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Long-Term Neuroplasticity and Functional Consequences of Single Versus Recurrent Early-Life Seizures

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"…If it were done but once"

John Donne, 1571–1631 (Easter Day)

The fundamental differences between the impact of a single seizure (of any duration) and that of recurrent seizures on the neonatal and infant brain are progressively being delineated. For single seizures, the absence of cell death and of significant synaptic reorganization, together with minimal cognitive deficits, distinguish the consequences of those occurring early in life from those in the mature CNS. However, single early-life seizures in several animal models have recently been shown to lead to more subtle functional changes, likely related to selective and persistent alterations in developmental programs of gene expression.

The consequences of *recurrent* short seizures during the neonatal or infancy periods are less well established. The paper by Chang and colleagues[1], in this issue adds significantly to our understanding of these long-term sequelae in an immature rodent model of recurrent febrile seizures. The data of Chang and colleagues, together with related recent work, delineate an array of potentially important molecular and functional consequences of recurrent early-life seizures. The putative mechanisms of these changes, their universality and their relevance to the human condition are discussed.

Single and Recurrent Early Life Seizures: The Problem

Much effort over several decades has been devoted to understanding the effects of single seizures on the developing brain. Because of the potential presence of factors pre-disposing to seizure occurrence or to the development of seizure-induced injury in human infants and children, and because of ethical issues inherent in human studies, animal models have been generated to address this question[2-4]. The comparison and interpretation of these animal studies have been confounded by their divergence in several important regards: First, these studies have varied in their definitions of the terms "neonatal", "infant", or "juvenile", and were conducted on rats aged 0-35 days (P0-P35). Thus, even ignoring the authors' disparate opinions of the correlation of hippocampal or "total brain" maturational states in rodents and humans on specific postnatal days[5] the many studies in the literature have covered a prolonged epoch of rapidly evolving cortical development. Therefore, age-specific factors governing vulnerability and resistance to seizures exist even *within* the group of studies focusing on "the developing brain", in addition to better-established distinctions between immature and mature CNS.

An additional complexity derives from the use of numerous and diverse convulsant agents, that elicit hyper-excitability and seizures from a variety of brain regions. Seizures evoked by the limbic convulsants kainic acid[6], hyperthermia,[1,7,8] hypoxia,[9] or tetanus toxin[10,11], should not be expected to influence the immature CNS in the same manner as generalized or mixed seizures provoked by flurothyl,[12,13] lithium-pilocarpine[14-16] or pentylenetetrazol,[17] particularly when the convulsants are administered at dissimilar doses and routes. Finally, the

methods chosen to assess seizure outcome, including cell death, learning and memory deficits, or the development of spontaneous seizures (epilepsy) have differed significantly among research groups.

In view of these confounders, it is quite remarkable that a broad consensus *has* emerged from the majority of these studies: single seizures, whether physiologically induced (models of asphyxia and fever) or provoked by toxins or drugs, do not generally kill neurons before the end of the second and perhaps third postnatal week.[7,18,19] [Note that extremely prolonged status epilepticus lasting many hours might lead to neuronal death in certain models.][20,21]

In the "real world", however, whereas seizures never recur in ~60% of children,[22] they may constitute an epileptic condition characterized by recurrent and often frequent seizures during infancy and childhood. Therefore, several groups have set out to reproduce recurrent early-life seizures in animal models, using hyperthermia[7,23] as reported by Chang and colleagues as well as flurothyl[19], kainic acid,[24] and tetanus toxin.[10]

Recurrent early-life seizures may differ from single seizures in their effects on long-term cognitive function. Critical parameters include age, the number of seizures, their severity and distribution

It is instructive to compare the long-term consequences of single and recurrent early-life seizures on neuronal function. Single seizures elicited by the prototypical limbic convulsant kainic acid, as well as by experimental fever, have been found to reduce seizure threshold later in life.[8,25] This effect was presumably mediated via permanent influence on a panoply of cellular components including neurotransmitter release mechanisms, ion channels and receptors[15,26-28] or post-receptor, "downstream" effects that are outside the scope of this editorial.

Interestingly, the majority of evidence suggests that a *single* prolonged limbic seizure (even status epilepticus, SE) does not lead to measurable deficits in hippocampus-mediated memory function if occurring during the first three postnatal weeks.[29] Limbic SE in older animals may result in long-term memory dysfunction,[29] highlighting the role of age (i.e., maturation of the limbic-hippocampal circuit). In addition, prolonged and generalized SE, evoked, for example by flurothyl, can induce hippocampal cognitive impairment even when inflicted at the end of the third postnatal week-[30] emphasizing the importance of seizure type and severity.

