NEUROANATOMY

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Introduction

In India, modern neuroanatomical studies have been pursued since the time medical schools came into existence here. A survey of the studies conducted until 1989 has been penned in an exhaustive review compiled by Professor Mahdi Hasan in the book 'Neurosciences in India: retrospect and prospect' (Hasan, 1989). An effort has been made again to place together the studies carried out on the neuroanatomical aspects of nervous system at various centres, colleges and institutions in India during the period of 1990 to 2006. A very wide spectrum of neuroanatomical studies has been reported. The reports range from descriptions on variations in the formation, branching patterns and distribution of peripheral nerves, histological, immunohistochemical and stereologically quantitated data on human fetal and adult brain to neurochemical and behaviour studies on experimental animals covering different aspects of the nervous system. Of the available works as many that could be collected are briefly outlined.

Aberrant size, course and branching of human cerebral arteries

Many original studies, atlases and treatises have collated the variations of cerebral arteries and their branches. Paul and Mishra (Paul & Mishra, 2004) at Maulana Azad Medical College, New Delhi reported *aberrant course and anastomosis between the anterior cerebral arteries* of both the hemispheres in 9 cases out of 50 human cadavers (Paul & Mishra, 2004). Most of the anastomoses were seen on the orbital surface of the frontal lobe. Arteries on both sides showed differences in their size.

Anatomical variations in the formation, branching patterns and distribution of human peripheral nerves

Knowledge about the variations of nerves is important in order to prevent injury to them while planning a surgery in the region or performing diagnostic and therapeutic invasive procedures. The variations may be clinically important because symptoms of nerve compression are often confused with more common causes, such as radiculopathies. Awareness of the variations is also helpful in avoiding incomplete denervation of the normal area of supply when required.

Roy and colleagues (2001, 2002, 2003, 2004) at department of Anatomy at the All India Institute of Medical Sciences, New Delhi reported a case of variation of the *median nerve* observed in a 55 years old Indian female cadaver (Chouhan & Roy, 2001). Three nerve roots formed the median nerve, one each from the lateral and medial cords and the third root originating from the musculocutaneous nerve. The third root received separate communication from the musculocutaneous nerve.

This abnormal root coming from the musculocutaneous nerve had a very close oblique course over the brachial artery. An unusual origin of the *inferior alveolar nerve* from the mandibular nerve by two roots with the second part of the maxillary artery passing between them was also reported [Roy et al, 2002a]. They also reported a rare variant of a muscular *branch of the brachial artery that penetrated the median nerve* in the lower part of the arm [Roy, 2003]. At the site of arterial penetration, the nerve displayed perineurial thickening, increased fasciculation and interfascicular connective tissue. Another rare variant in the axilla was described where the *medial cutaneous nerve of the forearm penetrated the axillary vein* thereby creating two narrow venous channels at the site of passage (Roy & Sharma, 2004).

Rao and Madhyastha (2001) from the department of Anatomy at Kasturba Medical College, Manipal described a rare case showing unusual course and distribution of the *posterior interosseous nerve* in the left hand [Rao & Madhyastha, 2001]. Udyavar (2001) reported an unusual *branch of the median nerve to the thenar muscles* given off in the lower part of the forearm, about 2-cm above the proximal margin of the flexor retinaculum [Udyavar, 2001].

Dhall and Kanta (2001) at the department of Anatomy, BD Sharma PGIMS, Rohtak demonstrated that in only 50% of the upper limb, the *nerve supply to extensor carpi radialis brevis* had the usual origin from the deep branch of radial nerve. In the rest 50% it arose from the superficial branch (35%) or from the angle of bifurcation of radial nerve (15%). This variation is not a rare occurrence and needs to be emphasized in view of the current use of this muscle in 'free functional muscle transfer' [Dhall & Kanta, 2001].

Kapur and associates (2003) from the department of Anatomy, Maulana Azad Medical College, New Delhi displayed *inter-communications between median and musculocutaneous nerves* with dual innervation of brachialis muscle in the left arm of a 60-years old male [Arora et al, 2003]

Prakash R and colleagues (2003) at the department of Anatomy, University College of Medical Sciences & GTB Hospital, Shahdara, Delhi reported variation of the *lateral cord of brachial plexus piercing the coracobrachialis muscle* [Abhaya et al, 2003].

Gupta and colleagues (2004) at Post-Graduate Institute of Medical Research, Chandigarh demonstrated *variations in the formation and distribution of posterior cord (PC) of brachial plexus* and its branches on both the sides [Gupta et al, 2001]. These variations were: i) prefixed brachial plexus on left side, ii) PC on both the sides was formed by the union of posterior divisions of only the upper (C5, 6) and middle (C7) trunks while the posterior division of lower (C8, T1) trunk joined the radial nerve directly and also gave contribution by two roots to left thoracodorsal nerve, iii) length of PC varied on both the sides; on right side it was 4cm while on left side it was 1.5cm long, iv) upper subscapular nerve on right side arose from PC far proximal to other branches and on its course communicated with lower

subscapular nerve before distributing to subscapularis while on left side it arose from posterior division of the upper trunk (C5, 6) only, v) on left side nerve to teres minor arose directly from axillary nerve trunk instead of arising from its posterior branch. The axillary nerve on both the sides divided into terminal branches before entering the quadrangular space of arm instead after traversing the quadrangular space, vi) branches of radial nerve, which normally arise in the radial groove, arose in the axilla on both the sides.

Wadhwa, Mehra, Khan and Kapur (2004) from the Department of Anatomy, Maulana Azad Medical College, New Delhi, reported the presence of an abnormal musculo-aponeurotic tunnel in the arm that originated as the ligament of Struthers but terminated as the brachiofascialis muscle of Wood. This may cause possible entrapment of the median nerve and brachial artery. This was associated with high origin of nerve to pronator teres within the tunnel [Jacob et al, 2005]. On histological examination the nerve was flattened and showed some perineurial thickening. In view of the flexor function of brachialis muscle, this anomalous musculoaponeurotic band may be considered a clinically important entity in the causation of idiopathic neurovasculopathy in the hand.

