Effects of deltamethrin on granule cell migration during postnatal development of rat cerebellum

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Deltamethrin (DLT; 0.7mg/kg/body wt/day, ip, dissolved in propylene glycol) administration during postnatal days 9-13 in Albino rat pups, resulted in a delayed appearance of radial glial fibers, that guide the migration of granule cells. Moreover, the radial glial fibers in the DLT-treated pups were disorganized, hypertrophied and heavily stained. Thus, it is being proposed that although after exposure to DLT the neuronal proliferation occurs at normal rate, the neuronal migration along the stumpy and crumpled radial fibers hamper the journey of the healthy neurons to their proper destination.

Keywords: Astrocytes, Cerebellar development, Deltamethrin, Glial fibrillary acidic protein, Granule cell migration, Radial glial fibers

The developing mammalian brain is highly vulnerable to environmental toxicants due to its extensive period of development and high susceptibility and the lack of self-renewing ability of the undifferentiated neurons. Development of brain broadly involves cytogenesis, histogenesis, morphogenesis and synaptogenesis. Migration of the neuronal precursors and neurons from the site of their differentiation is an important feature of the development. Thus, any disturbance during development leading to improper acquisition of position of the migrating neurons may cause congenital brain anomalies and alter various aspects of development.

In the developing mammalian brain the cerebellar granule cells originate superficially in the external granular layer (EGL) and after differentiation migrate actively through the developing molecular (ML) and the Purkinje cell layers (PCL) to lie deep in the internal granular layer (IGL) where they reside in the mature cerebellum. During both embryonic and early postnatal development, the neuronal migration to their final positions in different regions is mediated by glial fibers, which in turn provide a generic guidance for neuronal locomotion with the navigational instructions being coded by the neuron. Proper acquisition of the neuronal position, attained through active migration, ultimately affects the cell’s morphology, synaptic connectivity, and function. Radial glial cells, a specialized cell class of astroglia, appear transiently during development in the mammalian nervous system. These cells help in neuronal migration along their straight radial glial fibers extending from a point on the ventricular surface to the roof of the cortical plate.

The developing cerebellum has a unique class of radial glial cells called the Bergman glial cells. These cells position themselves midway in the cerebellar cortex located at the upper strata of the PCL and extend their processes both ways i.e. both towards the pial and the ventricular surface. The granule cell migration and assembly in the IGL is most prominent between postnatal day 2 to postnatal day 14 (P2-P14) and is concluded by the end of the third postnatal week in mouse cerebellum. An exposure to γ-irradiation in mice during this postnatal age has been observed to cause abnormal interaction between the neurons and glia leading to a crucial failure of granule cell migration to its proper destination in mice.

Extensive use of pyrethroid insecticides in agriculture, public health and public protection has enhanced the chances of human exposure to this class of insecticides. Deltamethrin, a type-II pyrethroid insecticide has an easy and rapid access to all tissues, thus even a very small dose is able to produce significant biological effects. Deltamethrin is reported to have outstandingly high safety factor for man because: (i) its non-penetration of keratin layer, (ii) detoxification by enzymes like esterases that have ability to break the ester bond in the deltamethrin
molecule, leaving an acid and an alcohol, both of which are non-toxic. Esterases hardly exist in an insect that is why the rate of detoxification is higher in humans than in insects and thus high insect/human toxicity ratio. Developmental neurotoxicology demonstrates frank pathological changes caused by commonly used drugs, pesticides, occupational hazards and other environmental agents otherwise claimed to be completely safe to the adult and mature brain. Keeping these benefits in view, the possible adverse effects of DLT in mammalian brain were studied and a significant delay and/or retarded formation of cells in the inner granular layer was observed.

The migration of neuronal precursors and neurons from the site at which they begin to differentiate to their final destination is an important feature of neuronal development. The migration of neuronal precursors helps in establishing the identity of the neurons as well as defining the functional properties and future connections of the neurons.

The granule cells, a class of excitatory interneurons in the cerebellum align themselves along the radial glial fibers during their migration from the external granular layer (where they are generated) to the internal granular layer. During this journey they pass through three distinct cellular compartments i.e. ML, PCL and IGL. Any disturbance or misalignment of the radial glial fibers will thus result in the inability of granule cell migration and their arrest in more superficial layers. Thus, the correct orientation of the radial glial fibers appears to be critical and a prime prerequisite for the normal migration of the granule cells to their proper position and placement.