Recurrent limbic seizures, when evoked by kainate, did not impair hippocampal-dependent learning and memory.[24] It should be noted that in this study, only four seizures were induced at 2 day intervals between P20-P28. In contrast, when starting at P10, recurrent seizures evoked by tetanus toxin injection impaired hippocampal function.[11] Finally, 25 short but severe generalized seizures provoked (by flurothyl) on P0-P5 led to significant long-term cognitive deficits.[31] Thus, for recurrent seizures- as for single ones—precise age within the "developmental period", and the number and type of seizures are critical determinants of outcome.

The study by Chang and colleagues illustrates these points. The authors relied on an established model of febrile seizures[7,8,27] to evoke limbic seizures on P10-12. They compared the effects of one, three and nine episodes, each lasting less than 5 minutes, on hippocampus-mediated learning and memory functions. Only animals sustaining nine seizures within 72 hours demonstrated cognitive impairment. A similar dose-effect of hyperthermia-evoked seizures was reported in a different model of febrile seizures.[23] It might be noted that Chang and colleagues,[1] did not find that recurrent or single seizures reduced seizure threshold to a convulsant challenge later in life, in contrast to earlier findings in the febrile and kainate models of limbic seizures.[8,25] The discrepancy might stem from the nature of the second convulsant

employed by the authors. Whereas both *in vivo* and *in vitro* susceptibility to chemical or electrical limbic excitation was described after early-life seizures,[8] Chang and colleagues challenged their animals with pentylenetetrazol, a GABA receptor antagonist eliciting generalized seizures.

In summary, the long-term consequences of recurrent early-life seizures differ from those of a single one in several models, including chemical and physiological convulsants. A common denominator of these models is a narrow developmental age (P10-12), and involvement of the limbic-hippocampal circuit. Indeed, when present, the consequences of either single or recurrent seizures in these models involve deficits or vulnerability of hippocampal function.

The mechanisms by which early-life recurrent seizures influence hippocampal function long-term do not involve “the usual suspects”

Clearly, understanding the mechanisms by which early-life seizures impact or endanger the hippocampus would be very helpful, because it would permit design of specific interventions. While global interruption of excitatory mechanisms can block many post-seizure excitotoxic processes, this approach may not be suitable to the developing brain, because it interferes with normal neuronal processes. Defining selective or circumscribed mechanisms for functional injury will permit the design of selective blockers, ideally without disruption of CNS maturation and function. Whereas the mechanisms underlying seizure-induced hippocampal dysfunction are not yet known, many of the prime “usual suspects”, potentially mediating hippocampal impairment in the adult, have been excluded.

Cell death is not generally a consequence of limbic seizures in any of the models leading to functional hippocampal deficits, including kainic acid,[6] hypoxia,[9] hyperthermia,[7,32] or tetanus toxin,[11] and was not found by Chang and colleagues. Although modest cell death as well as hippocampal impairment were found in the corticotropin releasing hormone (CRH) model, [33,34] the general absence of neuronal death in early-life seizure models indicates that it is not required for seizure-induced functional hippocampal deficits.

Altered neurogenesis has been implicated in the consequences of both adult[35] and early-life seizures. Both reduced and enhanced neurogenesis (likely dependent on the time of assessment) have been reported after generalized neonatal seizures.[13,31] Increased neurogenesis was found after kainate[32] and lithium-pilocarpine seizures[36] induced at the same developmental age studied here (P10; “equivalent” to infancy in terms of hippocampal development, see below). Chang and colleagues,[1] did not address this issue, but others reported that a single 20 minute long experimental febrile seizure did not alter neurogenesis.[32] The inconsistent relationship of neurogenesis and long-term deficiency of hippocampal mediated learning and memory excludes the former as a cause of the latter.

Abnormal neuronal connectivity and synaptic reorganization have been reported after single and recurrent limbic early-life seizures.[31,32] Chang and colleagues did not find this “sprouting” after recurrent experimental febrile seizures, whereas Bender and colleagues,[32] described modest sprouting after a single long seizure. Findings in other limbic models of recurrent seizures have been variable,[11] again, suggesting that sprouting might not be a key determinant of long-term hippocampal dysfunction. What, then might be the mechanisms that mediate the long-term deleterious alteration of hippocampal network function after *recurrent*, but not single seizures?

Recurrent Stereotyped Stimulation Engenders Plasticity in Developing Neuronal Circuits

Clues to the means by which repetitive neuronal hyper-excitability (seizures) modulate hippocampal function long-term may be derived from analogous neuroplastic processes requiring recurrent stimuli to influence the neonatal hippocampus long-term.