Jacob, Wadhwa, Paul and Das (2005) reported *anomalous branching of the thoracic sympathetic chain* [Wadhwa et al, 2004]. On the right side at the level of T3 ganglion, they showed an accessory sympathetic chain (ASC) descending anteromedial to the main sympathetic chain (MSC). Communication between MSC and ASC at the level of T9, T10 and T11 ganglion, indicate absence of the classical pattern of greater, lesser and least splanchnic nerves. The ASC may represent a higher origin of greater splanchnic nerve at the level of T3 ganglion. The branches from MSC at T9, T10 and T11 ganglion may be the lesser and least splanchnic nerves, which further joined the ASC (i.e presumably the greater splanchnic nerve) to form a common trunk. This common trunk pierced the right crus of diaphragm to reach the right suprarenal plexus after giving few branches to the celiac plexus.

Poornima and Venugopal (2006) from the department of Anatomy, JSS Medical College, Mysore reported the *absence of musculocutaneous nerve bilaterally* and the presence of a third head of biceps brachii in the left arm [Poornima & Venugopal, 2006]. Branches of median nerve in a single cadaver innervated all the flexors in both the arms.

Krishnamurthy and colleagues (2006) from the departments of Anatomy, Melaka Manipal Medical College and International Centre for Health Sciences, Manipal reported that the *superficial branch of radial nerve (SBRN)* which normally lies deep to the brachioradialis muscle (BR) in the forearm and passes deep to the tendon of BR before winding round the lateral side of the lower end of radius was found to pass between two slips of the tendon of BR before entering the dorsum of hand [Surendran et al, 2006].

Data on human adult brain and spinal cord

Corpus callosum

Koshi and colleagues (1997, 2003) in the Department of Anatomy at Christian Medical College, Vellore measured the size of the corpus callosum on midsagittal section in 100 (50 males, 50 females) normal adult Indians using magnetic resonance imaging (MRI) [Koshi et al, 1997; Suganthy et al, 2003]. The width of the trunk and genu decreased with age in males but not in females. The variables correlated significantly among each other but only callosal length and genu width correlated with gestation age. Significant absolute increase occurred in callosal length and genu width, whereas body and splenium widths remained the same. Simple regression equations to estimate the callosal length and genu width for a given age were derived.

Termination of the spinal cord

They also determined the vertebral level of termination of the spinal cord [Vettivel, 1991], the length of vertebral column and length of the spinal cord in 78 South Indian fetuses (42 male and 36 female) which varied from 40 to 330 mm CRL. Nine (2 male and 7 female) full-term neonates were also studied. Vertebral level of termination ranged from the 5th sacral to the 1st lumbar vertebrae, recession varied from 3 to 90 mm and ascent from 4 to 13 vertebrae. There was a rapid ascent of the conus medullaris up to the 120 mm CRL stage, when it reached the 4th or even the 3rd lumbar vertebra. Beyond that, the ascent was fairly uniformly gradual and the spinal cord terminated mostly opposite the 1st or 2nd lumbar vertebrae in the full-term neonates. The South Indian female fetuses had cords terminating at the same or often a higher level than male fetuses. The spinal cords of South Indian neonates terminated at a higher level than North Indian neonates and a vertebra or higher than the level quoted by Western textbooks. The correlation between crown-rump length, length of vertebral column, length of spinal cord, vertebral level of termination and recession of spinal cord among all males, females and both was statistically highly significant.

Ageing changes in the human retina

Nag and Wadhwa at department of Anatomy at the All India Institute of Medical Sciences, New Delhi studied ultrastructural changes in the photoreceptors of the human retina during normal ageing [Nag et al, 2006]. Retinas from 33 donors (age span 13-94 years) were examined by electron microscopy to see morphological changes in the cones with ageing. They showed mitochondrial alterations and occurrence of electron-dense globules in the cone inner segments from the fifth decade of life. The globules are more prevalent in the macular cones than those in the mid-peripheral or nasal retinas ($p < 0.05$) and peak in the sixth decade of life. Other type of inclusion seen is made up of bundled microtubules, which occur, exclusively in the macular cones at the eighth decade of life. Evidence suggests

that altered cone mitochondria with cristae remnants and dense matrix participate in globule formation in the ageing retina. Such mitochondrial changes may cause energy depletion, and bundling of microtubules (to form filamentous inclusions) could result in decreasing intracellular transport, in which case cones, may die in the long run. They also showed age-related decrease in the density of protein kinase C immunostained rod bipolar cells of the adult human retina [Aggarwal et al, 2007].

Data on human fetal brain and peripheral nervous system

Morphological and neurochemical development of the human retina

Wadhwa and colleagues (1994-2006) at department of Anatomy at the All India Institute of Medical Sciences, New Delhi have examined the spatio-temporal differentiation of neurons in fetal human retina by labeling the neuronal types with known antigenic markers. The neuronal differentiation in the central retina begins earlier than that occurring elsewhere, completes by midgestation (19-21 weeks) and continues in the periphery until 30 weeks of gestation (wg). During differentiation, the neurons express an array of molecules, such as neurotransmitters, neuropeptides and calcium-binding proteins. Although their precise involvement in retinal development is unclear, their patterns of expression indicate that many of them may be necessary for neuronal differentiation.

Ganglion cells differentiate at 8-9 wg and arrange into a ganglion cell layer (GCL) in the central retina at 13-14 wg. Immunoreactivity (IR) to markers such as GABA, tyrosine hydroxylase, calretinin, Trk B and Trk C is localized in the prospective ganglion cells from 10-11 wg onward [Jotwani 1995; Jotwani et al, 1994; Nag & Wadhwa, 1997; Nag & Wadhwa, 1999a; Nag & Wadhwa, 1999b]. DiI labeling showed the early ganglion cells to possess few dendrites between 7-11 wg. At 10-11 wg, some of them exhibit dendritic filiform processes and spines. They show progressive growth and dendritic elaborations at 17-18 wg. By 20 wg, three major cell types resembling alpha, beta and gamma classes of cat are identifiable in the human retina [Wadhwa et al, 1993]. Also, connections to visual centers via ganglion cell axons are partially formed at this time point [Wadhwa et al, 1988].

