Electrophysiological characterization of mature neurons derived from mouse embryonic stem cells by Sox-1 Lineage selection and directed differentiation

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Sx1TV2/16C is a mouse embryonic stem (ES) cell line in which one copy of the *Sox1* gene, an early neuroectodermal marker, has been targeted with a neomycin (G418) selection cassette. Directed differentiation with retinoic acid and G418 selection results in an enriched neural stem cell population that can be further differentiated into neurons. After 6-7 days post plating (D6-7PP) most neurons readily fired tetrodotoxin (TTX)-sensitive action potentials arising from the activation of tetrodotoxin (TTX)-sensitive Na^+ channels. Neurons approached their maximal cell capacitance after D6-7PP, however ion channel expression continued until at least D21PP. The percentage of cells receiving spontaneous synaptic currents increased with days in culture until 100% of cells received a synaptic input by D20PP. Spontaneous synaptic currents were reduced in amplitude and frequency by TTX, or upon exposure to a Ca^{2+}-free, 2.5 mM Mg^{2+} physiological saline. Synaptic currents of rapid decay time constants (<20 ms) were reduced in amplitude with membrane depolarization and preferentially blocked by the nonNMDA glutamatergic receptor antagonists, CNQX or NBQX. Ca^{2+} levels within ES cell-derived neurons increased in response to glutamate receptor agonists L-glutamate, AMPA, N-methyl-D-aspartate (NMDA) and kainic acid (KA) and to acetylcholine, ATP and dopamine. NBQX displaced the concentration-Ca^{2+} response curve to AMPA but not to glutamate or KA. NMDA evoked a cationic membrane current which reversed at -11 mV and displayed a Mg^{2+}-dependent outward rectification (block) at negative potentials. Glycine and GABA evoked Cl^--selective currents which reversed at -70 and 78 mV, respectively. It was concluded that ES-derived neurons fire action potentials, receive excitatory and inhibitory synaptic inputs and respond to various neurotransmitters in a manner similar to primary central neurons.