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TRANSLATIONAL BIOLOGY, MEDICINE, AND HEALTH GRADUATE PROGRAM

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“EFFECTS OF CONSTITUTIVE AND ACUTE CONNEXIN 36 DEFICIENCY ON BRAIN-WIDE SUSCEPTIBILITY TO PTZ-INDUCED NEURONAL HYPERACTIVITY”

TRANSFATIONAL BIOLOGY, MEDICINE, AND HEALTH GRADUATE PROGRAM
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Connexins are transmembrane proteins that form hemichannels allowing the exchange of molecules between the extracellular space and cell interior. Two hemichannels from adjacent cells dock and form a continuous gap junction pore, thereby permitting direct intercellular communication. Connexin 36 (Cx36), expressed primarily in neurons, is involved in the synchronous activity of neurons and may play a role in aberrant synchronous firing, as seen in seizures. To understand the reciprocal interactions between Cx36 and seizure-like neural activity, we examined three questions: a) does Cx36 deficiency affect seizure susceptibility, b) does seizure-like activity affect Cx36 expression patterns, and c) does acute blockade of Cx36 conductance increase seizure susceptibility. We utilize the zebrafish pentylenetetrazol (PTZ; a GABA(A) receptor antagonist) induced seizure model, taking advantage of the compact size and optical translucency of the larval zebrafish brain to assess how PTZ affects brain-wide neuronal activity and Cx36 protein expression. We exposed wild-type and genetic Cx36-deficient (cx35.5-/-) zebrafish larvae to PTZ and subsequently mapped neuronal activity across the whole brain, using phosphorylated extracellular-signal-regulated kinase (pERK) as a proxy for neuronal activity. We found that cx35.5-/- fish exhibited region-specific susceptibility and resistance to PTZ-induced hyperactivity compared to wild-type controls, suggesting that genetic Cx36 deficiency may affect seizure susceptibility in a region-specific manner. Regions that showed increased PTZ sensitivity include the dorsal telencephalon, which is implicated in human epilepsy, and the lateral hypothalamus, which has been underexplored. We also found that PTZ-induced neuronal hyperactivity resulted in a rapid reduction of Cx36 protein levels. 30 minutes and one-hour exposure to 20 mM PTZ resulted in a rapid reduction of Cx36 protein levels. This Cx36 reduction persists after one-hour of recovery but recovered after 3-6 hours. This acute downregulation of Cx36 by PTZ is likely maladaptive, as acute pharmacological blockade of Cx36 by mefloquine results in increased susceptibility to PTZ-induced neuronal hyperactivity. Together, these results demonstrate a reciprocal relationship between Cx36 and seizure-associated neuronal hyperactivity. Cx36 deficiency contributes region-specific susceptibility to neuronal hyperactivity, while neuronal hyperactivity-induced downregulation of Cx36 may increase the risk of future epileptic events.