Photoreceptor differentiation As seen by scanning electron microscopy, at 15 wg, the cilium develops as a small protrusion from the apical ends of cone inner segments. The latter become arranged into mosaic patterns by 18-19 wg, wherein few large cone inner segments (putative blue cones) stand out prominently from the mosaic of many small cone inner segments (prospective red/green cones). The rod inner segments are identified at 18-19 wg and show ciliary outgrowths. At 24 wg, the rod outer segments begin to develop from the distal ends of cilia [Narayanan & Wadhwa, 1998].

From the time of proliferation, cones express a number of molecules in a spatio-temporal pattern in human fetal retina. A good marker of cones is calbindin, a member of EF-family calcium-binding proteins (calbindin, parvalbumin and calretinin) [Nag & Wadhwa, 1999a; Nag & Wadhwa, 1996]. The central cones show calbindin and calretinin IR at 20-21 wg. In adults, all cones (except the foveolar), are brilliantly calbindin positive, but show weak calretinin IR. This suggests that calretinin plays a limited role for Ca^{2+} buffering in the cones of human retina. Parvalbumin does not express in the fetal retina till 25 wg, but expresses in the infant retinal cones. In adults, only few macular cones show parvalbumin IR [Nag & Wadhwa, 1996].

Synaptic development in fetal human retina By labeling with markers of synaptogenesis (synaptophysin and syntaxin-1) showed that IR to both markers is present in the central inner plexiform layer at 11-12 wg [Nag & Wadhwa, 2001]. This indicates that both proteins are incorporated into respective synaptic elements in parallel with their morphological differentiation at this time point. In the outer plexiform layer, however, IR becomes prominent by 16 wg, consistent with the electron microscope data of appearance of synaptic vesicles and ribbons in photoreceptors. These observations suggest that the inner plexiform layer matures before the outer plexiform layer in the human retina. Synaptogenesis of the human retina is not studied beyond 16 wg by electron microscopy.

Neuroactive substances and neuronal differentiation

Calcium-binding proteins. The distribution of calbindin, parvalbumin and calretinin was examined in the human fetal retinas [Nag & Wadhwa, 1999a; Nag & Wadhwa, 1996]. Parvalbumin-IR occurs in most ganglion cells, all horizontal and few amacrine cells, while calbindin-IR is found in some horizontal and ganglion cells from 13-15 wg. At 20-21 wg, calretinin IR is mainly localized in most differentiated amacrine and horizontal cells. The calretinin-positive horizontal cells are smaller and less frequent in distribution than the calbindin- and parvalbumin-positive cells. No horizontal cells are calretinin positive in the postnatal or adult retinas. These facts suggest a critical role for calretinin in the maturation of a type of horizontal cells, whose identity at present is unknown.

Gamma amino butyric acid (GABA) is expressed at 12 wg in many cells of the inner neuroblastic layer and few axons of nerve fiber layer, which suggests that some of the stained cells could be prospective ganglion cells [Nag & Wadhwa, 1997]. From 16 to 25 wg intense IR is found in most amacrine and horizontal cells in the central to mid-peripheral retina. In postnatal retina, some horizontal cells are moderately labeled, but no IR is found in the adult human retinas consistent with the known patterns in mammals. Thus, there is a possibility that GABA plays a role in horizontal cell differentiation in the human retina.

Nitric Oxide synthase and glutamate A transient increase of nNOS IR in ganglion cells occurs between 18-25 wg of fetal human retina [Wadhwa & Nag,

1999]. This coincides with the period of naturally occurring cell death in the GCL. Before this period, at 16-17 wg, IR to glutamate and parvalbumin (that binds glutamate released Ca^{2+}) increases in the GCL of fetal human retina [Nag & Wadhwa, 1996 and Jotwani et al, 1998]. Thus, cell death in the GCL is perhaps linked to excessive glutamate and nitric oxide activity, as proposed earlier.

Taurine. In human fetal retina, the ganglion cells and some of their axons show taurine IR at 16-17 wg. From 20-21 wg, IR develops in some amacrine, bipolar and horizontal cells, which increases with progressive fetal age [Nag et al, 1998]. In postnatal infant retina, taurine IR is present in some amacrine cells and strongly in photoreceptors, but absent in ganglion and horizontal cells. In human fetal retina, expression of taurine IR follows a sequence that parallels with neuronal differentiation in an inner to outer direction. It is likely that this amino acid is vital for retinal cell differentiation, as was shown in cultures.

Neuropeptides and aminergic transmitters

The earliest fetal age to show neuropeptide IR in the human retina is 10 wg, when substance P, and enkephalin- positive cells are seen in the outer neuroblastic layer and tyrosine hydroxylase and neuropeptide Y - positive amacrine cells in the inner nuclear layer. During 17-24 wg, IR appears in the outer and inner nuclear layers and inner plexiform layer. By 26-30 wg, neurons in the GCL are substance P and enkephalin-positive [Jotwani et al, 1994]. The IR to all neuropeptides occurs in a sequence from outer to inner layers of the developing human retina. All these reports indicate that neurotransmitters and neuropeptides play a role in neuronal differentiation.

The availability of antigenic markers has provided a fresh impetus and opportunity to the retinal researchers, to learn about the neurochemical development of the human retina. Our data demonstrate a changing IR pattern of the amino acids and their derivatives, neurotransmitter candidates and neuropeptides in the developing human retina, which is relevant to comprehend the neurochemical differentiation in human retinal transplants before use for therapeutic purposes. More in-depth studies are, however, needed to understand the involvement of various neurochemical substances in retinal cell differentiation.

Development and maturation of human olivo-cerebellar pathway

Nag and Wadhwa (1999d, 2004) at department of Anatomy at the All India Institute of Medical Sciences, New Delhi also worked on the development and maturation of the human cerebellum and inferior olivary complex [Nag & Wadhwa, 1999c; Nag & Wadhwa, 2004]. They showed the spatio-temporal expression of two calcium-binding proteins (calbindin and parvalbumin) in the olivo-cerebellar pathway during the process of cell differentiation and maturation. Of the two proteins, parvalbumin is found to decrease substantially in the principal olivary nucleus and olivo-cerebellar fibres, with ageing of the brain. Such a loss is likely to cause

impaired calcium functioning in active neurons, ultimately affecting the control of fine motor activities by this nuclear system in the elderly persons.

