Main achievements are summarized as follows:

(i) What is the cellular distribution of the anandamide-synthesising pathways?

1. All the enzymes implicated in anandamide (AEA) synthesis are expressed at mRNA level in rat naive dorsal root ganglia (DRG), however, inositol 5' phosphatase (Inpp5) and protein tyrosine phosphatase, non-receptor type 22 (PTPn22) are hardly detectable by reverse-transcriptase PCR (RT-PCR). The most abundant enzymes are glycerophosphodiester phosphodiesterase 1 (GDE-1) > α/β-hydrolase 4 (ABHD4) >/= *N*-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) in the DRGs. Similarly to DRG, GDE-1 > ABHD4 >/= NAPE-PLD are the most abundant enzymes in the spinal cord.
2. Three (GDE-1, PTPn22 and NAPE-PLD) out of the 6 enzymes are translated into proteins in detectable levels in naive DRGs as well as in the spinal cord of rats when examined with Western immunoblotting. However, NAPE-PLD protein is expressed just above the detection level in naive DRG.
3. Culturing of primary sensory neurons (PSN) has a significant impact on the transcription/translation of AEA synthesizing enzymes with known molecular identity. At mRNA level, culturing causes a significant down-regulation in the expression of ABHD4 and group 1b soluble phospholipase A_2 (sPLA2) transcripts, whereas it elevates the transcription of PTPn22. At protein level, NAPE-PLD, PTPn22 and GDE-1 proteins are upregulated following culturing of PSNs.
4. Cultured PSN express, in a cell-specific manner, five of the six enzymes, which have previously been implicated in AEA synthesis and these enzymes form several functional pathways.
5. Using immunohistochemistry only four (NAPE-PLD, Inpp5, GDE-1 and PTPn22) out of six enzymes implicated in anandamide synthesis are detectable in naive DRG. While antibodies raised against ABHD4 or sPLA2 produce positive staining in other tissues, these antibodies do not result in any immunoreactivity on rat DRG sections.
6. NAPE-PLD- and Inpp5 immunopositive neurons are almost exclusively small size cells, whereas GDE-1- and PTPn22 immunopositive cells are both small and large cells.
7. The four AEA-synthetizing enzymes (NAPE-PLD, Inpp5, GDE-1 and PTPn22) show restricted co-expression in rat naive DRG.
8. Inpp5 is mainly expressed by lectin IB4 (IB4)-containing cells. A larger proportion of NAPE-PLD-IR neurons is also IB4 positive. While PTPn22 immunoreactivity is predominantly found in the large neurofilament (NF200)-labeled subpopulation of DRG neurons, most of the GDE-1-IR cells express calcitonin gene-related peptide (CGRP).
9. More than half of NAPE-PLD-labeled cells have transient receptor potential vanilloid type 1 ion channel (TRPV1) staining and approximately 80% of Inpp5-positive neurons show TRPV1-IR.
10. NAPE-PLD- and PTPn22-positive neurons exhibit the highest level of coexpression with cannabinoid 1 receptor (CB1R).

(ii) Does inflammation of peripheral tissues or injury to peripheral nerves induce changes in the expression and activity of anandamide-synthesising enzymes?

1. No changes in the mRNA expression of any enzymes involved in AEA synthesis are found in ipsilateral DRG in prolonged peripheral inflammation which is evoked by subcutanous Complete Freund's Adjuvant (CFA) injection into one of the hind paws. However, CB1R transcription is reduced in the ipsilateral- and increased in the contralateral DRG when compared to the corresponding untreated controls. In addition, significant upregulation in mRNA expression of sPLA2 and CB1R are detected, in the spinal cord.
2. No CFA-induced alteration in the protein level of AEA-related enzymes and receptors are detected in rat DRG by western immunoblotting.
3. There are inflammation-related changes in the average proportion of immunoreactive DRG neurons for the enzymes implicated in AEA synthesis and AEA-responding receptors (TRPV1 and CB1R) neither ipsilateral nor contralateral to CFA application as compared to the control from naive animals.
4. The number of neurons immunopositive for NAPE-PLD, GDE-1 and CB1R is decreased in the population of small-size neurons, whereas increased in large-size neurons on both sides (ipsi- and contralateral) following CFA injection. Futhermore, CFA application also increases the proportion of TRPV1-, Inpp5- and, more interestingly, PTPn22-IR cells in the group of small neurons. In addition, the percentage of cells showing PTPn22-IR is slightly reduced in large neurons.
5. Western immunoblotting revealed that after unilateral axotomy (L5 spinal nerve ligation; SNL), all the detectable enzymes involved in AEA production as well as TRPV1 are significantly reduced in DRG exposed to ligation.
6. Based on cell-size analyses of DRGs of SNL- and sham-treated rats rightward shifts in the size of NAPE-PLD-, and TRPV1-IR neurons are observed in the ipsilateral injured DRG as compared with the corresponding sham-operated controls. There is a significant leftward shift in PTPn22 immunreactivity after axotomy in both ipsilateral DRGs (ligated and adjacent non-ligated) in comparison with DRGs from sham-treated animals.
7. Following unilateral nerve injury, the proportion of PTPn22- and CB1R-IR neurons in small-size neurons is increased both in injured L5 and uninjured L4 DRGs. In the injured L5 DRG, the relative number of NAPE-PLD- and GDE-1-IR large neurons are increased, whereas the proportion of PTPn22- and CB1R-IR large neurons is decreased. In the adjacent non-ligated L4 DRG of SNL rats, the percentage of PTPn22-, GDE-1- and CB1R positivity are reduced in large cells compared with their sham pairs. Regarding TRPV1, the number of small neurons showing TRPV1 immunoreactivity is reduced in the axotomized L5 DRG in comparison to sham operated L5 ganglia.

(iii) What is the role of anandamide-synthesising pathways in the development of pain in inflammation of peripheral tissues and injury to peripheral nerves?

1. Despite the siRNA-mediated down-regulation of NAPE-PLD, GDE-1 and sPLA2 transcripts at mRNA level, the corresponding protein levels failed to be reduced as determined by immunoblotting as well as immunofluorescent staining. These enzymes probably due to their long half-lives and low turnover difficult to down-regulate with siRNA. Thus, no experiments with intrathaecal siRNA delivery was done because of the failure of siRNA contructs to induce "silencing" of enzymes *in vitro*.

To sum up, these results suggest that phenotypes of sensory neurons undergo profound changes in response to either peripheral inflammation or injury of their peripheral axons, however, these alterations - at single cell level - have been masked by the net effect of changes induced by these peripheral pathologies, and thus, certain proteins seemingly may stay unaffected.