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.

The colony was infested with *Varroa destructor* and hence treated with formic acid in summer 2016 and oxalic acid in winter 2016. The experimental procedure was identical as described previously (Christen et al. 2016). Ten bees were placed in one PET bottle. Bees were fed either with sucrose solution containing 0.1% DMSO (solvent control, four bottles) or with 3 ng/bee clothianidin (four bottles) for 24 h. The selection of clothianidin concentration and exposure time is based on our previous study where the strongest induction of *vitellogenin* transcript was found after 24 h exposure to 3 ng/bee clothianidin (Christen et al. 2016). Per bottle, 3 bees were pooled to one hemolymph, brain, and fat body sample.

Hemolymph collection

Hemolymph collection was done according to Rutz and Lüscher (1974) and Randolt et al., (2008) with some slide modifications. In brief, hemolymph from frozen unexposed and exposed bees was collected from the dorsal part of the bees by using a sterile 10 µL micro tip. Individual bees were fixated between a pair of tweezers and the intersegmental membrane was slit slightly with the micro tip between the fourth and fifth tergite of the honeybee abdomen. Emerging clear hemolymph was collected with the micro tip which had been pre-wetted with PTU (N-Phenylthiourea, Sigma P7629) and protease inhibitor cocktail solution (Roche complete 04693124001) to prevent immediate melanisation and protein degradation. Turbid hemolymph was discarded. The hemolymph (5–10 µL per bee) was transferred into an Eppendorf tube containing an ice-cold mixture of 10% PTU and protease inhibitor cocktail. The samples were stored on ice during collection. The volume was adjusted to 60 - 80 µL with ice- cold PBS pH 7.4 and stored at –20°C until further analyses. Protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit (Pierce™ BCA Protein Assay Kit, Thermo Fisher) according to the manufacturer's protocol.

Fat body collection

To obtain abdominal fat body tissue, the frozen bee was briefly adjusted to room temperature. The thorax was cut off and the abdomen was fixated with needles to styrofoam. By gripping