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Crain S M. Development of "organotypic" bioelectric activities in central nervous tissues during maturation in culture. *Int. Rev. Neurobiol.* 9:1-43, 1966.
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This review summarizes my electrophysiologic studies demonstrating that neurons isolated in long-term cultures of embryonic mammalian cerebral and spinal cord tissues show progressive development of complex bioelectric activities with remarkable mimicry of organized synaptic network functions of the central nervous system *in situ*. [The *SCI*® indicates that this paper has been cited in over 135 publications.]

Neurons Organize Functional Networks in Tissue Cultures

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These studies of the intrinsic capacity of central nervous system (CNS) neurons to organize functional synaptic networks in culture were stimulated by my demonstration, during predoctoral studies at Columbia University in the early 1950s, that embryonic sensory ganglion neurons isolated for months *in vitro* could express characteristic membrane excitability properties, including the generation of propagated action potentials in response to local electric stimuli.[1] Prior to these electrophysiologic analyses, many neuroanatomists, including my collaborators at Columbia University, Edith R. Peterson and Margaret Murray, had shown that immature nerve cells isolated in culture could sprout long nerve fibers and develop many structural features resembling neurons *in situ*. However, some investigators had expressed concerns that nerve cell functions would probably "atrophy" following isolation from the stimulating trophic and hormonal factors provided within the living organism. Furthermore, electrophysiologic studies of CNS tissue cultures in other laboratories in the early 1960s led to premature conclusions that although nerve cells isolated *in vitro* could, indeed, generate action potentials, they appeared to be unable to establish functional synaptic connections with other neurons.

In contrast, electrophysiologic analyses of more critically prepared spinal cord tissues explanted from human as well as mouse embryos (in collaboration with Peterson, at Columbia) provided the first compelling data (in 1962) demonstrating that CNS neurons developing in culture could generate complex patterned "organotypic" bioelectric activities, i.e., resembling organized synaptic network discharges generated by the CNS *in situ*.[2] Shortly thereafter, in collaboration with Murray B. Bornstein, I showed that similar synaptic network discharges, including rhythmic electroencephalogram-like components, could be recorded during the maturation of fetal mouse cerebral neocortex explants in culture.[1]

My 1966 review of these and related electrophysiologic studies of CNS cultures was evidently the turning point in convincing neuroscientists that embryonic neurons isolated *in vitro* can provide remarkably reliable and powerful model systems for experimental analyses of cellular and molecular mechanisms underlying development and function of the nervous system. This review was published shortly after I moved my laboratory from Columbia University to the Albert Einstein College of Medicine, where I had just been honored with a five-year Kennedy Scholar Award by the Joseph P. Kennedy Foundation for Mental Retardation. During the following decade, dozens of laboratories, throughout the US and abroad, began to utilize similar culture techniques for electrophysiologic and pharmacologic analyses of neuronal functions, as reviewed in my 1976 monograph[1] (a Russian translation of which was published in the USSR in 1980).

Our research group has placed increasing emphasis on mechanisms underlying the development of *specific* synaptic connections in the CNS, utilizing cultures of hippocampal explants,[3] spinal cord explants innervated by sensory dorsal root ganglion neurons,[4] and tectal explants innervated by retinal ganglion cells.[4] After establishing that many of these neurons were able to express *in vitro* characteristic pharmacologic sensitivities to a wide variety of neurotransmitters and neuromodulators,[1] we have more recently focused on spinal cord-ganglion cultures for analyses of cellular and molecular mechanisms underlying opioid analgesia, tolerance, and addiction.[5,6]

1. Crain S M. *Neurophysiologic studies in tissue culture*. New York: Raven Press, 1976. 280 p. (Cited 150 times.)
2. Crain S M & Peterson E R. Bioelectric activity in long-term cultures of spinal cord tissues. *Science* 141:427-9, 1963. (Cited 25 times.)
3. Crain S M & Bornstein M B. Early onset in inhibitory functions during synaptogenesis in fetal mouse brain cultures. *Brain Res.* 68:351-7, 1974. (Cited 45 times.)
4. Crain S M. Role of CNS target cues in formation of specific afferent synaptic connections in organotypic cultures. (Pfeiffer S E, ed.) *Neuroscience approached through cell culture*. Boca Raton, FL: CRC Press, 1983. Vol. II p. 1-32. (Cited 5 times.)
5. Crain S M, Crain B & Peterson E R. Cyclic AMP or forskolin rapidly attenuates the depressant effects of opioids on sensory-evoked dorsal-horn responses in mouse spinal cord-ganglion explants. *Brain Res.* 370:61-72, 1986.
6. Crain S M, Shen K-F & Chalazonitis A. Opioids excite rather than inhibit sensory neurons after chronic opioid exposure of spinal cord-ganglion cultures. *Brain Res.* 455:99-109, 1988.