Glia fibrillary acidic protein (GFAP) is a major cytoskeletal protein of protoplasmic and fibrous astrocytes. The radial glial cells and fibers also react well with the GFAP antiserum, thus this antibody is widely used as a specific biochemical marker for glial differentiation in the developing brain. The present study has been designed to investigate the effects of DLT on postnatal cytokinetic behavior of the neuronal migration using anti-GFAP antibody.

Materials and Methods

Animals and treatment—Wistar rats obtained from the breeding colony of the Animal House of Neuroscience Centre were used for the present study. The animals were maintained in a closed room maintained at 25±2°C under an alternating 12:12 hr L:D schedule and were fed with standard rat pellet feed and water ad libitum. The first 24 hr following birth was considered as postnatal day 0 (P0). Rat pups of postnatal day 9, born to healthy mothers (average litter size for the study was chosen to be eight) were selected. Half of the pups of each litter served as controls and the other half were injected with DLT during postnatal day 9-13 (P9-P13) as per the experimental plan detailed out in Table 1. The experimental protocols were approved by the Institutional Animal Ethics Committee. The pups were weighed and sacrificed on postnatal days 12, 15, 21 and 30. On the respective days the brains were dissected out after intracardial perfusion with phosphate buffered saline (pH 7.4) followed by 10% phosphate buffered formalin (pH 7.4). Every care was taken to perfuse the control and treated pups similarly to avoid any perfusion-associated artifact. The perfusion pressure was maintained by reducing the recommended height of the containers for the saline and the fixative to 60 cm from 120-150 cm and by use of a narrow gauge needle of 26G. Cerebellum along with the brain stem was separated at tectal level and post-fixed in phosphate buffered formalin for 48 hr. The tissues were embedded in paraffin wax (m.p. 60-62°C) and serial sections were cut at 10 mm thickness using a rotary microtome. The sections were mounted on TESPA (Sigma No. A3648) coated slides.

Immunocytochemistry—Three to four slides (each containing 8-10 sections), selected randomly from the serial sections per parameter were deparaffinized with xylene, rehydrated in a down graded ethanol series and brought to water. The sections were treated with 1% hydrogen peroxide in methanol for 20 min to block endogenous peroxidase activity followed by washing in water. The sections were then rinsed with three changes of 0.1 M phosphate buffered saline (PBS), pH 7.4, prior to triton treatment (0.5% Triton-X in PBS) for 30 min. After washing in three changes of PBS for 5 min each, the sections were incubated with 1% donkey serum diluted with PBS for 40 min for non-specific protein blocking. The sections were

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<sup>a</sup>Vehicile only (equal injectable volume of propylene glycol).
<sup>b</sup>Deltamethrin dissolved in propylene glycol at a dosage of 0.7mg/kg/body/wt/day, ip.
<sup>c</sup>Five pups per age group, no mortality.
subsequently incubated overnight with rabbit anti-
GFAP IgG (diluted with 1% BSA in PBS, 1:4000,
Dakopatts, Denmark) at 4°C. Next morning the
sections were again rinsed with three changes of PBS,
5 min each and incubated with secondary antibody i.e.
biotinylated donkey anti-rabbit IgG (diluted with 1%
BSA in PBS, 1:100, Amersham, UK) for 40 min.
The sections were again washed thrice with PBS and
incubated with a streptavidin biotin–Horseradish
peroxidase complex (diluted with 1% BSA in PBS,
1:200, Amersham, UK) for 40 min and visualized
with 0.025% 3,3’-diaminobenzidine tetrahydro-
chloride and 0.03% hydrogen peroxide in PBS for 10
minutes. The sections were rinsed in distilled water,
counterstained with 0.1% cresyl fast violet in acetate
buffer (pH 3.5), dehydrated in ethanol series, cleared
in xylene and mounted in DPX. Control and treated
sections were processed together in the same manner.

