

Long-term patch recordings from adult spinal neurons herald new era of opportunity

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Submitted 26 September 2011; accepted in final form 26 September 2011

Hochman S. Long-term patch recordings from adult spinal neurons herald new era of opportunity. *J Neurophysiol* 106: 2794–2795, 2011. First published September 28, 2011; doi:10.1152/jn.00873.2011.—Recently, Andreas Husch, Nathan Cramer, and Ronald M. Harris-Warrick achieved a remarkable breakthrough in patch-clamp recordings of ventral horn neurons in the adult spinal cord slice preparation. This landmark study that breaks the “age barrier” is titled “Long-duration perforated patch recordings from spinal interneurons of adult mice” (Husch et al., in press). In it, the authors demonstrate the unprecedented ability to undertake day-long (up to 12 h), and utterly stable perforated patch recordings. A description of the methodology is detailed in their paper. Here, I give a brief overview before providing context to this extraordinary achievement.

spinal cord; perforated patch; interneuron

HUSCH ET AL. (IN PRESS) DESCRIBE THE INCORPORATION of two technical strategies that combine to enable stable and very long-duration recordings. The first technique emphasizes the surgical approach and preparation of spinal cord slices, and the second technique is on the use of the perforated patch approach with amphotericin B. Overall, while the method has notable challenges, when implemented successfully, it will undoubtedly open a tantalizingly broad window for experimentation, on neural function, neurological dysfunction, and aging in the adult spinal cord.

Isolating the spinal cord. The authors first limit hypoxia by optimizing removal of the spinal cord by a method similar to that undertaken by Bennett’s group in rat (Li et al. 2004). This is accomplished by cord exposure via laminectomy with subsequent local surface application of an ice-cold modified artificial cerebrospinal fluid solution to reduce metabolism. Further enhancing oxygenation by providing 100% O₂ for the mice to breathe was not included in this approach [cf. (Li et al. 2004), but may be needed to afford greater protection from hypoxia if motoneurons are targeted]. Carp and colleagues (2008) successfully recorded with sharp electrodes from adult rat motoneurons in spinal slice by a comparable method, but like their predecessors (Aghajanian and Rasmussen 1989; Hori et al. 2001), they also included transcardial perfusion of ice-cold modified artificial cerebrospinal fluid. Again, this added layer of neuroprotection may afford greater survival of motoneurons from hypoxia, but was clearly not necessary for the interneurons targeted in the Husch et al. study (in press).

Perforated patch-clamp recordings. Successful implementation of the perforated patch technique plainly requires meticulous attention to detail (Rae et al. 1991). Pore-forming molecules like amphotericin B prevent the formation of gigaohm seals, so the electrode tip must be filled in solution without it. As recordings in slices are typically below the surface, positive pressure is required to clear debris, so cell

targeting and subsequent membrane suction must occur before pore-forming molecules reach the electrode tip. However, if they are too far from the tip, pore formation would be too slow and incomplete to obtain the desired low series resistance. So, the right electrode tapers, the right amount amphotericin-free solution at the tip, and the right amount of positive pressure undertaken for the right amount of time all must be tightly calibrated to bring this approach to fruition. While not explored, the possibility of utilizing this technique in “blind” targeting approaches from thicker slices (Blanton et al. 1989) is also feasible within the same time constraints (Hall et al. 1999).

Time is a treasure. When it comes to cellular physiology, there are few luxuries greater than time and recording stability. With these luxuries come reproducible application of agonists and antagonists, time to study long-term changes, and dose/physiological-response relations can be undertaken without the worry of cell dialysis-induced time-dependent changes in function. A systematic pharmacological dissection of the prospective role of many metabotropic and ionotropic receptors within an individual neuron also becomes feasible. Moreover, due to the remarkable recording stability, as stated by Husch et al. (in press), “it is possible to discern even subtle effects of neuromodulators on the intrinsic properties of neurons, which were previously difficult to measure due to the rundown of various intrinsic currents in whole cell recordings.”

A window into disease, injury, and aging. Most central nervous system (CNS) disorders present in adulthood, and many include alterations in spinal circuit function. This includes neurodegenerative disorders (e.g., amyotrophic lateral sclerosis), epilepsies (e.g., spinal myoclonus), and injury-induced disorders (e.g., spinal cord injury). For instance, spinal cord injury imposes alterations in sensory, motor, and autonomic circuits with clear interneuronal involvement. How interneuronal properties are altered has not been examined in vitro in adult interneurons, and so has remained the territory of in vivo approaches with inherent limitations in neuron identity, recording stability, and drug access. The same limitations could be said for studies of other disorders with spinal cord involvement. Moreover, how aging-induced alterations in motor coordination reflect alterations in spinal interneuronal function could now be explored using this recording approach.

How generalizable are these findings, and what is the secret for long-duration recordings? In this study, the oldest animals were 3 mo old, but there is no reason to believe that older animals will restrict success. However, nothing is said about whether ensheathing perineuronal nets (PNNs), a likely physical barrier to membrane access, were encountered while using this method. Not all neurons are encapsulated in PNNs, and the genetically identified V2A interneuron population was selectively targeted. The spinal cord ventral horn has the greatest percentage of neurons enshrouded in PNNs (~60%) than any

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other CNS region (Bertolotto et al. 1996). In dorsal horn, less than 15% of neuron cell bodies are draped in PNNs. An intriguing possibility is that the patch electrode was able to locally breach an ensheathing PNN, but its continued presence (perhaps dependent on lack of cell dialysis) helped maintain structural integrity to greatly enhance recording duration and stability. If a relationship between PNNs and recording stability exists, this method would not be uniformly applicable to all spinal neurons and may differ after procedures that alter neuronal ensheathment [e.g., chondroitinase treatment-induced plasticity after spinal cord injury (e.g., Alilain et al. 2011)]. More generally, why consistently long-duration recordings have not been highlighted in other CNS regions, and whether this relates to an absence of PNNs (Bertolotto et al. 1996) and/or lack of use of amphotericin B (Kawamura and Wahler 1994) remains to be determined.

In summary, the Husch et al. (in press) paper will undoubtedly inspire a new era of experimentation. While neither the approach to spinal cord isolation nor the use of perforated patch recordings is technically novel, the combination leads to a landmark and absolutely transformative advance in the study of adult spinal cord interneurons implicated in motor control. It is no exaggeration to say that the present study breaks a critical barrier in electrophysiological studies on spinal neuronal function, providing for the first time a gateway into age-appropriate studies on spinal disease mechanisms. Moreover, the approach should generalize to many other spinal neurons. Last, the capacity for very long-term stable recordings moves characterization of a wide range of physiological properties in individual neurons from fantasy to reality.

GRANTS

The author is indebted to the following funding agencies: Christopher and Dana Reeve Foundation, Craig Neilsen Foundation, Paralyzed Veterans of America, National Science Foundation #0745164, National Institutes of Health awards EB-006179, NS-045248, NS-40893, and NS-40440.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S. H. drafted manuscript; S. H. edited and revised manuscript; S. H. approved final version of manuscript.

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