is also found in the non-reproductive worker castes of bees. It is produced in the fat body (a tissue functionally homologous to the white adipose tissue and liver of vertebrates) in the abdomen (Snodgrass, 1956). Vitellogenin is generally released from the fat body, circulates in the hemolymph and is taken up by the ovaries through receptor-mediated endocytosis (Amdam et al., 2003; Guidugli et al. 2005). Honey bee workers typically do not import vitellogenin to the ovaries, but vitellogenin can be taken up for example by the hypopharyngeal glands in the head of nurse bees that synthesize food jelly for young larvae, other workers and the queen (Amdam et al., 2003). Vitellogenin also shields cells from oxidative damage and protects both workers and queens from oxidative stress (Seehuus et al. 2006; Corona et al. 2007; Havukainen et al. 2013).

Several factors such as pathogens and plant protection products (PPPs, pesticides) can alter vitellogenin levels in honey bees. Infection of young nurse bees with *Nosema ceranae* decreased the expression of vitellogenin transcript (Antúnez et al. 2009) whereas infection of honey bee larvae caused increased vitellogenin titers in young adults (BenVau and Nieh 2017). Some PPPs, including neonicotinoids, the organophosphate chlorpyriphos and the pyrethroid cypermethrin led to increased levels of the *vitellogenin* transcript in the brain of mixed-aged honey bee workers exposed in the period between beginning of May and end of June (Christen et al. 2016, Christen and Fent 2017). In contrast, the organophosphate malathion and chlorantraniliprole decreased the *vitellogenin* transcript (Christen et al. 2017).

The aim of our present study is to assess whether the induction of vitellogenin mRNA upon exposure to the neonicotinoid clothianidin is paralleled on the protein level. First, we generated and characterised an *Apis mellifera* vitellogenin antibody and evaluated its presence in different tissues. Second, we analysed vitellogenin protein levels in different tissues upon exposure to the neonicotinoid clothianidin and compared them with mRNA levels. We detected tissue-specific expression patterns of vitellogenin proteins. Our study demonstrates that previously reported alterations in vitellogenin mRNA are accompanied by changes in protein

levels and may result in physiological consequences, including disturbed transition of worker bees and decreased life-span.

## MATERIALS AND METHODS

### *Chemicals*

Clothianidin (purity > 99%), dimethyl sulfoxide (DMSO), dithiothreitol (BioXtra grade) and iodoacetamide (HPLC grade) were from by Sigma–Aldrich (Buchs, Switzerland). DMSO was used to prepare clothianidin solutions. Stock solutions were diluted into 20% sucrose solution to obtain final DMSO concentrations of 0.1%. Acetonitrile (LC-MS Grade) and formic acid (LC-MS grade, Honeywell-Fluka) were from Fisher Scientific (Reinach, Switzerland).

### *Antibody generation*

The generation of a polyclonal *Apis mellifera* vitellogenin antibody was performed by Davids Biotechnology (Regensburg, Germany). In brief, 15 mg of two peptides (peptide 1: KGKHIGKSGKVDVINAAKE, located at N-terminus, and peptide 2: EKNEAAMKLKKRIEKGANPD, located at C-terminus) were synthesized by solid phase synthesis. Peptide sequences were chosen by the company based on prediction programs. The quality of the peptides was analysed by HPLC and mass spectrometry. Peptide purity was > 80%. Before immunization, a short KLH sequence was conjugated to the peptides. Two New Zealand white rabbits were immunized with a mixture of the two peptides (concentration of each peptide: 10 mg/mL) at day 1, 14, 28, 42 and 56. At day 63, the final bleed was taken from each rabbit (40-90 mL final serum) and the polyclonal antibody was purified by affinity purification revealing two polyclonal vitellogenin antibodies with concentrations between 0.78 and 1.17 mg/mL.

### *Experimental design of laboratory exposures*

Mixed aged adult forager honey bees (*Apis mellifera carnica*) were collected from frames from an outdoor colony located in an area without farming activity and pesticide application in the Black Forest (Germany, GPS: N 47.7667, E 7.8333) between May and August 2017.