Results

Radial glial fibers were stained deep brown both in
control and DLT-treated cerebellar preparations by
employing GFAP-immunocytochemistry using
streptavidin biotin-HRP method. GFAP-positive
radial glial fibers of various lengths were clearly seen
projecting radially between the proliferative zone in
the EGL and the ventricular zone. In the control
preparations the radial fibers observed were thin and
straight that ran perpendicular to the pial surface. But
in the DLT-treated cerebella these fibers were more
heavily stained, hypertrophied and presented an
undulating course. In P12 rat cerebeHurri the
GFAP-immunoreactive radial glial fibers ran straight
and perpendicular to the pial surface in the controls.
In P12 rat cerebeHurri the GFAP-immunoreactive radial glial fibers ran straight
and perpendicular to the pial surface in the controls,
but the fibers were disorganized, hypertrophied and
heavily stained in DLT-treated preparations. As the
glial fibers following DLT exposure was
observed in ML of P12 and P15 DLT-treated
preparations, suggesting a delayed active migration
during this phase. Throughout their migration the
granule cells remained closely associated with the
Bergman glial processes. A number of brighty
stained protoplasmic astrocytes with ramified
processes were common in all the treated preparations
as compared to the astrocytes with fewer processes in
their respective controls.

Proliferation and migration of cerebellar granule
cells in control animals occurs during PO-P21. The
very presence of EGL with mitotic figures even after
P21 indicates delayed proliferation and subsequent
migration of granule cells in the IGL of the cerebellar
cortex of DLT-exposed pups. This was based on the
persistent presence of EGL along with several mitotic
figures even at 21-day postpartum in contrast to the
complete depletion of EGL in the respective
controls. This was evidenced through stereological
measurement of the various layers. The migration
along the strongly GFAP-labeled, hypertrophied and
crumpled/undulated radial glial fibers in P15 and P21
DLT-treated pups in contrast to the faintly stained
radial fibers in controls (Figs 3, 4) recorded in the
present investigation further confirms the delayed and
abnormal granule cell migration following DLT-
treatment.

Discussion

In the cerebellum, cellular interactions with
Bergman glial fibers traversing through ML has been
considered essential for the proper acquisition of the
position of granule cells 16. In the present study,
GFAP-immunoreactive radial glial fibers ran straight
and perpendicular to the pial surface in the controls,
but the fibers were disorganized, hypertrophied and
heavily stained in DLT-treated preparations. As the
granule cell migration is guided by the glial fibers, the
disorganized and crumpled fibers seen after DLT-
exposure may have significantly contributed to
abnormal migration via a disoriented pathway.
Moreover, the retention of the EGL beyond postinatal
day 21 in DLT-treated cerebella suggests that
although the neuronal proliferation/production occurs
at a normal rate, the neuronal migration along the
disorganized, hypertrophied and crumpled radial glial
fibers hamper the journey of the healthy neurons to
their proper destination. Whether or not this is a
prominent disruption of neuronal migration or results
simply in a delay remains to be determined.

An enhanced GFAP expression and hypertrophy of
the radial glial fibers following DLT exposure was
Figs 1-4—The photomicrographs of the sagittal sections of cerebella of rat pups at different postnatal ages showing well-developed GFAP-immunoreactive radial glial fibers (brown). [All preparations were immunolabeled with GFAP-antibody and counterstained with cresyl fast violet. P15 control preparations showing thin and lightly stained radial glial fibers (arrows) running straight through the cerebellar cortex (1); whereas the DLT-treated preparations of the same age presenting hypertrophied and undulating course of radial glial fibers (2); Scale bar=20 μm. The straight radial glial fibers (arrows) continued to run perpendicular to the pial surface even in P21 controls (3); however these fibers are seen disorganized, crumpled and hypertrophied in DLT-treated group preparations (4); scale bar = 10 μm].

also an important and consistent feature of the present investigation. The radial glial cells are considered as glial precursor cells due to their astroglial traits and later transformation into astrocytes during brain development. Reactive gliosis or astrogliosis, a response of the astrocytes to nervous system damage has been well documented. The hallmark of the astrogliosis is the accumulation of glial filaments, the major component of which is the GFAP. GFAP has also been used as a sensitive indicator for identifying sites of toxicant-induced damage in cases of TMT- and MPTP-induced neurotoxicity. Thus, the enhanced GFAP expression and hypertrophy of the radial glial fibers reported in the present investigation may also be considered as an indication of DLT-toxicity. Thus, it is proposed that the deltamethrin is causing extensive cell damage and an increased GFAP expression in the radial glial cells (the future astrocytes) is an early indicator of such effects. In addition to this, an early appearance of numerous protoplasmic astrocytes in the DLT-treated cerebella as compared to their respective controls perhaps
indicate an acceleration of maturation of astrocytes in the developing cerebella due to DLT-exposure associated neuronal damage and/or neurotoxicity.

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