

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

lighter vitellogenin form has been found in the hypopharyngeal glands of workers, where vitellogenin constituents are processed into food secretions for larval feeding (Amdam et al. 2003a). This lighter vitellogenin is not found in the unexposed honey bee brain (Fig. 2B, 3B). As nurse bees show higher vitellogenin titers and are responsible for the feeding of the larvae (Amdam et al. 2003a, b), the lighter vitellogenin in the brain may be a marker of nurse bees. The exposure of foragers to clothianidin induced the light vitellogenin in the foragers, suggesting the presence of a “nurse-bee”-like phenotype. Vitellogenin plays such an essential role in hormone signalling and in transition of nurse bees, which have high vitellogenin levels, to foragers, which have lower vitellogenin levels. Vitellogenin is also involved in stress management by protecting against oxidative stress and regulation of life-span; long-lived queens show high vitellogenin levels (Amdam et al. 2004b, Nelson et al. 2007, Seehuus et al. 2006). On this basis, we conclude that altered levels of vitellogenin may have significant physiological consequences in exposed worker bees. Further studies are needed to link vitellogenin alteration to physiological outcomes and to demonstrate whether vitellogenin may serve as a biomarker candidate for the exposure of honey bees to neonicotinoids and other pesticides. It should be noted that possibly other factors may also affect vitellogenin protein levels and that they may fluctuate or become increased in a compensatory manner, or induction may be transient. More detailed investigation should thus be devoted to such questions before vitellogenin can be regarded as a biomarker for pesticide exposure. The advancement of our newly developed antibody is the fact that it represents a good tool to analyse in detail the physiological role of vitellogenin in honey bees and to analyse the potential effects of plant protection products on vitellogenin expression and function.