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Trimethyltin Induced Hippocampal Lesions at Various Neonatal Ages

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Organotin compounds, particularly the trialkyltins, have been used for both industrial and agricultural purposes, including chemosterilants, fungicides, and plastic stabilizers (Luijton 1972; Smith and Smith 1975). Among the alkyltin compounds, triethyltin (TET) and trimethyltin (TMT) are particularly neurotoxic, with TET being primarily a myelinotoxicant (Watanabe 1977, 1980) and TMT being a potent neuronotoxicant inducing lesions primarily in the limbic system (Brown et al. 1979; Bouldin et al. 1981; Chang et al. 1982 a,b,c, 1983a).

Although investigations have been performed to explore the neurotoxic effects of TMT, adult animals were used in most of these investigations (Brown et al. 1979; Chang et al. 1982a,b,c, 1983a); studies on the effects of this potent neurotoxicant on the developing nervous system are relatively few (Bouldin et al. 1981; Miller et al. 1982; Reuhl et al. 1983). However, there are strong indications that the developing nervous system is extremely sensitive and vulnerable to TMT toxicity (Reuhl et al. 1983) and the toxic influence of TMT on the brain may be age dependent (Reuhl and Mackenzie 1983). Our present investigation was designed to study the neurotoxic effects of TMT on the rat hippocampus when animals were subjected to a single exposure of TMT at different neonatal ages.

MATERIALS AND METHODS

Sprague-Dawley rats were used in the experiment. Young virgin females were mated and allowed to deliver at term. The day of birth was designated as postnatal day 1 (PND 1). Pups from each litter were randomly selected for each treatment. Animals were individually weighed, and injected (i.p.) with trimethyltin chloride at a dose of 6.0 mg/kg b.w. between PND 1-30. Control pups were similarly injected with equal volumes of saline solution. Animals in groups of eight were sacrificed weekly between PND 15 and 45. At sacrifice, animals were anesthetized and perfused intracardially with saline solution followed by 2.5% buffered glutaraldehyde. Brains were carefully removed and further immersion fixed in 10% buffered formalin, dehydrated with graded ethanols, and embedded in paraffin. Sections were made at parasagittal planes and were stained with hematoxylin-eosin (H&E).

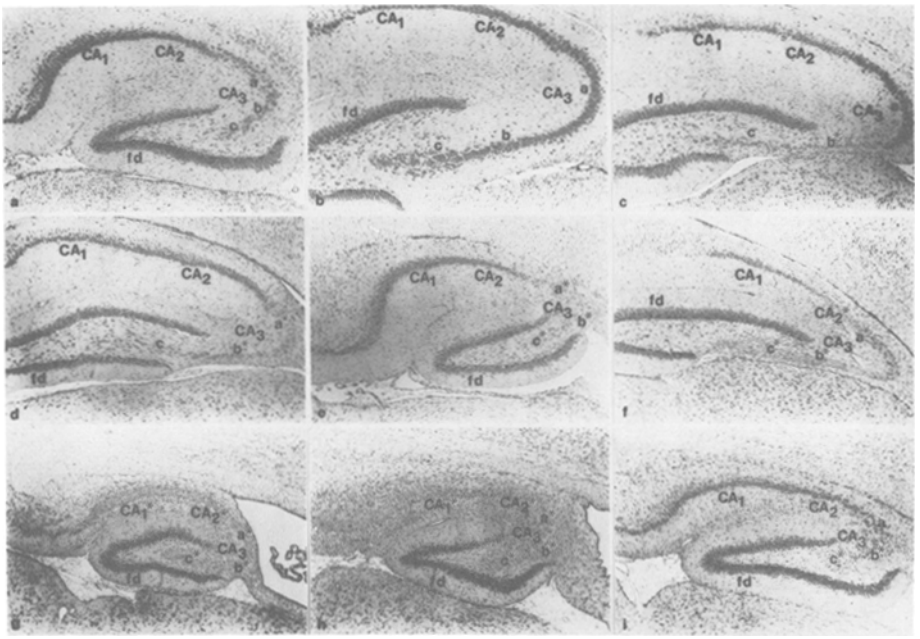

Figure 1. Patterns of injury induced by TMT in the hippocampal formation at different postnatal ages. a-h, sections of brains sampled on postnatal day (PND) 21. a, control; b, injected on PND 3 (iPND 3) - no damage; c, iPND 6 - lesion in CA_{3b} field of Ammon's horn; d, iPND 7 - lesion in $CA_{3a,b}$; e, iPND 9 - destruction of entire $CA_{3a,b,c}$; f, iPND 11 - destruction of entire CA_3 with involvement of CA_2; g, iPND 13 - destruction of CA_1, CA_2 and CA_3; h, iPND 15 - destruction of the entire Ammon's horn; i, pups injected on PND 20, brains examined on PND 31 - damage limited only to $CA_{3b,c}$. No significant damage in the fascia dentata (f.d.) was observed in any of the treatment groups.

