UNIVERSITÀ DI PISA

DEVELOPMENT AND DIFFERENTIATION OF THE NERVOUS SYSTEM

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Anno accademico	2018/19
CdS	NEUROSCIENCE
Codice	414EE
CFU	6

Moduli	Settore/i	Tipo	Ore	Docente/i
DEVELOPMENT AND DIFFERENTIATION OF THE NERVOUS SYS	BIO/06	LEZIONI	48	ROBERT VIGNALI

Obiettivi di apprendimento

Conoscenze
Il corso si propone di illustrare le modalità con cui si sviluppa il sistema nervoso centrale (SNC), con particolare riguardo ai vertebrati. Verranno studiate le interazioni tissutali e genetico-molecolari che progressivamente costruiscono la complessa organizzazione strutturale e funzionale del sistema nervoso, inclusi i meccanismi generali relativi al differenziamento delle cellule del SNC, alla crescita e navigazione degli assoni. Particolare attenzione verrà dedicata al controllo genetico-molecolare del patterning antero-posteriore e dorso-ventrale del tubo neurale e della specificazione e differenziamento neuronale.

Modalità di verifica delle conoscenze
La verifica delle conoscenze consiste in un colloquio orale, secondo le indicazioni date nel campo specifico.

Capacità
Lo studente sarà in grado di orientarsi nell'ambito delle problematiche dello sviluppo del sistema nervoso e acquisirà capacità critiche per l'analisi critica della letteratura pertinente e per l'ideazione di progetti di ricerca sulle problematiche dello sviluppo. Lo studio di articoli chiave originali della letteratura scientifica sarà la base per tali acquisizioni.

Modalità di verifica delle capacità
Una parte dell'esame di profitto è dedicata alla discussione di esperimenti e di articoli scientifici ed alla teorica programmazione di esperimenti con cui il ricercatore potrebbe chiarire interrogativi ancora aperti dello studio dello sviluppo del sistema nervoso. Lo studente dovrà fornire adeguate motivazioni alle strategie che intende adottare nelle sue proposte sperimentali.

Prerequisiti (conoscenze iniziali)
Sono importanti buone conoscenze di:
- Citologia ed Istologia; Biologia Cellulare
- Anatomia generale del sistema nervoso
- Fisiologia del Sistema Nervoso (conoscenze di base)
- Biologia Cellulare
- Biologia Molecolare
- Genetica
- Biologia dello Sviluppo

Prerequisiti per studi successivi
Questo insegnamento costituisce un pre-requisito per la comprensione dell'anatomia e della fisiologia del sistema nervoso.

Indicazioni metodologiche
Le lezioni frontali prevedono l'uso di presentazioni Power Point. I problemi vengono sviluppati attraverso passaggi in cui il docente stimola gli studenti a porsi successive domande per la risoluzione delle problematiche biologiche in esame, indicandone, ove possibile, i possibili approcci sperimentali.
Vengono segnalate attività seminariali cui i ragazzi possono partecipare.
Il docente fornisce il materiale didattico (slides Power Point) al termine di ogni lezione ed indica i testi di riferimento. Ricevimenti sono tenuti in giorni settimanali indicati all'inizio del corso previo appuntamento concordato per email o telefono.

Programma (contenuti dell'insegnamento)

Introduction to the course and suggested textbooks. Early phases of nervous system development: formation of the neural plate and of the neural tube (neurulation). Formation of the three initial vescicles (prosencephalon, mesencephalon, rhombencephalon); five-vesicle stage (telencephalon, diencephalon, mesencephalon, metencephalon, myelencephalon). Spinal cord. Fundamental (basal) and alar plate (lamina); sulcus of His; roof plate and floor plate. General description and definitions of central and peripheral nervous system (CNS and PNS); autonomous nervous system. General contribution of the neural tube, neural crest and ectodermal placodes to the formation of the nervous system. Principal animal model systems used for the study of the development and differentiation of the nervous system. Main issues of the development of the nervous system.

Fate maps and specificarion maps. Concepts of commitment, specification, determination. The initial evidence for neural induction: the organizer experiment (Spemann and Mangold). Regional specific induction: Otto Mangold's einsteck experiments. Artificial inducers. Vertical and planar signals in neural induction: conjugation and exogastrulation experiments by Holtfreter. Nieuwkoop and the activation-transformation model; Eyal-Giladi and the demonstration of the activation-transformation model. Xenopus as a model system.

Functional screening of the organizer genetic activities: the cloning of noggin and chordin and their expression and functional screening. Evidence that active signals prevent neural differentiation: animal cap dissociation experiments. TGF-beta signal transduction pathways. Inhibition of activin signalling, and the discovery of BMP as epidermalizing factors. BMP/noggin and BMP/chordin antagonism.

