Neuroanatomical Research Methods

Why do we need tracing methods? Exact information about connections between neurons is elusive. The path that a single neuron takes can be elucidated by studying its activities, such as axonal transport or glucose/oxygen metabolism. Viruses that cross synapses can also be used as a tracers and afford information about pathways.

Problem: Clinico-pathological correlations and functional imaging don't tell us much about how neurons are connected. Histology sounds like a natural alternative, but histological material makes direct tracing impossible. The methods that allow for tracing include those based on degeneration, axoplasmic transport, transport in membranes, substances that cross synapses (viruses), and cell metabolism. Other methods include those that exploit the properties of neural transmission (neuronography) or use toxic substances.

Degeneration. This is an old method. Basically, the method entails lesioning an axon and then staining those that undergo degeneration as a result of the damage. Problem: many neurons fail to exhibit the characteristic changes of interest. Distal degeneration is the most reliably observed result. Marchi's method involves staining degenerating myelin with osmium tetroxide. Unmyelinated materials aren't visible using this technique. Silver methods are used for unmyelinated material. These aren't suited for study of humans, as degenerating axons are observable only only during a window of time some 4 to 8 days later. These techniques are for postmortem studies.

Axoplasmic transport. This method is much newer. It reveals cells of origin and the sites of axon termination. Useful! One effective method in this category uses tritium, which is radioactive. Leucine, tagged with tritium is injected into a site. The amino acid is imbibed by neurons and incorporated into proteins, which are transported to axon terminals. A day or two later, the animal is sacrificed and a chemical is used to freeze the proteins in place. High concentrations of silver grains (tritium) are seen at the site of injection, along the axon and at its terminal.

Why is this better than the old methods above? Labeled molecules only enter the cell bodies and dendrites. Axons passing through the injection site don't imbibe the tracer and are thus undetected. Precision!

Related methods rely on uptake and axonal transport or a histochemically detectable protein (like peroxidase or lectin; these can be found by a protein tracer) or something fluorescent (this can be seen using special microscopes). If one's interested in tracing how axons branch, use two different colours of fluorescent materials that are transported retrogradely. One then asks, "Are both colours present in one cell body?"

Problem: proteins and dyes are also swallowed up by injured axons that happen to be passing through, so care must be taken when injecting these substances.

Membrane probes. Cyanine dyes (e.g. DiI) enter lipid membranes of cells and diffuse along the cell (even in dead cells). Diffusion is slow, of course - it takes months to trace just one centimetre. A useful advantage of this slow tracing method is that it can be used in living cells; it's good for studying developmental issues.

Transsynaptic tracing of pathways. The rabies virus spreads through the nervous system, jumping across synapses. Viruses are great tracers. They replicate within neurons, travel along axons and jump across synapses. Viruses can allow for a sort of custom-designed labeling, as their genetic material can be modified to, say, harbour a histochemically detectable protein.

Metabolic methods. Consider 2-deoxy-D-glucose. It's just like ordinary D-glucose, except that it isn't metabolized. It can be radioactively labeled and is thus a useful tracer. Given that neurons *in use* are metabolically active, this tracer is great for *functional* tracing. Cytochrome oxidase, an enzyme, also tends to pool in active neurons and can be detected histochemically. Another *functional* tracer, of sorts.

Physiological methods. There are other ways of exploring connections between neurons... Measurement of time elapsed between stimulation and recording provides information about the number of synapses in a pathway. This is neuronography.

Toxic substances can tell us a lot about pathways. Nicotine blocks synapses and thus reveals their locations. It can also stimulate some neurons that normally respond to acetylcholine. For those interested in studying such neurons, nicotine can help to establish where they are and where they go. Excitotoxins are similarly useful. Kainic and ibotenic acid are toxic. They're analogues of glutamic acid (excitatory). These acids result in abnormally long and destructive activation of only the postsynaptic cells. This is a very selective lesion. There are toxins that specifically target dopamine or noradrenaline or serotonin...

Some poisonous lectins are swallowed by axon terminals and injured axons of passage, transported retrogradely and halt protein synthesis, ultimately killing the affected neurons. This is yet another selective lesion.

