased when trains of stimuli at 10 Hz or faster were used. Importantly, when the frequency was increased to 200 Hz, just 3 to 5 stimuli were sufficient to achieve charge transfer comparable or even stronger than in the control (AAV-EGFP) neurons, although the onset of the response was delayed by several milliseconds. Thus, while the temporal precision of transmission suffered, downstream neurons still responded to high-frequency spikes. Even long-term potentiation was retained in Syt1-infected animals.

When the mice were tested in a contextual fear conditioning paradigm, the results with TetTox injections largely confirmed previous investigations using more traditional methods. Recent memory was impaired in animals with the virus injected in the hippocampus and entorhinal cortex, whereas remote memory (tested several weeks after fear conditioning and the virus injection) was affected only in the prefrontal group. However, the results with Syt1-infected mice were surprising. While recent fear memory was seriously impaired after entorhinal Syt1 knockdown, Syt1 hippocampal mice performed just like the controls. Animals with Syt1 infections in the prefrontal cortex were comparable to their TetTox peers. In summary, high-pass frequency filtering of spikes by Syt-1 did not matter much in the hippocampus but was devastating in both the entorhinal cortex and prefrontal cortex. On the basis of these spectacular findings, Xu and colleagues (2012) suggest that different spike coding mechanisms are at work in the three different brain regions. Hippocampal circuits can rely on bursts of spikes only, whereas the paleo- and neocortex networks need high temporal precision of single spikes for coding, at least for the mediation of contextual fear memory.

The authors' account of their findings may indeed be right. Yet, one might also consider the possibility that it is not necessarily the precision of spikes that matters, but rather the extent to which each structure is able to communicate via high frequency bursts, and thus overcome the genetic manipulation. As the authors point out, cortical neurons can fire both single spikes and complex spike bursts and the bursts may be critical for spike transmission under certain conditions (Lisman, 1997). Unfortunately, there is no natural frequency border between single spikes and spike bursts and the interspike interval statistic reflects a renewal process where spiking history is critical (Harris et al., 2001). Traditionally, a spike burst is defined as three or more spikes with < 8 ms intervals (Ranck, 1973). In the hippocampus, spike doublets and triplets of pyramidal cells at such short intervals occur 14% and 3% of all spikes during exploration. A burst of 4 spikes is rare (0.4%) and 5 or more spikes is super rare (0.06%) although these fractions can increase several-fold during sleep. Importantly, burst fractions in the hippocampus are almost an order of magnitude higher than in the entorhinal cortex (Mizuseki et al., 2009) or the prefrontal cortex (Fujisawa et al., 2008). In the entorhinal cortex, layer II stellate cells are the best bursters but still far less efficient than their hippocampal peers. One may therefore speculate that these intrinsic differences in the propensity of bursting can explain why Syt1 knockdown had so much less of an impact on behavior in the hippocampus than in other areas.

In addition to the properties of pyramidal cells, consider the mossy terminal, one of the largest synapses in the mammalian brain connecting the dentate granule cells with CA3 pyramidal cells. This giant synapse has hundreds of release sites. A single spike in a granule cell can only discharge inhibitory interneurons. On the other hand, a burst of spikes in one granule cell is sufficient to bring its target pyramidal cells to spike threshold (Henze et al., 2002). Since the mossy terminal relies on high-frequency communication under physiological conditions, one may predict that the dentate-CA3 communication is perhaps not seriously impaired in Syt1 mice, although this conjecture needs to be tested. Thus, assuming everything else being equal, the high propensity of bursts in the hippocampus and the burst-dependent nature of the mossy synapse may explain why high-pass frequency filtering by Syt1 knockdown was well tolerated by the hippocampal networks. Other circuits, such as the entorhinal cortex and prefrontal cortex, failed simply because their neurons do not generate enough high frequency bursts in the first place under physiological conditions.

Another potential consideration when interpreting the findings is the complexity of neural network dynamics and the resilience of cortical networks to injury/manipulations. For example, it is possible that other types of compensatory mechanisms are also at play in Syt1 knockdown mice. Indeed, Syt1 is often colocalized with Syt2, especially in the hippocampus (Fox and Sanes, 2007). Proper timing in cortical circuits often depends on oscillations, supported by the large family of interneurons (Freund and Buzsáki, 1996). Inhibitory terminals are also equipped with Syt1 but their genetic elimination is less remarkable than in excitatory terminals (Kerr et al., 2008), perhaps because of the high-frequency firing of interneurons or because other $Ca^{2+}$ sensors are more important in the control of inhibitory terminals than Syt1. Furthermore, dendrite-targeting but slow firing inhibitory neurons are efficient burst controllers (Royer et al., 2012), so that failure of Syt1-mediated inhibition of dendritic $Ca^{2+}$ influx can lead to stronger bursting in pyramidal cells. Thus, in circuits with both inhibitory and excitatory synapses the overall spike output from pyramidal cells may depend deeply on the wiring details and synapse dynamic. To explain

the interesting results of Xu et al. (2012) by these more complex explanations, one needs to assume that the basic inhibitory/excitatory network dynamics are different in the hippocampus and other cortical areas.