Molecular mechanism of chordin and noggin action. Other BMP inhibitors. Posteriorizing factors in induction of the nervous system. Role of FGF as a posteriorizing agent and/or a direct neural inducer. Wnts as posteriorizing factors of the CNS.

Wnt inhibitors and reduction of the rostral regions of the embryo CNS: Dickkopf (Dkk) and Freezbee (Frzb-1). Cerberus, a gene that promotes head development. Cerberus and the triple inhibition model: demonstration of the anti-BMP, anti-Wnt and anti-Nodal activities. Insulin-like growth factors in the induction of rostral regions of the CNS. The mouse as a mammalian model system for analysis of development of the nervous system.

Genetic analysis of noggin and chordin roles in the mouse: single and double knockouts. Effects of triple inhibition of noggin, chordin and follistatin in Xenopus neural induction. Other genes involved in the anterior CNS development: Hesx1 inactivation leads to absence of the rostral brain. Dkk1 knockout and the deletion of the head region. Otx2 expression and its role in the anterior CNS: analysis of knockout effects.

Evolutionary conservation of the Otx/otd function: the Drosophila otd gene rescues the knockout effects of Otx2. Translational control of Otx2 protein synthesis in the neuroectoderm. Secondary organizers and CNS regionalization: the mouse anterior neural ridge and the “row1” cells in zebrafish and their role in telencephalon induction. Zebrafish as a model system for studying CNS development.

Role of Tlc in the “row1” cells and in induction of the telencephalon in zebrafish. The isthmic organizer: inducing properties. FGF8 mimicks the isthmic organizer activity. Other genetic activities at the isthmus: Wnt1, en1, en2. Gbx2 and Otx2 activities in the positioning of the isthmic organizer.

Genetic modification of Otx2 and Gbx2 expression misposition the isthmic organizer. The prosomeric model of the rostral brain organization: prosomeres, neuromeres and segmentation of the anterior CNS. Segmental organization and compartmentalization of the hindbrain. Cell lineage analysis and rhombomere transplantations experiments. Cell sorting properties of rhombomeres. Genes involved in rhombomere segmentation and specification: Krox-20.

Genes involved in rhombomere segmentation and specification: Kreisler. Role of Hox genes in hindbrain segmentation and in rhombomere specification. Hoxa1 and Hoxb1 functional inactivation. Hoxb1 ectopic expression. Hox genes and patterns of connectivity of branchiomotor nerves to visceral arches.

Role of retinoic acid (RA) as a posteriorizing factor for the CNS. Use of dominant negative or constitutive RA receptors in Xenopus and effects on embryonic patterning and development. CYP 26 and Raldh2 roles in hindbrain patterning. Ephrins and Eph receptor expression in hindbrain; role of the ephrin and Eph receptors in cell mixing/cell sorting properties of cells of adjacent rhombomeres.

Expression of ephrins and their receptors in the hindbrain and their influence on cell mixing/cell sorting properties of adjacent rhombomeres. Spinal chord histogenesis. Roof plate and floor plate. Ventralizing activity of notochord and floor plate. Transplantation of notochord and floor plate: effects on differentiation of ventral motor neurons. Sonic hedgehog expression and function: knockout effects.

Dorsoventral organization of the neural tube: basal and alar laminae; florr plate and roof plate. Dorsoventral patterning effect of sonic hedgehog. Homeobox patterning genes: Nkx2.2 and Nkx6.1 as downstream effectors of sonic hedgehog in ventral neuron specification. Dorsalizing activity of the roof plate.

Genetic ablation of the roof plate: effects on dorsoventral patterning and on the specification of dorsal interneurons. The derhere mutant and Lmx1a. Dorsalizing activity of the roof plate: Wnt1 and Wnt3a. Columnar organization of the spinal motor neurons and their targets. Hox genes in the specification of the lateral motor column (LMC) and the Column of Terni (CT) in the chick spinal cord.

Role of Hox genes in the specification of motor neuron pools and of their projection patterns. Combination of transcription factors and specification of pools of motorneurons. Conservation of molecular mechanisms in the development of the nervous system: CHD/BMP and sog/dpp antagonism in the Vertebrates and Drosophila, respectively. Neurogenic ectoderm and proneural clusters in Drosophila. Proneural and neurogenic genes. Lateral inhibition in proneural clusters: Delta-Notch signaling.

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Proneural genes. bHLH factors and their general mechanism of action. Conservation of proneural and neurogenic genes in the Vertebrates. Primary neurogenesis in Drosophila: neurogenin and the identification of proneural columns. Hierarchical interactions among neurogenein, Delta and Notch and the selection of primary neurons from proneural columns in Xenopus. Delta-Notch signalling inhibitory action on neurogenesis. General action of bHLH factors in neuronal specification in the spinal cord and telencephalon.

