

## SA1

**Progenitor domains in the developing spinal cord: molecular control of neuronal diversity**

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The control of motor behaviour in the spinal cord depends on the coordinated activity of motor neurons (MN) and local circuit interneurons. Anatomical and physiological studies have revealed the existence of distinct classes of interneurons. They differ for their transcription factor profile, migration pattern, settling position and axonal trajectory, neurotransmitter phenotype and synaptic connectivity. During development distinct classes of interneurons are generated in a precise number and location in adjacent domains within the neural tube and subsequently are redistributed to their final destination by migration. Distinct neuronal identities are acquired through a progressive restriction in the developmental potential of progenitor cells in response to local environmental signals. In the last few years genetic and molecular approaches have begun to elucidate how these diverse aspects of interneuron phenotype emerge during development. Moreover, it has been possible to start to unravel the relationship between early genetic programs in progenitor cells and differentiated functions of interneuronal classes in the adult. We have shown that the spatially-restricted expression of a single progenitor HD protein, Dbx1, contributes to coordinate diverse phenotypic features that underlie interneuron identity and function within the developing spinal cord. These results begin to suggest a direct link between early programs of gene expression and differentiated features of interneuronal classes. In addition, they have allowed to genetically identify spinal interneurons that are involved in coordinating left-right motor activity during locomotion and, therefore, to relate early specification of neuronal identity with the formation of specific circuits in the mature spinal cord.

## SA2

**Using Mouse Genetics to Label Subcellular Structures of Neuronal Subpopulations**S. Arber<sup>1,2</sup>, M. Sigrist<sup>1</sup> and I. Kramer<sup>1,2</sup><sup>1</sup>*Cell Biology, Biozentrum, Basel, Switzerland* and <sup>2</sup>*Friedrich Miescher Institute, Basel, Switzerland*

An ever more challenging problem in neurobiology will be to identify distinct subpopulations of neurons to study their function in neuronal circuit assembly and function. In order to be able to visualize subpopulations of neurons and distinct subcellular compartments within those neurons we have taken a mouse genetics approach. We have used different spectral variants of the enhanced green fluorescent protein (eGFP) and have made fusion constructs to target the resulting proteins to distinct subcellular locations. These fusion proteins include the generation of a construct to tag eGFP to membranes by linking an amino-terminal membrane tagging extension to eGFP and the generation of a construct in which eGFP is fused to Synaptophysin to label presynaptic structures selectively. We have used these fusion

constructs to generate transgenic mice in which these genes are expressed under the control of Tau or Thy1 regulatory elements to achieve neuron specific expression at different developmental stages. Moreover, we have used the same strategy to express these fusion proteins conditionally by using a Cre-LoxP based genetic system in the mouse. We have tested our strategy extensively in the spinal cord and dorsal root ganglia (DRG) neurons and results from this analysis will be presented. In summary, the combination of conditional activation of eGFP based reporters by Cre recombinase represents a powerful approach to label distinct subpopulations of neurons.

## SA3

**Electrophysiological analysis of genetically defined interneurons in the mouse spinal cord**R.M. Brownstone<sup>1</sup>, J. Wilson<sup>1</sup>, I. Lieberam<sup>2</sup>, Y. Yoshida<sup>2</sup> and T. Jessell<sup>2</sup><sup>1</sup>*Surgery (Neurosurgery) and Anatomy & Neurobiology, Dalhousie University, Halifax, NS, Canada* and <sup>2</sup>*Columbia University, New York, NY, USA*

The neuronal organization of the spinal cord, and the elucidation of properties that permit the integration of proprioceptive, descending and intrinsic segmental neural activity to produce movement, have challenged neuroscientists for decades. The excitation and inhibition of motoneurons during locomotor activity is controlled by spinal interneurons. Classical *in vivo* experiments have led to the classification of interneurons by their location, segmental input, and axonal projections (Jankowska, 1992). However, using these criteria it has not been possible to provide a comprehensive classification scheme that unambiguously defines the functional roles and properties of ventral horn interneurons. The generation of transgenic mice in which enhanced green fluorescent protein (eGFP) is expressed in specific populations of interneurons has provided the methodology that enables the identification and study of spinal interneurons. In Hb9-GFP mice, eGFP is expressed under the control of the murine promoter of the homeodomain protein HB9. These mice (Wichterle et al., 2002) carry an estimated 5-10 copies of a transgene comprised of a ~9 kb fragment with the 5' upstream region of the murine HB9 gene followed by a 5' splice substrate, an eGFP gene, and a polyadenylation signal. In these Hb9-GFP mice, some ventral interneurons, in addition to motoneurons express GFP. Of note are a discrete, clustered population of GFP+ interneurons which are located in medial lamina VIII throughout the spinal cord, and which, unlike other GFP+ interneurons, are indeed Hb9 positive. Electrophysiological and anatomical investigations were carried out on this genetically defined population of interneurons. To assess whether the medial Lamina VIII cells are involved in locomotor activity, adult mice were subjected to a 90 minute overground locomotor task. Following an intraperitoneal injection of ketamine and intracardiac perfusion, the spinal cords were removed, post-fixed and immunohistochemically processed for the activity-dependent immediate early gene *c-fos*. Double labeling of *c-fos* and GFP in the medial Lamina VIII cells indicates that these interneurons are active during locomotion. To assess the transmitter phenotype of these cells, we used fluorescent *in situ* hybridization with a mRNA probe for the