Development of substantia nigra

Sailaja and Gopinath (1994) at the department of Anatomy, All India Institute of Medical Sciences, Ansari Nagar, New Delhi studied the midbrains from 43 fresh human embryos and fetuses at 8-22 weeks of gestation after processing for routine histology, Golgi staining, tyrosine hydroxylase (TH) immunolabelling and retrograde tracing with the fluorescent dye DiI [Sailaja, et al, 1994]. They showed that the nigral neurons in the human migrate and mature until mid-gestation. In 1996, they also studied the ultrastructure of the developing substantia nigra in 14 human embryos/fetuses of 8-24 weeks of gestation and showed that synaptogenesis starts at 8 weeks and continues beyond 24 weeks of gestation [Sailaja & Gopinath, 1996]. Sailaja (1996) also reported the biparietal diameter to be a useful measure for determining gestational age of human abortuses [Sailaja et al, 1996].

Cochlea, cochlear nerve, cochlear nuclei and inferior colliculus

Roy and colleagues at the department of Anatomy, All India Institute of Medical Sciences, Ansari Nagar, New Delhi studied the development of the *human cochlea* during fetal life [Mishra, 2001]. Hair cells begin maturation by the 16 weeks of gestation. Globule formation on the hair cells indicates the onset of stereociliogenesis, which later disintegrate to give rise to the stereocilia. The stereocilia are initiated earlier on the IHC. Tunnel of Corti opens up by GW 20. To conclude, the cochlea is capable of receiving auditory stimulus by 20-22 weeks of gestation, but complete maturation is not observed as the kinocilium is still seen at this age. On the other hand kinocilium regresses at 30 week and the stereocilia show adult pattern of arrangement on the OHC at GW 30. Therefore, the cochlea is mature to receive the adult pattern of auditory stimulus before birth.

Development of the *human cochlear nerve* showed that at 14-16th fetal week the nerve was thin with aggregations of Schwann cells between the unmyelinated nerve fibers [Ray et al, 2005a]. By the 22nd fetal week, fascicular arrangement was distinct, blood vessels appeared in the endoneurium and the total nerve fibers reached a maximum value. Myelinated cochlear nerve axons were present between the dense array of Schwann cell clusters. The total fibers decreased and thick myelinated fibers started appearing by 24th fetal week. Schwann cells started decreasing after 26 fetal weeks. Myelinated fibers increased during the third trimester and by 37th week total fiber count reached adult level. The results demonstrate that the distal auditory nerve starts maturation during midgestation and the fiber counts reach adult level at term but the majority of the fibers are thin and unmyelinated. This signifies that postnatal sound stimulation has major role in maturation and development of the finer functional components of the auditory process.

The maturation of the *human cochlear nucleus* during gestation and early postnatal period showed a steady increase in the volume of the nucleus, the size of the neurons and neuronal count was observed with increasing ages, while the cell density decreased at the 28 WG [Mishra, 2001]. Parvalbumin was noted during 14th WG, the intensity of which increased with ageing and by PND 40 it was maximal. Synaptophysin was observed first in the cell soma and axons at 20 WG. Adult pattern of synaptogenesis was observed at 37 WG and PND 40. This study shows that morphological and functional maturation of the cochlear nucleus in human takes place during midgestation and it continues through term and postnatal period.

Sympathetic neurons

Kiran (2002) at the department of Anatomy, Kamineni Institute of Medical Sciences, Narketpally, Nalgonda, Andhra Pradesh reported histogenesis of human sympathetic neurons demonstrating proliferation, migration, differentiation and maturation from 90 samples of human fetal sympathetic chains ranging from 8th week to full term [Kiran, 2002].

Histopathological changes in human eyes in systemic lupus erythematosus.

By electron microscopy and immunohistochemistry, Nag and Wadhwa (2005, 2006) at the department of Anatomy, All India Institute of Medical Sciences, Ansari Nagar, New Delhi have shown various pathological changes occurring in the eyes in systemic lupus erythematosus. Ultrastructurally, the ganglion cell axons were swollen, and contained unusual accumulation of cytoskeletons. There were degenerative changes in the smooth muscle cells of blood vessels. Retinal capillary lumen was partially obliterated, and contained IgG, which was detected also in the lumen and wall of choroidal arterioles. The latter and Bruch's membrane showed fibrin deposition. The optic nerve showed infiltrated mononuclear cells near the degenerated axons, these axons lacked IR to calbindin and parvalbumin. Dense GFAP IR was observed surrounding the injured wall of central retinal artery. These pathological changes are due to impaired blood circulation caused by haemorrhage and vasculitis, and vessel occlusion by fibrin [Nag & Wadhwa, 2005; Nag & Wadhwa, 2006].

Nitric oxide and neurotrophic receptors in human brain tumors and peritumoral edema

In a collaborative work at the Department of Anatomy, Pathology and Neurosurgery at AIIMS, New Delhi, Bakshi et al (1998) showed a pattern of localization of nitric oxide in human brain tumors (astrocytomas and glioblastomas) and peritumoral edema. By localizing the three different isoforms of nitric oxide synthase (NOS), namely neuronal (nNOS), endothelial (eNOS) and macrophage-derived (mNOS) [Bakshi et al, 1998]. In another study, they showed the expression profiles of Trk A and Trk B receptors in adult human astrocytomas and glioblastomas

(Wadhwa et al 2003). Both receptors express highly in low-grade benign tumors (grade I and II), but down-regulate with transformation into malignant form (grade IV) [Wadhwa et al, 2003].

Data on brains of experimental animals

Khanna and Sengupta (2001) from the department of Anatomy, University College of Medical Sciences, Shahdara, Delhi demonstrated *asymmetry in the total volume of olfactory bulb and volume of outer stratum,* which was greater on the right side [Khanna & Sengupta, 2001].

Prakash and colleagues (2001, 2002) carried out morphometric analysis of the nucleus proprius of lumbar dorsal spinal horn and medial geniculate body in the rat [Bhardwaj et al, 2001 and Dada et al, 2002].

Dhar, Mehra, Sidharthan and Sharma from the Department of Anatomy at AIIMS, New Delhi described the distribution pattern of calcium binding proteins in area MT of rhesus monkey [Dhar et al, 2001].

Tracing techniques in the brain

Mishra, Gupta and Sengupta (2000) studied the effect of vincristine on axoplasmic flow by fluorescent tracer fast blue [Mishra et al, 2000].