Zic and Sox genes and the response to neural induction. Eye development in Drosophila and the vertebrates: conservation of the genetic network. Eyeless, toy and other genes involved in eye development of Drosophila are conserved in the vertebrates. Genes involved in eye filed specification: Pax6, Six and Rx1. Rx1 in eye specification and retinal progenitor proliferation.

The Ciliary marginal zone of fishes and amphibians as a model for the study of commitment and differentiation in the retina. Molecular mechanisms of retinal specification. bHLH and homeobox transcription factors in retinal cell commitment. Ath5 and retinal ganglion cell specification: knockout of ath5 and the zebrafish lakritz mutant. Xbh1 homeobox factor in retinal ganglion cell commitment. Otx2 and Otx5 in the specification of bipolar and photoreceptor neurons.

Molecular domains involved in determining the specific actions of Otx2 and Otx5 for Xenopus bipolar and photoreceptor cell specification, respectively. Translational control of specific mRNAs as a key event for the appropriate timing of retinal cell commitment: role of micro RNAs in the regulation of translation of Otx2 and Vsx1 genes in the Xenopus retina. Affecting cell cycle affects translation of key mRNAs.

Asymmetric cell division in Drosophila and the Vertebrates: Par6, Numb and other molecular players of apico-basal cell polarity (general aspects). Histogenesis of the CNS: cell divisions and histogenesis in the spinal cord. Pseudolayered organization of the neuroepithelium, cell cycle and interkinetic migration of nuclei within the neural tube wall. Sidman experiments and labeling of precursors: assessing cell cycle lenght during development. Cerebral cortex histogenesis. "Birthdating" experiments; cortical neurons. "inside-out" model of cortical development. della corteccia. The radial glia cells and their significance. Ventricular and subventricular zone. The changing picture of cortical histogenesis. The contribution of subpallial structures to the cortex neurons. Tangential migration of GABAergic neurons: role of Dlx1 and Dlx2.

Organization and histogenesis of the cerebellar cortex. "The rostral migratory stream". Secondary neurogenesis; the hippocampus. Axon extension: the growth cone. Ultrastructure of the growth cone. Role of the ctìytoskeleton (actin filaments and microtubules); lamellipodia and philopodia. Molecules involved in axonal navigation (general aspects); stepwise navigation. Chemoattraction and chemorepulsion, at distance and by contact. Role of netrins and their receptors.

Chemoattraction and chemorepulsion, at distance and by contact. Main classes of molecules and receptors involved in navigation. CAMs, fasciclines, extracellular matrix, integrines; semaphorines, plexins and neuropilines (general aspects). Role of netrins and their receptors. Commissureless and roundabout mutants in Drosophila; Slit and Robo in Drosophila and the Vertebrates. Commissural axons as a paradigm. Morphogens that influence axonal extension (Wnt). Roger Sperry and the chemoaffinity hypothesis; the stripe assay. Gradients of Ephrins and Eph receptors in the tectum and topographic projections of retinal axons. General aspects on Reelin and cortex layering.

Bibliografia e materiale didattico

Sanes et al., Development of the Nervous System, Academic Press
trad. Italiana: Sanes et al., Lo Sviluppo del Sistema Nervoso, Zanichelli
Gilbert, Developmental Biology, Sinauer
Kandel et al. (eds.), Principles of Neurosciences, McGraw-Hill
Sono inoltre forniti come pdf articoli scientifici e reviews chiave per la comprensione degli argomenti trattati.

Indicazioni per non frequentanti

Non esistono differenze nei contenuti o nelle modalità di esame per gli studenti che non frequentano. Eventualmente, dietro richiesta, possono essere tenuti ricevimenti per chiarire aspetti del corso e risolvere dubbi allo scopo di consentire il migliore studio possibile agli studenti che non hanno seguito le lezioni.

Modalità d'esame

La verifica delle conoscenze consiste in un colloquio orale, nell'ambito del quale vengono discusse le problematiche presentate a lezione.
Il candidato dovrà dimostrare la acquisizione delle nozioni essenziali dello sviluppo del sistema nervoso, ma anche la capacità critica su come si è arrivati a chiarirne gli aspetti più significativi. Dovrà anche eventualmente dimostrare capacità di progettazione e di analisi di esperimenti virtualmente realizzabili per rispondere alle domande biologiche che emergono nel corso della discussione.
A titolo puramente indicativo, la durata del colloquio è di circa 30 minuti.

Note

Le lezioni si tengono in lingua Inglese. Gli esami possono essere tenuti in Inglese o Italiano, a scelta dello studente.

Ultimo aggiornamento 04/10/2018 12:46