vesicular glutamate transporter vGlut2. Data revealed that the eGFP+ medial lamina VIII interneurons are glutamatergic. To identify the electrophysiological properties of these cells, we obtained whole cell patch clamp recordings from medial lamina VIII eGFP+ neurones. Experiments were done at a developmental stage where the mice could weight bear and walk with their abdomens pendant (> postnatal day 8). To obtain spinal cord slices, mice were anaesthetized with ketamine, decapitated and their spinal cords removed. 200  $\mu$ m thick slices of the upper lumbar spinal cord were cut on a vibrating microtome and equilibrated for an hour prior to recording. GFP positive interneurons in medial lamina VIII were identified and patch-clamped using IR-DIC optics and epifluorescence. Two distinct electrophysiological classes of neurones were evident. The first class (n= 40) consisted of neurones with very small somata (mean  $\pm$  sdev:  $9.3 \pm 1.4$  pF), high input resistance ( $1012 \pm 321$  M $\Omega$ ) and a prominent post-inhibitory rebound (PIR) giving rise to action potential doublets (100%). The PIR, a property important in rhythm-generating neurones, is mediated by a nickel-sensitive transient calcium current. Many of these cells (58%) were spontaneously firing. The second class of Lamina VIII GFP positive interneurons (n=8) were larger ( $16.8 \pm 2.2$  pF,  $p < 0.002$ , student's t-test), and had lower input resistances ( $520 \pm 200$  M $\Omega$ ,  $p < 0.002$ ), prominent hyperpolarisation-activated inward currents (I<sub>h</sub>, 100%). These cells had spontaneous biphasic oscillations of membrane potential characteristic of electrotonic coupling (100%). This study demonstrates that advances in developmental biology combined with genetic technology provide the methodology to identify classes of spinal interneurons and to study their anatomical and physiological properties. This is necessary for the characterisation of spinal motor networks.

Jankowska E. (1992) Prog Neurobiol. 38:335-78

Wichterle H, Lieberam I, Porter JA, Jessell TM. (2002) Cell 110:385-97.

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#### SA4

### Physiological and genetic approaches to locomotor circuits in mammals

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Intrinsic spinal networks control much of the rhythmic motor neuron activity that underlies locomotion. These spinal rhythm-generating networks are known as central pattern generators (CPGs), and are thought to function as local command and control centres for the phasic activation of motor neurons during locomotion in all vertebrates, including man. The precise organization of the mammalian CPG is, however, so far unknown. In this talk I will review findings that have identified CPG neurons involved in the left-right alternation during locomotion. These neurons play an important role in coordinating flexor and extensor activity between the two sides of the body. I will also review findings showing that knockout of the axon guidance molecule EphA4 results in an abnormal hopping gait in mice. These dramatic changes in gaits are due to a genetic reconfiguration of the CPG, which can be explained by aberrant crossing of excitatory

CPG interneurons in mutants. I will furthermore review data that have shown that many interneurons expressing EphA4 in lumbar spinal cord are rhythmically active during locomotion. A subset of these neurons provide monosynaptic excitation of motor neurons on the same side defining them as being involved in generating the local ipsilateral rhythmic motor activity. A large number of EphA4-positive interneurons are derived from the V2 interneuron population defined by expression of Chx10 in the embryonic spinal cord. These results suggest that the axon guidance molecule EphA4 in combination with the transcription factor Chx10 defines a population of ipsilateral locomotor-related interneurons.

Kiehn, O., Butt, SJB. (2003). Physiological, anatomical and genetic identification of CPG neurons in the developing mammalian. *Progress in Neurobiology*, 70: 347-361.

Kullander K., Butt S.J.B., Lebet J., Lundfald L., Restrepo E., Klein R., Kiehn, O. (2003). Role of EphA4 and EphrinB3 in local circuits that control walking. *Science*, 299: 1889-1892

Butt, SJB, Kiehn O. (2003). Functional identification of interneurons responsible for left-right coordination of hindlimbs in mammals. *Neuron*, 38, 953-963

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#### SA5

### Primitive inhibitory interneurons in the developing frog spinal cord

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Understanding the neuronal networks in the spinal cord is hampered by the huge diversity of neurons and their connections. Transcription factors are beginning to provide a key to resolving classes of spinal interneuron and revealing homologies between different vertebrates. In developing zebrafish and frog, a discrete population of spinal interneurons with very characteristic axonal projections, express engrailed (En-1; Higashijima et al., 2002). We have used whole-cell recordings from pairs of neurons together with neurobiotin filling to study the responses and connections of these ascending interneurons in *Xenopus* tadpoles. We show directly that these neurons produce glycinergic, negative-feedback inhibition that can limit firing in neurons of the swimming central pattern generator. They may therefore be functionally related to mammal Renshaw cells that are also thought to express En-1. In the frog tadpole, these ascending interneurons have a second role. They are the modulatory neurons producing glycinergic inhibitory gating of cutaneous sensory pathway interneurons during swimming (Li et al., 2002). These discoveries raise the possibility that some classes of interneuron with distinct functions later in development may differentiate from an earlier class where these functions are shared.