Saraswathi (2003) from the department of Anatomy, Stanley Medical College, Chennai used horseradish peroxidase (HRP) retrograde tracing technique in the monkey (Macaca radiata) to find the nuclear origin of the constituent motor fibres of the hypoglossal nerve [Saraswathi 2003]. She showed that the constituent somatomotor fibres of the hypoglossal nerve originate bilaterally from the hypoglossal nucleus and the perihypoglossal nuclei and the visceromotor fibres come from the ipsilateral dorsal motor nucleus of the vagus

Estrogen receptor (ER) subtypes in the normal adult and the aged female rat hippocampus

Mehra RD and colleagues (2005) at the department of Anatomy of the All India Institute of Medical Sciences, New Delhi, have studied the distribution pattern and levels of expression of two estrogen receptor (ER) subtypes in the female rat hippocampus to establish baseline data and the changes that occur during aging [Mehra et al, 2005]. The two ER subtypes co-exist in the same hippocampal neurons. ERalpha and ERbeta positive neurons are present in all subfields of the hippocampus with maximum presence in the stratum pyramidale of CA3. Some stained neurons in CA3 exhibited pyramidal neuron like characteristics while all other immunoreactive neurons show non-pyramidal neuron features. Neuronal counts revealed a significant decrease in the number of immunoreactive neurons in CA3-CA1 of aged hippocampus. The percent decrease in counts of the immunoreactive neurons/mm^2 area in the aged rat (compared to the adult) was 78% for the ERalpha

and 88% for the ERbeta ($P < 0.001$) in CA3. In CA1, it was 56% ($P < 0.001$) and 41% ($P < 0.01$) respectively. The OD of immunoreactivity was significantly decreased ($P < 0.01$) in CA3 but increased ($P < 0.01$) in the CA1 immunoreactive neurons. Western blot analysis also showed a significant decline ($P < 0.01$) in the levels of the ERalpha and ERbeta proteins with age.

Stress, environment and the developing brain

Stress, neurogenesis, dendritic remodeling

Vidita Vaidya and her colleagues (2000-2006) at Tata Institute of Fundamental Research, Bombay are engaged in understanding the pathways that contribute to the regulation of basal hippocampal neurogenesis, and may be recruited by stress and antidepressant treatments to regulate structural plasticity [Vaidya, 2000; Vaidya & Duman, 2001]. The two major projects in the laboratory address (a) the regulation of adult hippocampal neurogenesis and (b) studies to identify important trophic and signaling molecules targeted by stress/antidepressant treatments to regulate adult structural plasticity.

Recent studies from their group and others have suggested that norepinephrine but not serotonin, influences adult hippocampal neurogenesis [Kulkarni et al, 2002; Jha et al, 2006]. They are also examining the influence of critical neurohormones such as thyroid, that have been strongly implicated in the pathogenesis of mood disorders, and addressing the effects of thyroid on adult hippocampal neurogenesis [Desouza et al, 2005]. Their studies use both in vivo perturbations and in vitro adult hippocampal progenitor cultures. Elucidating the pathways that regulate adult neurogenesis will significantly add to understanding of this complex form of adult structural plasticity.

They are also examining the regulation of the developmental signaling molecules and growth factors by stress and antidepressant treatments. The brain derived neurotrophic factor (BDNF) gene generates multiple transcripts and their studies have shown that antidepressants rather than converging on one specific promoter regulate distinct promoters [Dias et al, 2003; Sathanoori, et al, 2004]. In addition, they are examining the role of other important developmental signaling pathways of the sonic hedgehog [Banerjee et al, 2005], Wnt and bone morphogenetic protein families by paradigms that alter adult hippocampal neurogenesis. By using approaches to address regulation of these molecules at the transcript and protein level their goal is to examine the contribution of these molecular targets of stress/antidepressant treatments to adult structural plasticity, such as hippocampal neurogenesis.

Chattarji (2002) at National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore has shown that chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons [Vyas et al, 2002]. In agreement with previous reports, chronic immobilization

stress (CIS) induced dendritic atrophy and debranching in CA3 pyramidal neurons of the hippocampus. In striking contrast, pyramidal and stellate neurons in the basolateral complex of the amygdala exhibited enhanced dendritic arborizations in response to the same CIS. Chronic unpredictable stress (CUS), however, had little effect on CA3 pyramidal neurons and induced atrophy only in BLA bipolar neurons. CIS, but not CUS, reduced open-arm activity in the elevated plus-maze. These findings raise the possibility that certain forms of chronic stress, by affecting specific neuronal elements in the amygdala, may lead to behavioral manifestations of enhanced emotionality. Thus, stress-induced structural plasticity in amygdala neurons may provide a candidate cellular substrate for affective disorders triggered by chronic stress.

Vyas, Bernal and Chattarji (2003) examined the effects of chronic immobilization stress (CIS) on neuronal morphology of central and extended amygdala neurons of rats. While dendritic arborizations in central nucleus of the amygdala (CeA) neurons remained unaffected, it increased in bed nucleus of stria terminalis (BNST) in the extended amygdala after CIS [Vyas et al, 2003]. These results suggest a role for dendritic remodeling of BNST neurons in stress-induced facilitation of anxiety. Chronic stress enhances anxiety and consolidation of aversive memories [Mitra et al, 2005a and Vyas & Chattarji et al, 2004].

Chattarji S and colleagues (2004) showed that even after 21 days of stress-free recovery, animals exposed to chronic immobilization stress (CIS) continue to exhibit enhanced anxiety, as manifested by a significant reduction in open-arm exploration and risk-assessment behavior in the elevated plus-maze [Vyas et al, 2004]. At the cellular level, CIS-induced dendritic remodeling in the amygdala is also as long lasting as enhanced anxiety after 21 days of recovery. Indeed, long-lasting facilitation of CIS-induced anxiety is accompanied by a persistent increase in dendritic arborization of basolateral amygdala (BLA) spiny neurons. Moreover, CIS-induced BLA hypertrophy is distinct from hippocampal CA3 atrophy, which is reversible within the same period of stress-free recovery. These findings on persistent dendritic remodeling in the amygdala, in addition to highlighting important differences with hippocampal structural plasticity, may provide a cellular basis for examining anxiety and mood disorders triggered by chronic stress.