Major Themes/Questions

- What is the value of these techniques?
- What *can't t*hese techniques tell us?
- How are neurons affected using different tracing methods?
- Which techniques are suitable for living tissue?
- Which techniques can tell us about disease?
- Which techniques can tell us about pathways?
- What is the relative precision of each technique?

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Somatic Sensory Pathways

Two big systems: the spinothalamic system, which conveys pain, temperature and light touch and the medial lemniscus system (posterior column system/dorsomedial system), which conveys discriminative touch and proprioception. The first system combines with the spinoreticulothalamic system, which includes the reticular formation, and conducts cutaneous touch signals. Together, these systems are the central mechanism for pain conduction and can collectively be referred to as the ventrolateral or anterolateral system.

Sensations from the head are conducted via the trigeminothalamic pathways, which are akin to the two big systems described above.

Important note: the general way of thinking about these pathways is that they're comprised of 1st- 2nd- and 3rd-order neurons with cell bodies in sensory ganglia, the spinal cord/brain stem and the thalamus... It's not that simple! There are many, many, smaller-scale topographical differences that one must attend to. These are detailed in the lecture materials. Also, the role of descending input cannot be ignored... Remember this...

SPINOTHALAMIC SYSTEM

Pain *receptors*. Recall that this system is concerned with pain. There are two types of pain receptors, corresponding to two types of pain. The thin, myelinated group A and unmyelinated group C axons convey sudden, sharp onset of pain and a slow, dull throbbing pain, respectively. How does pain work? Injured cells release "mediators". These dilate blood vessels, which leak plasma and make an injured area red and swollen. Stimulation of the receptors described above convey the pain message. The messages also propagate antidromically and result in the release of substance P, which enters interstitial tissues in the dermis, further dilating vessels and triggers release of more mediators.

Temperature *receptors*. These aren't well understood. They're probably free endings, similar to the pain receptors.

Light touch *receptors*. Merkel, Meissner, Ruffini, Pacini... Medium-sized, myelinated group A fibres carry touch messages from these specialized receptors.

Synapses and interneurons in the dorsal horn. Let's imagine the voyage that one of the above signals would embark upon on its way up to the brain... 1st-order neurons enter through medial division of the dorsal rootlet and enter the dorsolateral tract, branching in ascending and descending directions. The dorsolateral tract neurons synapse in the substantia gelatinosa in the tip of the dorsal horn, in Lamina II. Cells in this "gelatinous substance" ascend and descend short distances, branching off and synapsing with tract cells (2nd-order), whose axons ascend in the spinothalamic tract. This is an important region, as modification of sensory signals takes place here. Dendrites here are not only contacted by 1st-order afferents, but also by descending reticulospinal inputs, like those from the raphe nuclei in the medulla. Up from here...

Spinothalamic tract. Tract cells have their nuclei in the nucleus proprius (Laminae IV, V, and VI). The axons decussate in the ventral white commissure. Through the ventral horn of spinal grey matter and then ascension, through the spinothalamic tract. Continuing upwards, axons are continually added to the internal bits of the tract. Near the neck, fibres from sacral segments are most superficial. Cervical fibres are closest to the centre and to the grey matter.

Consider the pathway as it reaches the medulla... The tract traverses the lateral medullary zone at the level between the inferior olivary nucleus and the trigeminal nucleus, close to the lateral surface of the medulla. The spinal lemniscus (comprised of our spinothalamic tract plus the spinotectal tract) continues through the ventrolateral bits of the pons. Spinothalamic axons branch off and terminate in the reticular formation (at both the medulla level and the level of the pons) - some so-called spinoreticular fibres stop here.

Thalamus and cerebral cortex. Next, the pathway proceeds to the thalamus... Most axons wind up in the VPl region of the thalamus (medial lemniscus goes here, too) and in the VPm region, which receives trigeminothalamic input. Somatotopy is observed here. The contralateral lower limb is represented dorsolaterally and the contralateral upper limb is represented ventromedially in the VPl. The opposite side of the head is represented in the VPm.

