smaller 150 kDa vitellogenin, a 200 kDa band was detected in fat body, hemolymph and brain lysates. This band represents an uncharacterised honey bee protein, which shows cross-reactivity with the vitellogenin antibodies.

In the fat body, hemolymph and in the brain of workers, we detected full length vitellogenin slightly below 180 kDa (Fig. 2). Corona et al. (2007) localized the vitellogenin mRNA not in the brain of bees themselves but in fat cells tightly bound to the brain. As these cells cannot be separated during dissection, our analysis covers the brain and these fat cells together. In addition to the full length vitellogenin, we also detected a band slightly below 150 kDa vitellogenin in the fat body and in the hemolymph (Fig. 2). According to Havukainen et al. (2011) this band likely represents the processed vitellogenin in the fat body and a degradation product in the hemolymph. The detected vitellogenin bands were both slightly below the published sizes of 180 and 150 kDa (Havukainen et al. 2011), potentially due to anomalous migration of the protein standard or samples as a result of incomplete denaturation or binding of detergent to the protein (Dolnik and Gurske 2011). In the brain, the lighter vitellogenin band is almost not detectable. As the fat body is the major source of vitellogenin synthesis and the hemolymph is the distributor of vitellogenin in the honey bee body, the occurrence of both vitellogenin forms in these tissues makes sense. In the brain and associated fat cells and in the hypopharyngal glands, the lighter vitellogenin form is only present in nurse bees, which are responsible for the larval feeding (Amdam et al., 2003b).

In addition to full length and processed vitellogenin, we detected a band at 70 kDa in the hemolymph (Fig. 2). Havukainen et al. (2011) used ion exchange purified hemolymph to detect different vitellogenin bands of 40, 150 and 180 kDa. However, in raw hemolymph extract a band around 70-75 kDa was detected, which is similar to the 70 kDa band detected in our present work (Fig. 2). This protein may represent a degradation product of the full length or 150 kDa vitellogenin. Both the full-length and the 150 kDa fragments are post-translationally modified by phosphorylation and glycosylation (Havukainen et al. 2011). The loss of

phosphorylation or glycosylation during our protein extraction may explain the smaller size of the full length and processed vitellogenin detected in our work (Fig 2). The functionality of the processed vitellogenin is not currently understood. We could not detect the 40 kDa fragment because it maps between residues 53 to 294 of the N-terminus of the known vitellogenin protein sequence. However, the N-terminal peptide used to immunize rabbits in the present study maps between residues 409 to 427. Therefore, the generated vitellogenin antibody is unable to detect the 40 kDa fragment. In contrast to most insects, where vitellogenin expression is tissue-specific and vitellogenin receptors have only been detected in ovaries (Chen et al. 2004; Tufail and Takeda 2005, 2007; Ciudad et al. 2006), in honeybees vitellogenin receptors were observed in head, fat body and ovaries of worker bees (Guidugli-Lazzarini et al. 2008). This may be related to the multiple regulatory function of vitellogenin in the social life of honey bees.

Vitellogenin induction by clothianidin

We previously observed that different classes of pesticides altered the expression of vitellogenin mRNA. The four neonicotinoids acetamiprid, clothianidin, imidacloprid and thiamethoxam induced the vitellogenin transcript in the brain at environmentally relevant concentrations (Christen et al. 2016). Alterations in vitellogenin expression in the brain of workers was also observed after exposure to the organophosphates chlorpyrifos and malathion, to the pyrethroid cypermethrin and to the ryanodine receptor activator chlorantraniliprole (Christen and Fent 2017). In our present study, we evaluated whether clothianidin not only leads to induction of the transcript but the protein as well. Indeed, we observed a 3-fold induction of full-length vitellogenin protein in the fat body and of the lighter vitellogenin in honey bee brain (Fig. 3B and C). These data complement our previous data that showed a fourfold induction of vitellogenin mRNA by clothianidin and other neonicotinoids (Christen et al. 2016). The