A well-studied example of such process is repetitive neonatal handling: daily removal of immature rats from the home cage and mother for 15 minutes results in profound plasticity of the hippocampal-hypothalamic pituitary adrenal system.[38-40] At hippocampal levels, this recurrent stimulus causes permanent changes in learning and memory functions as well as in the expression of specific receptor and other genes.[38-40] It seems that returning the pups to home cages elicits intense sensory input from the mother (grooming, licking).[41] Whereas a single handling followed by maternal stimulation is insufficient to alter hippocampal function, recurrence of this sequence for a week (P2-P9) suffices to program the neuroplastic process involving altered gene expression in hypothalamus and hippocampus, and associated functional enhancement of hippocampal tasks.[5,42]

Both handling (and the resulting sensory hyperstimulation) and recurrent early-life seizures are characterized by critical periods of vulnerability as well as by a dose effect: a minimum of 5-7 days is required for the handling effect.[40] Chang and colleagues suggest that more than 4 bouts of hippocampal activation are required for provoking permanent deleterious cognitive effects. An attractive hypothesis for the nature of this need for recurrence suggests that each individual (seizure) stimulus leads to transient cellular changes such as calcium fluxes[26,43] or phosphorylation states. Repeated activation of these cellular pathways finally leads to their permanent ‘imprinting’ or programming, perhaps via transcriptional mechanisms.[39,42,44]

In summary, repetitive limbic seizures, similar to other forms of stereotyped recurrent activation of the limbic circuit, if occurring in sufficient number during a critical developmental window, will lead to permanent measurable alteration of hippocampal network function.

Relevance of Experimental Models to Human Early-Life Recurrent Seizures

Whereas Chang's data are thought-provoking, their application to the understanding and management of infants and children is not straightforward. First, these data should be reconciled with available human studies. In addition, important differences of CNS maturation in rodent and human should be considered.

The cognitive consequences of recurrent febrile seizures in children have been addressed in large prospective epidemiological studies (see Hirtz for recent review),[45] that did not detect general behavioral or learning difficulties. Papers involving individuals with preexisting conditions or retrospective analyses of selected patient populations (i.e., those from epilepsy clinics) are far more difficult to interpret because of multiple confounders.[45,46] More recently, a controlled prospective study focused specifically on hippocampus-dependent learning and memory functions in a smaller cohort of children with febrile seizures.[47] This study, involving intellectually normal children, found no impairment (indeed, somewhat superior scores) in children with febrile seizures- with the exception of one group: Infants younger than one year seemed to not fare as well.

The differing outcome in infants versus older children highlights again the ‘within group’ variability of the “immature brain”: this term encompasses a broad spectrum of maturational states. Therefore, the distinct age and specific maturational stage of the brain as a whole and of specific seizure-vulnerable regions are important determinants of seizure outcome, in human

as well as in rodent, and should be considered. Data interpretation and experimental conclusions regarding vulnerability or resistance should be qualified for the precise ages studied. Given these facts, can one compare, with any degree of precision, the immature rodent CNS to that of human infants and children?

The developing brain can be considered as overlapping modules, each developing with unique velocity. In addition, the precise nature of these modules, as well as their rates of development varies across species. While older studies considered the brain as a whole, more recent work recognized the different velocities and sequences of maturation of specific CNS regions in humans and rodents, advocating selective regional comparisons. In the context of recurrent febrile seizures, shown, (at least in the rodent model) to involve the hippocampal formation[8], the relative maturational stages and velocities of this structure should be compared in human and rat. This is particularly true in view of the hippocampus-dependent nature of the cognitive deficits described in the rodent[1] as well as in human infants in prospective studies.[47] A summary of structural and functional maturational milestones of human and rat hippocampus has recently been published,[5] and concluded that even comparative analyses limited to the hippocampal formation cannot provide *precise* correlation of human and rodent "ages". However, in the aggregate, multiple structural and functional milestones suggest that the first week of life (or days 0-5) in the rat could be comparable to the third trimester gestational period of the human. The first year of human life might correspond roughly to days 7-14 in the rat, whereas 'early preschool years' might correlate with the rat's third postnatal week.

In summary, the potential consequences of recurrent early-life seizures on an otherwise normal infant and young child are an enormously important problem that is virtually impossible to directly solve in human studies. Immature rodent models offer important advantages, including control of pre-existing factors, and of seizure number, duration, severity and location. They also, as shown by Chang and colleagues, allow testing of intervention strategies. However, animal models are limited by inherent differences between human and rodent brain as well as disparate maturational sequences and velocities among these species. These caveats notwithstanding, careful design and analysis of immature animal experiments can produce important information about the nature and mechanisms of seizure-related transient and permanent alteration in CNS structure and function. The novel mechanisms uncovered using animal models provide critical information not only about the nature of epileptogenesis, but also about the general fundamental workings of neuronal networks and their maturation.

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