

## Scientists succeed in making the spinal cord transparent

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A spinal cord as if made of glass: The new method enables scientists to see nerve cell in the intact cellular network. © MPI of Neurobiology / Ertürk

(Medical Xpress) -- In the event of the spinal cord injury, the long nerve cell filaments, the axons, may become severed. For quite some time now, scientists have been investigating whether these axons can be stimulated to regenerate. Such growth takes place on a scale of only a few millimetres. To date, changes like this could be determined only by cutting the tissue in question into wafer-thin slices and examining these under a microscope. However, the two-dimensional sections provide only an inaccurate picture of the spatial distribution and progression of



the cells. Together with an international team, scientists at the Max Planck Institute for Neurobiology in Martinsried have now developed a new method by virtue of which single nerve cells can be both examined in intact tissue and portrayed in all three dimensions.

The spinal cord is the most important pathway for relaying information from the skin, muscles and joints to the brain and back again. Damage to <u>nerve cells</u> in this region usually results in irreversible paralysis and loss of sensation. For many years, scientists have been doing their best to ascertain why nerve cells refuse to regenerate. They search for ways to stimulate these cells to resume their growth.

To establish whether a single cell is growing, the cell must be visible in the first place. Up to now, the procedure has been to cut the area of the spinal cord required for examination into ultra-thin slices. These are then examined under a microscope and the position and pathway of each cell is reconstructed. In exceptional cases, scientists could go to the trouble of first digitizing each slice and then reassembling the images, one by one, to produce a virtual 3D model. However, this is a very timeconsuming endeavour, requiring days and sometimes even weeks to process the results of just one examination. Even worse, mistakes can easily creep in and falsify the results: The appendages of individual nerve cells might get squashed during the process of slicing, and the layers might be ever so slightly misaligned when set on top of each other. As Frank Bradke explains: "Although this might not seem dramatic to begin with it prevents us from establishing the length and extent of growth of single cells." Bradke and his team at the Max Planck Institute of Neurobiology have investigated the regeneration of nerve cells following injuries to the spinal cord. Since July he has been working at the German Centre for Neurodegenerative Diseases (DZNE) in Bonn. "However, since changes on this crucial scale are precisely what we need to see, we worked meticulously until we came up with a better technique", he continues.



The new technique is based on a method known as ultramicroscopy, which was developed by Hans Ulrich Dodt from the Technical University of Vienna. The Max Planck neurobiologists and an international team of colleagues have now taken this technique a step further. The principle is relatively straightforward. Spinal cord tissue is opaque due to the fact that the water and the proteins contained in it refract light differently. Thus, the scientists removed the water from a piece of tissue and replaced it by an emulsion that refracts light in exactly the same way as the proteins. This left them with a completely transparent piece of tissue. "It's the same effect as if you were to spread honey onto textured glass", Ali Ertürk, the study's first author adds. The opaque pane becomes crystal clear as soon as the honey has compensated for the surface irregularities.

The new method is a leap forward in regeneration research. By using fluorescent dyes to stain individual nerve cells, scientists can now trace their path from all angels in an otherwise transparent <u>spinal cord</u> section. This enables them to ascertain once and for all whether or not these nerve cells recommenced their growth following injury to the spine – an essential prerequisite for future research. "The really great thing is the fact that this method can also be easily applied to other kinds of tissue", Frank Bradke relates. For example, the blood capillary system or the way a tumour is embedded in tissue could be portrayed and analysed in 3D.

**More information:** Ali Ertürk, Christoph P. Mauch, Farida Hellal, Friedrich Förstner, Tara Keck, Klaus Becker, Nina Jährling, Heinz Steffens, Melanie Richter, Mark Hübener, Edgar Kramer, Frank Kirchhoff, Hans Ulrich Dodt, Frank Bradke, 3D imaging of the unsectioned adult spinal cord to assess axon regeneration and glial responses after injury, *Nature Medicine*, online publication, December 25, 2011



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