Whatever the final answers to these many remaining questions will be, the experiments by Xu and colleagues (2012) clearly demonstrate that the newly emerging molecular tools (Fenno et al., 2011; Magnus et al., 2011; Nakashiba et al., 2009) for blocking or enhancing synaptic activity open new possibilities to examine neuronal communication in the behaving animal. The findings of Xu et al. (2012) are an important milestone in this direction. A perceived handicap of molecular biological tools, compared to electrophysiological methods, is their slow time resolution. However, it has become increasing clear not only that efficient timing in the brain depends on fast acting chemical mechanisms but that such processes can be precisely explored by targeted molecular biological approaches, such as demonstrated Xu et al. (2012). Who would have thought just a few years ago that words like "high-pass filtering" and "oscillations" might become part of the everyday discourse in molecular biology labs?

## REFERENCES

Fenno, L., Yizhar, O., and Deisseroth, K. (2011). Annu. Rev. Neurosci. *34*, 389–412.

Fox, M.A., and Sanes, J.R. (2007). J. Comp. Neurol. *503*, 280–296.

Freund, T.F., and Buzsáki, G. (1996). Hippocampus *6*, 347–470.

Fujisawa, S., Amarasingham, A., Harrison, M.T., and Buzsáki, G. (2008). Nat. Neurosci. *11*, 823–833.

Harris, K.D., Hirase, H., Leinekugel, X., Henze, D.A., and Buzsáki, G. (2001). Neuron *32*, 141–149.

Henze, D.A., Wittner, L., and Buzsáki, G. (2002). Nat. Neurosci. *5*, 790–795.

Kerr, A.M., Reisinger, E., and Jonas, P. (2008). Proc. Natl. Acad. Sci. USA *105*, 15581–15586.

Lisman, J.E. (1997). Trends Neurosci. *20*, 38–43.

Magnus, C.J., Lee, P.H., Atasoy, D., Su, H.H., Looger, L.L., and Sternson, S.M. (2011). Science *333*, 1292–1296.

Maximov, A., and Südhof, T.C. (2005). Neuron *48*, 547–554.

Mizuseki, K., Sirota, A., Pastalkova, E., and Buzsáki, G. (2009). Neuron *64*, 267–280.

Nakashiba, T., Buhl, D.L., McHugh, T.J., and Tonegawa, S. (2009). Neuron *62*, 781–787.

Ranck, J.B., Jr. (1973). Exp. Neurol. *41*, 461–531.

Royer, S., Zemelman, B.V., Losonczy, A., Kim, J., Chance, F., Magee, J.C., and Buzsáki, G. (2012). Nat. Neurosci., in press.

Xu, W., Morishita, W., Buckmaster, P.S., Pang, Z.P., Malenka, R.C., and Südhof, T.C. (2012). Neuron *73*, this issue, 990–1001.

# Stop and Be Fair: DLPFC Development Contributes to Social Decision Making

**Aidan Makwana[1] and Todd Hare[1,*]**
[1]Laboratory for Social and Neural Systems Research, Department of Economics, University of Zürich, Blümlisalpstrasse 10, 8006 Zürich, Switzerland
*Correspondence: todd.hare@econ.uzh.ch
DOI 10.1016/j.neuron.2012.02.010

**In this issue of *Neuron*, Steinbeis et al. (2012) show that DLPFC structure and functions are associated with strategic social choices during an economic task and relate to impulse control abilities in both age dependent and independent manners.**

Interpersonal interactions frequently involve balancing the desires of another person with one's own interests in order to achieve a mutually satisfactory outcome. Take the example of a storeowner or street vendor. The seller will try to name a price that the customer is willing to pay, but not any less, in order to maximize profit. Strategic actions such as this price setting are common in economic transactions and the neural mechanisms that mediate the balancing of self versus other's goals are of great interest to scientists studying the neurobiology of decision making. Previous reports have indicated a role for prefrontal cortex in strategic social decisions (Bhatt et al., 2010; Coricelli and Nagel, 2009; Spitzer et al., 2007). Given the relatively late maturation of prefrontal regions (Durston et al., 2006; Giedd et al., 1999), developmental studies of strategic behavior could provide insights into the role of prefrontal cortex in decision making. Clearly, the causal nature of child development and brain maturation is complex, and both age-dependent and -independent changes in neural systems may be linked to specific aspects of behavior. In this issue of *Neuron*, Steinbeis and colleagues (2012) have examined how age and developmental differences in impulsivity along with the structure and function of prefrontal cortex relate to strategic decision making. These results provide novel insights about the development of prefrontal cortex and its role in strategic economic decisions. Moreover, the findings raise several interesting questions for future research.