They also showed that stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala (2005). Aversive experience in the form of acute immobilization stress (AIS) and chronic immobilization stress (CIS) modulates spine density in the basolateral amygdala (BLA) of male rats. By modulating the duration of immobilization stress, it is possible to induce the formation of new spines without remodeling dendrites. However, unlike CIS-induced spine formation, the gradual increase in spine density 10-d after a single exposure to AIS is localized on primary dendrites. This delayed induction of BLA spinogenesis is paralleled by a gradual development of anxiety-like behavior on the elevated plus-maze 10 d after AIS [Mitra et al, 2005b]. These findings demonstrate that

stressful experiences can lead to the formation of new dendritic spines in the BLA, which is believed to be a locus of storage for fear memories. Their results also suggest that stress may facilitate symptoms of chronic anxiety disorders like post-traumatic stress disorder by enhancing synaptic connectivity in the BLA.

They have developed an algorithm that uses statistics from precise morphometric analyses to systematically remodel neuronal reconstruction (2005). The distribution function of the ratio of two normal distributed random variables has been used to specify the probabilities of remodeling along various regions of the dendritic arborizations. These probabilities are then used to drive an iterative algorithm for manipulating the dendritic tree in a region-specific manner. By applying this framework to a well-characterized example of dendritic remodeling stress-induced dendritic atrophy in hippocampal CA3 pyramidal cells the showed that the pruning algorithm is capable of eliciting atrophy that matches biological data from rodent models of chronic stress [Narayanan et al, 2005].

Sahai A and colleagues (2001) at the department of Anatomy at King George's Medical College, Lucknow showed 48hrs later *immobilization–induced light microscopic changes in the cerebellar Purkinje cells* of male albino rats [Rani et al, 2001]. Both control and stressed groups exhibited differential stainability of Purkinje cell somata. Quantification revealed statistically significant increase in the ratio of dark to total Purkinje cells with stress.

Effects of environmental factors and stimulation on brain

Singh and Singh (2002) at the department of Anatomy at the Institute of Medical Sciences, Banaras Hindu University, Varanasi, have demonstrated changes induced by magnetized water in adult rat brain [Singh & Singh, 2002]. Histologically, the treated group revealed marked spongiform changes leading to neuronal degeneration in cerebral and cerebellar cortices. No change was observed in the size of the ventricles. This study further proves that powerline exposure induces stable changes in water structure affecting the biomechanism of tissue fluids.

Rao BSS and colleagues (1998a,b, 1999, 2008) at the department of Neurophysiology at National Institute of Mental Health and Neurosciences, Bangalore subjected rats to self-stimulation experience (SS). They demonstrated alterations in the density of excrescences in CA3 neurons of hippocampus on both the main shaft and sub branches of the apical dendrites in SS experienced group as well as following self-stimulation-rewarding experience [Shankaranarayana et al, 1998a and Shankaranarayana et al, 1998b]. The increased number of excrescences in CA3 neurons could be due to an enhancement in the synaptic transmission in the mossy fiber pathway following the SS experience. They also showed long-lasting structural changes in CA3 hippocampal and layer V motor cortical pyramidal neurons following self-stimulation-rewarding experience [Shankaranarayana et al, 1999a]. The SS experience induced structural changes are sustainable, even in the absence of rewarding experience. They also showed

that SS rewarding experience restores stress-induced CA3 dendritic atrophy, spatial memory deficits and alterations in the levels of neurotransmitters in the hippocampus [Ramkumar et al, 2008].

They compared the impact of chronic restraint stress on spine density of medium spiny stellate neurons in MeA in wild-type mice with mice in which the tPA gene is disrupted (tPA-/-). In wild-type mice, chronic stress caused significant reduction in MeA spine density, which was in contrast to enhanced spine density in the neighboring basolateral amygdala (BLA). Strikingly, tPA-/- mice exhibited significant attenuation of stress-induced spine retraction in the MeA, but BLA spinogenesis was not affected. Therefore, tPA-dependence of stress-induced modulation in spine density was restricted to the MeA. Further, MeA neurons in tPA-/- mice, even when challenged with repeated stress, were able to maintain levels of spine density that were comparable to that of wild-type mice without stress. Our findings provide novel evidence for a permissive role for tPA in amygdalar spine plasticity elicited by behavioral stress [Bennur et al, 2007].

They demonstrated enriched dendritic arborizations in prefrontal cortical neurons following (-) deprenyl treatment (1999) and considered that this may be responsible for the enhancement of cognitive functions in Alzheimer disease patients on deprenyl [Shankaranarayana et al, 1999b].

The subiculum is a major source of output projections from hippocampus to cortical and subcortical regions on lesion causes dendritic atrophy in CA1 and CA3 pyramidal neurons of the rat hippocampus (2001) [Shankaranarayana et al, 2001]. This might be responsible for the impairments in operant and spatial learning tasks in these rats observed in earlier studies.

Raju and colleagues (2000) at the department of Neurophysiology at National Institute of Mental Health and Neurosciences, Bangalore examined the metabolic activity of rat retinal ganglion cells during postnatal development in vivo using cytochrome oxidase histochemistry [Govindaiah et al, 2000]. They showed increased levels of cytochrome oxidase at the same time, when neuronal maturity and synaptogenesis reach their peaks. They also examined the effect of glutamate on survival, differentiation and metabolic activity of cultured rat retinal ganglion cells at 3 days in vitro (2002). It was observed that enhanced metabolic activity coincided with survival and differentiation of cultured rat retinal ganglion cells exposed to glutamate [Govindaiah et al, 2002]. An increase in the metabolic activity indicates an enhancement in the electrical activity. Their results are consistent with the hypothesis that glutamate is critically involved in the regulation of electrical activity in developing rat retinal ganglion cells.

