Data processing and statistical analysis

One-way ANOVA and Bonferroni's multiple comparison test were applied to compare means of exposed and unexposed samples. Data are shown as means ± standard error of means. Statistically significant limits were: one asterisk at $0.05 > p > 0.01$, two asterisks at $0.01 > p > 0.001$ and three asterisks at $0.001 > p > 0.0001$.

RESULTS

Characterisation of vitellogenin antibody

Two rabbits were immunized using custom-synthesized peptides according to the vitellogenin peptide sequences. One peptide was based on the sequence located at the C-terminus and one based at the N-terminus (Fig. 1A). After 63 days of immunization, the final bleed was taken from each rabbit and the polyclonal antibody was purified by affinity purification revealing two polyclonal vitellogenin antibodies. Initially, the binding of the generated antibodies to honey bee vitellogenin from different tissues was analysed by dot blot analysis with a mixture of the two polyclonal vitellogenin antibodies. Figure 1B shows that the vitellogenin antibody mixture clearly detected vitellogenin in the hemolymph, fat body and brain tissue (Fig. 1B).

To further analyse the exact binding properties of the vitellogenin antibody, Western blot analysis was performed. The vitellogenin antibody detected the full length vitellogenin in fat body, hemolymph and brain (Fig. 2). According to our molecular size marker used, the size of the detected band was slightly lower than 180 kDa, representing the full length vitellogenin. In addition, the antibody recognized a lighter vitellogenin protein with a size slightly below 150 kDa in the hemolymph and fat body (Fig. 2A). However, in the brain, this smaller vitellogenin band was only detectable when overexposing the membrane (data not shown). In hemolymph, a band of approximately 75 kDa was detected in addition to the full-length and the lighter vitellogenin. In fat body, hemolymph and brain, an approximately 200 kDa band was detected in addition to 180 kDa vitellogenin (Fig. 2). No signal was detected in honey

stomach lysates (Fig. 2A). Mass spectroscopy was performed to identify exactly the detected bands. The vitellogenin antibody recognized the following proteins: uncharacterized protein at 200 kDa and vitellogenin at approximately 180 and 150 kDa (Fig. 2C). In hemolymph, an additional band was detected. Mass spectroscopy demonstrated that this band belongs to 6-phosphofructokinase, alpha-glucosidase, aconitate hydratase and transferrin. Which of these proteins was recognized by the antibody remains un-identified. Details of the mass spectroscopy analysis are presented in Table 1.

Induction of vitellogenin by clothianidin

As a proof of concept, we determined whether clothianidin leads to induction of vitellogenin. We previously showed that exposure of honey bee workers to clothianidin and additional neonicotinoids led to induction of *vitellogenin* mRNA in the brain (Christen et al. 2016). Here we extended our analysis onto the protein level and aimed to assess the induction of the vitellogenin protein. By Western Blot analysis we quantified vitellogenin in the hemolymph, brain and fat body of bees exposed to 3 ng/bee clothianidin for 24 h. In the hemolymph, vitellogenin levels (full-length and smaller vitellogenin) did not differ between solvent control and clothianidin exposed bees (Fig. 3A). In the brain, exposed bees showed no alteration of the full-length vitellogenin, but the smaller vitellogenin was significantly increased (Figs. 3B, C). In the fat body, a three-fold increase in the amount of full-length vitellogenin occurred in clothianidin exposed bees compared to control bees (Fig. 3D, E). These data indicate that clothianidin led to differential induction of vitellogenin protein in different tissues.

DISCUSSION

Vitellogenin influences hormone signalling, behavioural transition, stress resistance and longevity in honey bee workers (Amdam et al. 2004b, Nelson et al. 2007, Seehuus et al. 2006). Exposure of honey bees to PPPs including neonicotinoids, pyrethroids and organphosphates