RESULTS AND DISCUSSION

The patterns of injury in the hippocampal Ammon's horn were dependent on the neonatal age at which animals were exposed to the toxicant. No lesion was observed in animals exposed to TMT between PND 1-4. After PND 5, however, increasing damage in Ammon's horn was observed in animals exposed to the same dose of TMT. The extent of involvement progressed with exposure at later neonatal ages. The general patterns and loci of involvement were: PND 5-6, CA_{3b}; PND 7, $CA_{3a,b}$; PND 8-10, entire CA_3 ($CA_{3a,b,c}$); PND 11-12, CA_2, CA_3; PND 13-15, entire Ammon's horn (CA_1, CA_2, CA_3). The sensitivity or vulnerability of Ammon's horn pyramidal neurons to TMT toxicity, however, became greatly reduced again after PND 20 mimcking those of adult animals. These patterns were very consistent among pups in the same treatment groups and were duplicable with

Table 1. CORRELATION BETWEEN HIPPOCAMPAL DEVELOPMENT AND TMT-INDUCED LESIONS IN NEONATAL RATS

	PND 1-4	PND 5-6	PND 7	PND 8-10	PND 11-12	PND 13-15
Mossy fiber and synaptic development*	+ (CA_{3b})	++ (CA_{3b})	++ ($CA_{3a,b}$)	+++ ($CA_{3a,b,c}$)	+++	++++
Functional efficiency* (Electrical stimulation response)	None	Weak	Stronger response	Responsive but inconsistent	More mature and responsive	Strong and consistent
Damages in Ammon's horn as a result of TMT exposure	None	+ (CA_{3b} only)	++ ($CA_{3a,b}$)	++ ($CA_{3a,b,c}$)	+++ (CA_2, CA_3)	++++ ($CA_{1,2,3}$)

*Data interpreted from: Bliss et al. 1974; Stirling and Bliss 1978; Cowan et al. 1980.

pups from different litters. Representative lesions induced at various neonatal ages are illustrated in Figure 1.

It is of interest to note that the distribution of injury observed correlates extremely well with the morphological development and functional maturation of the hippocampal formation in rats, and the functional responsiveness of pyramidal neurons (Bliss et al. 1974; Stirling and Bliss 1978; Cowan et al. 1980). This correlation is summarized in Table 1. From these data, it becomes apparent that induction of lesions by TMT in the neonatal Ammon's horn was closely associated with and heavily dependent upon functional maturity and integrity of the neurons and circuitry in the hippocampus. This observation strongly suggests that damages induced in Ammon's horn may not be simply a direct toxicity of TMT on the pyramidal neurons, but, rather, may be the result of altered functional interactions between the granule cells and pyramidal neurons under the influence of TMT. This concept of "functional toxicity" is also supported by observations obtained from adult mice and rats treated with TMT.

In acutely intoxicated mice, rapid destruction occurred in the fascia dentata within 48 hours of TMT exposure. Little or no damage was observed in Ammon's horn (Chang et al. 1982a,b,c). Similar findings were observed in adult rats acutely exposed to a high dose (12.5 mg/kg b.w.) of TMT. At a lower dose (7.5 mg/kg b.w.), however, the most prominent change was found in Ammon's horn while only very limited damage was present in the fascia dentata granule cells (Chang et al. 1983b; Chang and Dyer 1983a). Furthermore, an opposed topographical relationship of lesion development between the granule cells and pyramidal neurons in TMT intoxication has also been described (Chang and Dyer 1983a,b). Thus, the development of Ammon's horn lesions under the influence of TMT apparently requires the presence and functional integrity of granule cells.

The present investigation clearly demonstrates that only little or no damage in Ammon's horn is induced when the mossy fibers are still immature and non-functional. Increased lesion development still occurs when the development and functional states of mossy fibers (granule cell axons) projecting to Ammon's horn (CA_3) approach maturity (PND 7-9). Involvement of pyramidal neurons in other fields ($CA_{1,2}$) after PND 10 may indicate the full establishment and functional maturity of the CA_3 pyramidal cell axons (Schaffer collaterals) projecting to and synapsing with pyramidal neurons in $CA_{1,2}$ (Chronister and White 1975). In view of the excitatory function which granule cells exert on pyramidal neurons, it may be postulated that a hyperexcitable state in granule cells is induced by TMT, leading to hyperstimulatory damage to Ammon's horn neurons.

The proposed role of hyperexcitatory and hyperstimulatory states modulating damage to hippocampal neurons by TMT is not entirely without foundation. It has recently been observed that hyperstimulation and hyperexcitatory states of granule cells lead to a reduction of zinc in hippocampal mossy fibers (Sloviter 1984). Indeed, reduction and depletion of zinc in mossy fibers was also

observed as early as 24 hours after TMT exposure (Chang and Dyer 1984) suggesting that a similar hyperfunctional phenomenon may have occurred in granule cells under the influence of TMT.

The precise reason for the reduction of lesion development (sensitivity) in animals approaching adult age (after PND 20) is still unknown. Several possibilities could be suggested (1) mature function of inhibitory cells and systems (e.g., hippocampal basket cells and interneurons) in the more mature brains would provide an inhibitory or dampening effect on the excitatory state of the neurons; (2) differences in metabolism or distribution of TMT in neonatal and adult brains; and (3) the capability of other organs in adult animals to detoxify and eliminate TMT. These possibilities, needless to say, are speculative and require further investigation.

In sum, the present investigation demonstrates, for the first time, a direct correlation of lesion production with developmental and functional maturity of nerve cells. This concept of "functional toxicity," if confirmed, would add a new dimension to the thinking and understanding of mechanisms of many toxicants. Moreover, the approach may be useful as a model for selective destruction of specific populations of Ammon's horn neurons. Behavioral studies performed in later life on other treated animals may also elucidate the specific functional significance of lesions in individual segments of Ammon's horn.

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