Wadhwa and colleagues (1999-2006) have investigated the effects of experience driven neural activity on developing chick auditory nuclei [Wadhwa et al, 1999; Alladi et al, 2002; Alladi et al, 2005a; Alladi et al, 2005b], higher auditory association area - mediorostral neostriatum hyperstriatum ventrale [Panicker et

al, 2002] and the hippocampus [Chaudhury et al, 2006; Chaudhury et al, 2007 and Chaudhury et al, 2008]. Stereological methods for quantitation, immunohistochemistry and Western blot procedures have been used to determine the quantitative morphological and neurochemical changes consequent to enhanced prenatal auditory stimulation. The studies have shown that prenatal auditory enrichment by species-specific sounds and sitar music modifies the structural components of the areas studied and enhances the expression of immediate early genes, synaptic density and proteins as well as calcium binding proteins (CaBP's). These studies are aimed to help understand the basis of better neonatal performance of the chicks in response to prenatal auditory cue [Jain et al, 2004]. Such a paradigm may also help reduce the deficits in disorders related to language acquisition and learning in children as well as improve the learning capabilities of normal children.

Neurotoxicity studies

Nicotine toxicity

Roy TS and colleagues at the department of Anatomy at the All India Institute of Medical Sciences, New Delhi conducted studies on effects of various toxicological agents in the developing brain. They have demonstrated that prenatal nicotine exposure reduces the brain and body weight of the rat fetus but there is no change when continuous nicotine exposure is given during the gestational period using minipump [Roy et al, 2002b]. Regaining the normal body and brain weight in the postnatal period takes nearly three weeks. In both animal models, the pyramidal neurons of the somatosensory cortex [Roy & Sabherwal, 1994] as well as the pyramidal and granule cells of the hippocampus [Roy & Sabherwal, 1998] show reduction in size, increase in neuronal density, dendritic branches and spines. Increase in glial cell population was noted in both models. In the whole rat embryo culture, after 48 hours in a medium enriched with nicotine Roy and colleagues have shown that rate of cell death increases in the neural tube [Roy et al, 1998a]. An increase in mitotic figures indicates that compensatory repair goes on simultaneously. From both in vitro and in vivo studies, it can be concluded that nicotine is teratogenic to the animal if it is exposed during embryonic as well fetal life.

Chlorpyriphos toxicity

Chlorpyriphos (CPF), an organophosphorus compound, because of its anticholinergic action, affects the neurons of the developing brain on prenatal or postnatal exposure. Studies on whole rat embryo culture by Roy and colleagues [Roy et al, 1998b] showed increased cell death, and mitotic activity in the cranial neural tube with dispersion and disorientation of the mitotic layer. In addition, cytoplasmic vacuolation, enlargement of intercellular spaces, and the presence of a significant number of apoptotic cells evidenced cytotoxicity. These alterations were evident even at the lowest concentration of chlorpyrifos, which produced no dysmorphogenesis. Chlorpyrifos specifically targets brain development at low

concentrations, indicating the need to reevaluate the safety of this compound for exposure in vivo. Chlorpyrifos (CPF) exposure on postnatal days (PN) 11-14, a regimen that is devoid of systemic toxicity, elicits long-term cognitive impairment and disruption of cholinergic, catecholaminergic, and serotonergic synaptic function. On PN15 and 20, the septal nucleus, striatum, somatosensory cortex and hippocampus showed a significant decrease in the number of glial cells and region-specific alterations in the number and type of neurons, and neuronal perikaryal dimensions [Roy et al, 2005; Roy et al, 2004]. The results indicate that there are subtle morphological changes in the juvenile rat brain after neonatal CPF exposure that are detectable only with quantitative analysis and that correlate with regional and cell-specific targets identified earlier in neurochemical studies.

Arsenic toxicity

Dhar and colleagues at the department of Anatomy at the All India Institute of Medical Sciences, New Delhi studied chronic exposure of adult Wistar rats to sodium arsenite and revealed downregulation of oxidative enzymes (cytochrome oxidase and succinyl dehydrogenase) in the spinal motor neurons [Dhar et al, 2005]. Sodium arsenite exposure during early postnatal period showed defective migration and delayed maturation of Purkinje cells in the developing rat cerebellum [Dhar et al, 2007].

Copper chloride toxicity

Tariq Zaidi and colleagues (2002) at the department of Anatomy, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh studied the effects of copper chloride toxicity on the corpus striatum of rat brain [Tariq et al, 2002]. The tissue showed degenerative changes in the form of concentric spaces around the nerve fibers bundles. Components of corpus striatum showed differential effects of copper toxicity, which may be due to their variable vascularity and cellularity.

Effects of multiple intracerebroventricular injections of methotrexate on brain

Madhyastha and colleagues (2002, 2005) from the Department of Anatomy at Kasturba Medical College, Manipal showed that following multiple intracerebroventricular injections of methotrexate in male Wistar rats, the animals demonstrated convulsions but no anxiolytic activity. Behaviour tests showed impairment of learning and memory. Brain monoamines were disrupted in the hippocampus alongwith severe cytotoxic effect on CA4 hippocampal neurons. There was reduction in the concentration of all three-brain amines (norepinephrine, dopamine and serotonin) in the frontal cortex and brain stem. Hypothalamus did not show any significant change. No structural changes were observed in the frontal cortex neurons [Madhyastha et al, 2002; Madhyastha et al, 2005]. The outcome of the study may have implications in the management of childhood lymphoblastic leukemia with methotrexate.

Neural transplantation studies

Shetty, Gopinath and Tandon (1991a) at the department of Anatomy and Neurosurgery at the All India Institute of Medical Sciences, Ansari Nagar, New Delhi grafted 16 –17 day fetal nigral transplants in the lateral ventricle of adult rats. A long-term morphological study until 360 days showed that degenerative changes were slow to develop in the partially integrated transplants thereby leading to conclude that target tissue interaction is necessary for prolonged survival of the grafted tissue [Shetty et al, 1991a].

Fetal nigral grafts in the anterior eye chamber of adult rats (1991b) observed over a long-term suggested that the maturation of nigral neurons is independent of specific afferent input, whereas target influence is necessary for the continued maintenance of the mature neurons [Shetty et al, 1991b]. Large glial processes, very prominent during the 4- to 6-month period became less significant afterward but continued to be present until the end of the period studied. Though there was no morphological evidence of lymphocytic infiltration, this might suggest an immunologic reaction.

In the long term transplants of fetal substantia nigra grafted to intact striatum of adult rat (1991c) there is morphological evidence for rapid ageing of neurons raising fresh doubts regarding permanent survival of grafted neurons in the host brain [Gopinath et al, 1991]. They also performed a long-term study (1991d) to see the growth changes of adrenal medullary autografts in anterior eye chamber, lateral ventricle and striatum of adult rats [Shetty et al, 1991c].