Higashijima S., Mandel G., and Fetcho J.R.. (2002) Generation of transgenic zebrafish expressing a genetically encoded fluorescent calcium indicator, cameleon, in spinal cord. Program No. 67.17. 2002 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience.

Li, W.-C., Soffe, S.R and Roberts, A. (2002) The spinal inhibitory neurons that modulate cutaneous sensory pathways during locomotion in a simple vertebrate. *J. Neurosci.* 22: 10924-10934.

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## SA6

**Transmitter phenotype and connectivity of lineage traced neurons in the spinal cord**

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Recently, it has been shown that different gene expression patterns delineate five progenitor domains in the developing ventral neural tube. Four of these domains (p0-p3) give rise to interneurons (V0-V3), while the other (pMn) gives rise to motoneurons. Although the cell migration patterns for these domains have been characterised in the embryo, the final somatic location and axonal connections of cells derived from each domain have yet to be determined in the adult. We have investigated the transmitter phenotype and connectivity of the V0 and V2 interneurons, by using expression of GFP driven by markers that are transiently expressed in the p0 and p2 domains (Dbx1 and Lim3 respectively). The relationships between progenitor-domain derived interneurons and their targets were examined with immunohistochemistry and confocal microscopy. To determine the transmitter phenotype, tissue sections were cut from mid-lumbar segments and reacted with antibodies against glutamic acid decarboxylase (GAD65, GAD67) and the neuronal glycine transporter GLYT2 (to identify GABAergic and glycinergic axon terminals), and against the vesicular glutamate transporters VGLUT1 and VGLUT2 (to identify glutamatergic axon terminals). Animals were anaesthetized with sodium pentobarbitone (1ml I.P.), killed humanely and fixed by perfusion. Dbx1-GFP cells made up approximately 40% of ventral horn neurons (excluding motoneurons) and included populations of inhibitory and excitatory interneurons. The Dbx1-GFP cells in the ventral horn occupied a region extending laterally from the central canal, and also passing down the medial and lateral aspects of the ventral horn. Fewer Dbx1-GFP cells were found in the central part of lamina VII, and they were rare in the motor nuclei. Dbx1-GFP axon-terminals were distributed throughout the ventral horn and innervated the motor nuclei. Immunofluorescent labelling of GFP and ChAT (choline acetyl transferase) suggests that the large cholinergic terminals on motoneurons, C-boutons (Boidan 1966, Conradi 1969, Nagy et al 1993), are derived from the Dbx1-expressing cell population and probably originate from a group of cells lateral to the central

canal in medial lamina VII and lamina X. Cholinergic cells in this area were previously shown to be immunoreactive for c-Fos following induction of fictive locomotion (Huang et al 2000). We also used GFP linked to a gene expressed in motoneurons (ISL-1) as an exclusion-marker, to show that C-boutons are not motoneuron collaterals. This was previously suspected because of the different vesicle constituents compared to motoneuron axon terminals. In contrast to the Dbx1 interneurons, the Lim3-GFP cells were less numerous and most of their axon terminals were VGLUT2-immunoreactive. Some were immunoreactive for GLYT2 but they were very rarely positive for GAD. Lim3 axon-terminals were mainly distributed in the ventral horn, including the motor nuclei and around the central canal, although they were less evident in the central part of lamina VII. In the dorsal horn, axon terminals were distributed evenly across lamina VI with some axonal arbors in medial and central lamina V. In addition to examining cell populations derived from progenitor domains, we also assessed the transmitter phenotype and somatic location of cells labelled by an HB9-GFP construct. Previous studies of cells expressing HB9-GFP during chick embryo development indicated that HB9 expression was restricted to motoneurons. (Arber et al 1999). However, in our HB9-GFP construct (Wichterle et al 2002), GFP expression was also observed in interneurons throughout lamina VII, Lamina VIII and Lamina X in the adult mouse spinal cord. In particular, the transgene highlighted a population of interneurons in lamina VIII that receive GABAergic contacts on their somata and proximal dendrites. There also appeared to be glutamatergic interneuron contacts on proximal dendrites of motoneurons but not on their somata. In summary: These data suggest that progenitor domains in the developing neural tube give rise to functionally distinct populations of cells in the adult mouse. They also show that reporter genes can assist in the targeting of functional clusters for anatomical analysis. Using these methods, we have identified a population of cells lateral to the central canal that probably give rise to the large cholinergic terminals on motoneurons. We have also shown that HB9-GFP expression can be used to identify a novel cluster of interneurons in lamina VIII.

Arber S et al, 1999, *Neuron* Vol 23: pp659-674Boidan D, 1966, *Bull Hopkins Hosp* 119: pp16-45Conradi S, 1969, *Acta Physiol Scand*, 332(supl): pp49-64Nagy et al, 1993, *Synapse* Vol 15: pp17-32Wichterle H et al 2002, *Cell* Vol 110: pp385-397

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