

## 7 Summary

In the first step, the localization of AmNR1 transcript was studied by *in situ* hybridization. The AmNR1-1 was detected in all somata regions of the brain, in which high expression levels were observed in the protocerebral lobe, sub-oesophageal ganglion, dorsal lobe, antennal lobe, and lobula, medulla and lamina of the optic lobe. In the mushroom body, AmNR1 transcript was detected preferentially that indicate in different subsets of the Kenyon cell population, which may be involved in a diverse range of functions including synaptic plasticity and different forms of memory such as acquisition, consolidation and memory recall.

In the second step, immunohistochemical analysis was performed for the localization of AmNR1 protein with two different antibodies (NR1-pan and NR1-mab363). Both antibodies yielded similar staining in all brain neuropils, except nerve tracts. Strong AmNR1 immunoreactivity was detected in the suboesophageal ganglion, dorsal lobe, protocerebral lobe, antennal lobes, and the optic lobes compared to mushroom body. High levels expression of AmNR1 transcript and protein together with the presence of glutamate-like immunoreactivity and glutamate transporter gene in the optic lobes and in the ocellar system indicate that functional involvement of glutamate as a neurotransmitter in the visual system of honeybee.

In the third step, the localization of AmNR1 transcript was investigated at different developmental stages using *in situ* hybridization with DIG-labeled sense and anti-sense RNA probes. The AmNR1 transcript was highly detected in the pupal brain even in 1 day old pupae and also in young bee (1 day and 2 day old bees). The high levels expression of AmNR1 transcript at different developmental stages of the honeybee

strongly support that this transcript is developmentally regulated, which may proceed through common genetic mechanism like other genes of other insects.

In the fourth step, the localization of AmNR1 protein was investigated at different developmental stages of the honeybee using immunoenzyme histochemical analysis with NR1-mab363 primary antibody. The AmNR1 protein was increased with ages. Age related increase of the AmNR1 protein suggests that this protein may be involved in the behavioral maturation underlying learning and memory.

In the fifth step, a comparative study for the localization of AmNR1 transcript was studied between summer and winter bees with DIG-labeled sense and anti-sense RNA probes to clarify the seasonal effects on the expression of AmNR1 transcript in the adult bee. No difference was detected for the localization of AmNR1 transcript in the summer and winter bees suggesting the expression of AmNR1 transcript is not influenced by season.

In the sixth step, immunoenzyme histochemical analysis was performed in the bees that were collected from different seasonal stages and hives with NR1-mab363 primary antibody. No difference was identified with the expression of AmNR1 protein in different seasonal stages and hives. In both cases, strong AmNR1 immunoreactivity was detected in the optic lobes, antennal lobes, suboesophageal ganglion, protocerebral lobe, and central body compared to mushroom body neuropils. These suggest that expression of AmNR1 protein is not affected by seasons and colony types.

In the seventh step, immunofluorescence was studied to detect the co-localization between AmNR1 and DLG on brain vibratome sections. Immunofluorescent signal reveals that both proteins are nicely co-localized at the same region of the bee brain suggesting these two proteins are co-expressed in the honeybee.