Tandon, Gopinath and colleagues (1992) confirmed that bilateral kainic acid (KA) injection at the caudate produces aphagia and adipsia in rats. The reduction in food and water intake was fatal after a higher dosage of the drug. To test the effect of transplantation on the mortality rate, KA was first injected in the left caudate, in one set of rats. After a gap of three days, fetal striatal tissue was unilaterally transplanted at this lesioned site, along with a second injection of KA in the right caudate. Successful transplantation, as ascertained morphologically, did not significantly alter the mortality rate. The morphometric study revealed that the neurons of the transplant were larger in size, and their numerical density lower than that of the caudate of normal rats. Only very few neurons of the transplant developed functional connectivity with the host, as demonstrated by electrophysiological studies [Tandon et al, 1992].

Gopinath and colleagues (1996a) studied the cell surface molecules (NCAM and L1) in intrastriatal transplants of embryonic mesencephalon in rats [Gopinath et al, 1996]. Prolonged expression of these molecules by the grafted neurons indicated delay in the maturation of these cells due to absence of adequate target sites for synaptic connections. Some of the smaller cells expressing these molecules after 30 days of transplantation could be either proliferating or reactive astroglia. They also reported the ultrastructural changes in long-term nigral

transplants in rat striatum (1996b). At the end of 2 years follow-up, in the transplant and interface region the changes observed suggest premature ageing or a slow rejection process. Slow rejection is a possibility, because these rats are only stock-bred, not inbred, and hence they are not completely immunologically compatible [Gopinath et al, 1996b].

In 1997, Gopinath, Tandon and colleagues [Sable et al, 1997] transplanted fetal dopaminergic neurons to the normal striatum of neonatal and adult rats and to the denervated striatum of adult rats. In the three transplant groups, tracing the immunolabeled tyrosine hydroxylase (TH) positive neurites was easy because they were thicker and coarser than other elements. No apparent glial reaction occurred in the neonates. The growth and maturation of dopaminergic neurons seemed to vary in different environments. The most conducive environment appeared to be neonatal brain in which growth factors are readily available.

Kayalvizhi (2003) from the department of Anatomy, Dr A.L.M. PGIBMS, University of Madras, Chennai reported experimental transplantation of human embryonic cortical tissue after vascular lesion in the motor cortex of bonnet monkey (Macaca radiata). She exhibited survival of the transplant implying its suitability for intra-cerebral transplantation in primates [Kayalvizhi, 2003].

Opioid targets in pain

Basu Ray at the department of Anatomy of the All India Institute of Medical Sciences, New Delhi has focussed his studies on therapeutic targets in pain. The expression of mu opioid receptors, which bind endogenous opioid peptides, was studied in human fetal spinal cords (12-13 to 24-25 wk of gestational age) using autoradiography [Ray & Wadhwa, 1999]. A selective increase was noted in lamina I-II of the dorsal horn in all age groups. In the adult rat spinal cord somewhat similar receptor expression is noted [Ray, 1996]. The adult pattern of receptor distribution was observed relatively late in ontogeny [Ray & Wadhwa, 2004]. Mu receptor expression can be correlated with the maturation of the pain modulatory pathway in the newborn. It is well known that prolonged administration of morphine leads to development of tolerance and dependence. Though various hypotheses have been framed, the exact cause is not definitely known [Ray & Wadhwa, 2001]. Changes in mu receptor density to increased activity of anti-opioid peptides have been proposed. Though a significant increase in expression of mu receptor was observed in the spinal cord after morphine tolerance [Ray et al, 2004], no such increase was noted in the density of opioid receptor-like 1 (ORL1) receptor [Ray et al, 2005b]. The anti-opioid peptide nociceptin binds to ORL1 receptors. Basu Ray and colleagues have also shown that coadministration of nimodipine, a L-type VGCC antagonist, along with morphine leads to greater pain relief than morphine alone [Verma et al, 2005]. It was observed that nimodipine continues to potentiate the analgesic effect of morphine, even 12 hr after its administration and concurrently, reduces the development of tolerance on long-term administration. The dosage of

nimodipine used did not produce any side effect. Additionally, nimodipine is more effective than nifedipine in attenuating morphine tolerance on chronic co-administration [Ray et al, 2008]. Loperamide, a synthetic diphenylpiperidine mu-opioid receptor agonist, which blocks mutiple VGCCs (L-, N- and P/Q-types) on intrathecal administration, produced significantly higher analgesic effect that also lasted for a much longer duration than morphine + nimodipine [Ray et al, 2005c]. It is also hypothesized that topical administration of loperamide could be effective in the treatment of burn pain as peripheral nerves start expressing mu receptors during inflammation [Ray, 2006].

Nitrergic myenteric neurons in the defunctionalized rat colon

Shariff and colleagues (2004) demonstrated significant ($P < 0.0001$) diminution in the area, perimeter and volume-weighted mean volume of soma and nuclei of nitrergic myenteric neurons in the defunctionalized rat colon after 45 days of complete diversion colostomy [Chaudhury et al, 2004]. In addition, there was a significant reduction in the neuronal density of the myenteric neurons with an increase in distance between the ganglia and myenteric glial atrophy. There was accompanied significant reduction ($P < 0.001$) in the volume fraction of the muscularis externa, the prime targets of these neurons. The disturbances in the microecology of the colon may jeopardize the finely orchestrated functioning of the components of the enteric nervous system (ENS) leading to colonic dysfunction. These observations may explain the bowel dysmotility in humans on restoration of colonic continuity after colostomy.

It is evident from this overview that there is a wide and unlimited range of interests in the neuroanatomical field in India. However increasingly, the investigators feel the need to study and present data using various methods and techniques with a view to provide comprehensive information on the research problem being studied. The resultant is that it is difficult to sort information pertaining to only neuroanatomical studies. Hence there may be data, which inadvertently has not been incorporated. However, with the other chapters covering the various other aspects of neuroscience there is every chance that most of the work being carried out would find mention. Neuroanatomical studies would continue to form an important aspect of the ever-expanding vistas of neuroscience though future trend would find neuroanatomical data as one of the components of the multifaceted and multidimensional neuroscience researches.

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