

Maturation of neuron excitability and membrane conductances in the superficial dorsal horn of the mouse spinal cord

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Neurons in the superficial dorsal horn (SDH; laminae I-II) of the spinal cord play a critical role in processing nociceptive, thermal and tactile information. As in other CNS regions the output of SDH neurons is determined by the combined action of synaptic inputs, and intrinsic membrane properties. It is well established that synaptic inputs in the SDH, especially those of primary afferents, undergo extensive reorganization during early postnatal development. It is unclear, however, whether or how interneuron excitability and membrane conductances change during development. In this study we assessed membrane properties and whole cell currents in SDH interneurons during late embryonic (E15-17) and early postnatal (P0-25) development. Mice (C57Bl/6) were anaesthetised with Ketamine (100 mg/kg, i.p.) and decapitated. Transverse slices were prepared from the lumbar spinal cord (segments L3-L5). Whole-cell patch-clamp recordings were obtained from SDH neurons at 32°C using a KCH_3SO_4 based internal solution. Data were grouped into one embryonic and five postnatal developmental stages (E15-17, $n = 51$ neurons; P0-5, $n = 60$; P6-10, $n = 49$; P11-15, $n = 54$; P16-20, $n = 49$; and P21-25, $n = 52$). Several passive and active membrane properties changed dramatically over this period. For example, input resistance decreased from over 1000 M Ω ; at E15-17 to ~ 400 M Ω ; in P21-25 neurons, and resting membrane potential became more hyperpolarized (-53.8 ± 1.5 to 63.4 ± 1.4 mV) over the same period. Action potential (AP) properties such as spike amplitude and afterhyperpolarization amplitude increased (24.9 ± 1.8 versus 43.2 ± 1.6 mV; 10.1 ± 1.8 versus 31.0 ± 0.9 mV, respectively), whereas AP half-width decreased (2.7 ± 0.4 versus 1.2 ± 0.1 ms). Comparisons of mean values for the above variables in the six age groups revealed that the transition from embryonic/neonatal to adult-like passive and active membrane properties occurred around P6-10. We next examined the discharge properties of SDH neurons in response to step-current injection (800 ms, 20 pA increments). Neurons were assigned into five categories according to AP discharge patterns: *tonic firers* fired spikes throughout the step duration; *initial bursters* fired several spikes at step onset; *single spikers* fired one or two spikes at step onset; *delayed firers* did not fire spikes until well after step onset; and *reluctant firers* did not fire APs during current steps. Before birth (E15-17) *single spikers* dominated the sample ($>50\%$), and *delayed firers* were not observed. Soon after birth (P0-10) *single spikers* still dominated ($\sim 41\%$) and *delayed firers* were rare ($< 1\%$). In older animals (P11-25) *initial bursters* dominated ($\sim 40\%$) and all five firing patterns were well represented. Investigation of the major subthreshold currents that contribute to the above discharge categories showed the rapid A-type potassium current (I_{Ar}) dominated at all ages examined. In embryonic neurons almost all neurons ($> 95\%$) had I_{Ar} . This decreased to $\sim 50\%$ of neurons after P10. Together, these data indicate that the membrane properties and excitability of SDH neurons undergo major alterations during development, with the critical period for these changes occurring around P6-10.