From here, the thalamocortical portion of the pathway traverses the posterior limb of the internal capsule and onto S1 in the parietal lobe. The contralateral half of the body is represented in an inverted fashion. Beginning ventrally, the somatotopy includes, hand, arm, trunk, thigh, and so on.

Some axons of the spinal lemniscus end in posterior and intralaminar thalamic nuclei and in mediodorsal nuclei. These project to insula/posterior parietal areas, frontal/parietal areas/striatum, and frontal lobes, respectively. Collectively, these pertain to alertness, affect, and decision making.

Pain. Now that we've considered the route that this system takes, let's turn our attention to one of its main signals, pain, and how it demonstrates the importance of not only ascending pathways, but descending pathways as well.

As hinted above, pain signals are modified in the dorsal horn. It's not a one-way transmission of information. Large-diameter afferents (for touch and deep pressure) have branches that synapse in the substantia gelatinosa (Lamina II). Impulses from these large cells stimulate the gelatinosa cells. This in turn causes these interneurons to inhibit tract cells (2nd-order) concerned with pain. This is a general pain suppression mechanism. This inhibition can be overcome by a very painful stimulus, which provides a sufficiently large input to the pain receptors and thus to the tract cells. This is the *gate control theory of pain*. A similar mechanism likely exists in the caudal part of the spinal trigeminal nucleus.

Note that pain is also transmitted through a simple, more direct pathway. Large neurons (Waldeyer cells) at the tip of the dorsal horn afford this pathway. These are activated by 1st-order neurons (pain receptors) and have axons that travel in the spinothalamic tract, all the way to the ventral posterior and mediodorsal thalamic nuclei.

What transmits the pain signal? Impulses that convey pain information are transmitted upwards in the spinothalamic and spinoreticular tracts. There are additional axons that complement these, present in the dorsolateral funiculus. So, what happens when the ventrolateral region of the cord is cut? What happens to the pain signal? Pain signals from the opposite part of the body are completely lost, for a

while. Over the course of weeks, pain perception returns. How? The nervous system increasingly turns to intact, alternate pathways to experience pain. What happens if the midline of the cord is cut? What happens to the pain signal then? This results in prolonged analgesia in the segments affected by the lesion.

Forget about the cord for a minute... What would happen to the pain experience if S1 were lesioned? Is pain felt at all? Yes, pain is still felt, but it's poorly localized. Spinothalamic and reticulothalamic afferents to the intralaminar and mediodorsal thalamic nuclei are responsible for the persistence of the pain experience. These thalamic nuclei are connected to most of the neocortex, including prefrontal and cingulate areas. Imaging studies have shown that a painful stimulus on just one side of the body results in increased blood flow to the cingulate areas, bilaterally. The ventral posterior nucleus of the thalamus, combined with S1, likely contribute to the localization of pain.

The pain experience is modified by descending input. Pain and the occurrence of reflexive, defensive actions can be suppressed, after all. How? This is mediated by corticospinal input, originating in the parietal lobe and ending in the dorsal horn. The reiculospinal pathways (i.e. raphespinal) exert control of this nature. The unmyelinated axons of the raphespinal tract traverse the forsal part of the lateral funiculus of the spinal cord. They rely on serotonin. In fact, the substantia gelatinosa contain more serotonin-containing synaptic terminals than most areas. The raphe nucleus itself is affected by descending input from the periaqueductal grey.

So, what happens when these two areas are stimulated? The result is profound analgesia. Can this be reversed? Yes, by cutting the dorsolateral funiculus or administering drugs that antagonize opioids, like enkephalin or morphine. The gelatinosa cells, unsurprisingly, contain receptors for opioids as well. The periaqueductal grey and the raphe also contain such receptors. Morphine works by stimulating these receptors. A clinical application of this pain pathway involves the use of chronically implanted electric stimulators in the periaqueductal grey. The stimulation relieves pain.

Major Themes/Questions

- Where does distal inhibition take place?
- What are the effects of different lesions? How might these effects differ depending on the height and location of the lesion?
- How might one block or enhance a _____ signal?
- How do sensory signals originating from the head differ from those originating elsewhere?
- What is the significance of descending inputs? How do these bear on different kinds of sensory information?