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The effects of acute stress on learning and memory in bumblebees

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ABSTRACT

Stress has complex effects on learning and memory, depending on both the type of stress and when the animal experiences it. Honeybees and bumblebees are agriculturally important pollinators for whom the effects of stress are extremely relevant. These pollinators are often transported long distances during which colonies experience severe physical disturbance, causing stress to individuals prior to their release for pollination. Under natural foraging conditions, bees are excellent at learning about the flowers they forage from, including associations between floral stimuli and rewards. However, it is not clear how stress might affect bees' abilities to learn and remember floral features. Here we address the effects of acute stress on learning and memory in the bumble bee, *Bombus impatiens*. Using the Proboscis Extension Response (PER) protocol, we look at stress effects on learning and memory in three experiments. After being trained to a conditioned stimulus, we addressed: (1) the effect of 24 h and (2) 30 min of stress on the recall of this learned association and (3) the effect of stress on subsequently learning an association. We found that 24 h of stress after learning appeared to improve memory recall, and there was a trend in the same direction for 30 min of stress. However, bees that were stressed prior to learning an association did not differ from unstressed bees in their ability to learn or remember an association. Our finding that stress has effects on memory in the bumblebee could have implications for their use in behavioral experiments and for promoting the success of both managed and wild populations.

Stress affects all animals, but can do so in many different ways. Stress encompasses a broad range of experiences, including nutritional stress, physical stress (pain), social stress and psychological stress. Stressors elicit a variety of physiological responses, but can also be seen through behavioral responses. How stress affects an individual's ability to learn and remember is diverse, there being no simple relationship between stress and learning (Schwabe, Joëls, Roozendaal, Wolf, & Oitzl, 2012). The effects of stress on learning and memory depend on both the type of stress (chronic e.g., Pravosudov, 2003; or acute e.g., Shors, 2001), when the stress occurs (before or after learning, and how long before or after (Schwabe, Wolf, & Oitzl, 2010), and individual factors such as sex and age (Shors, 2006). In the current study, we address the effect of stress on learning and memory in a case not before investigated, that of the bumble bee.

As generalist foragers, honey bees and bumblebees are extremely adept at learning associations between floral features and nectar rewards, both in order to learn the most highly rewarding flowers to forage on (Chittka, Thomson, & Waser, 1999) and to learn how to handle flowers effectively (Chittka & Thomson, 1997; Heinrich, 1979; Laverty, 1980, 1994). Bees have

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emerged as a model system for the study of learning and memory (Giurfa, 2007), using both free-flying behavioral assays (Papaj & Lewis, 1993), and the Proboscis Extension Response (PER) paradigm (Bitterman, Menzel, Fietz, & Schäfer, 1983; Riveros & Gronenberg, 2009; Takeda, 1961). In the PER protocol, an individually harnessed bee is presented with an initially neutral stimulus (the conditioned stimulus; CS, e.g., an odor), paired with a sucrose reward (the unconditioned stimulus; US). After the animal learns this association, it will extend its proboscis (in order to drink the sugar reward) in response to the conditioned stimulus alone (the conditioned response; CR). PER has been used extensively in research on learning and memory in honey bees (reviewed in Giurfa & Sandoz, 2012) and, more recently, in bumble bees (Laloi et al., 1999; Riveros & Gronenberg, 2009). However, despite extensive study of learning and memory processes in bees (Menzel & Giurfa, 2001), and a parallel literature on their physiological stress responses (reviewed in Even, Devaud, & Barron, 2012), we know little about what effects stress might have on learning and memory in this system.

Honeybees and bumblebees are perhaps the most important insects for human agriculture, due to the pollination service they provide around the world (Gallai, Salles, Settele, & Vaissière, 2009). These commercial pollinators face a number of stressors, many of which have been linked to their recent decline, including but not limited to habitat loss and fragmentation, use of agrochemicals, pathogens, parasitic mites and climate change (Potts et al., 2010). Aside from these diverse stressors, one form of stress for commercial pollinators comes from their long-distance transportation (Ahn, Xie, Riddle, Pettis, & Huang, 2012). For example, honey bees are transported from Florida to California in the spring for almond pollination, and from Michigan to Florida over winter before being brought back for apple and cherry pollination. Bumble bees pollinate a wide variety of economically important crops, including kiwifruit, tomatoes, strawberries, eggplant, sweet peppers, cranberries and blueberries, and are shipped from the east to the west of America (e.g., Whittington & Winston, 2004). While being packed for this transportation, during transportation and in unpacking afterwards, colonies are likely to experience physical disturbance (i.e., vibration). Bees within a colony are sensitive to such physical disturbance, and respond with behavior such as hissing (Kirchner & Röschard, 1999) and production of alarm pheromone (Jandt, Robins, Moore, & Dornhaus, 2012). Such disturbance (i.e., by shaking) also causes pessimistic cognitive biases in bees (Bateson, Desire, Gartside, & Wright, 2011). Once a colony reaches its destination and bees begin foraging, they will learn associations between features of the flowers they visit and the reward the flowers offer, as well as learning how to handle these flowers effectively. These same bees may then be transported again to the same or a different crop, thus experiencing more stress. Even among wild populations of bees, there is some evidence that queens prefer relatively "undisturbed" nest sites (e.g., those away from intensively managed fields; Barron, Wratten, & Donovan, 2000). However, we do not know what effects stress by physical disturbance has on bee learning and memory.

Stress is known to have both positive and negative effects on learning in animals, depending on a number of factors (reviewed in Joëls, Pu, Wiegert, Oitzl, & Krugers, 2006; Sandi & Pinelo-Nava, 2007). One factor that might be of particular relevance to commercial bees that are transported to the crops they pollinate is that of timing. At least in rats, which have largely been used as the model system for understanding stress effects on learning, when stress is encountered immediately after learning, it often has positive effects on memory consolidation, as opposed to when it is experienced prior to recalling a previously learned stimulus, where it is more likely to have detrimental effects (Roozendaal, 2002, 2003; Schwabe et al., 2012). To our knowledge, the only other study looking at stress effects on learning and memory in a bee addressed the effects of chronic nutritional stress on a honey bee colony (Mattila & Smith, 2008), a very different type of stress from the acute stress likely to also be experienced by commercial pollinators.

Here we tested the effects of stress on learning and memory on the Common Eastern bumble bee, *Bombus impatiens*, a species encountered across Eastern North America and sold commercially across the USA and Canada. Specifically we looked at the effect of the timing and duration of one stressor (physical disturbance by shaking), on a learning and memory task. We made the assumption that physical disturbance by shaking would be acutely stressful to bumble bees, as bees that encounter physical disturbance show behavior that is likely to indicate stress, such as hissing (Kirchner & Röschard, 1999) and production of alarm pheromone (Jandt et al., 2012). Furthermore, shaking in honeybees has been shown to have physiological effects, as it changes the bees' levels of biogenic monoamines, reducing constitutive levels of octopamine, dopamine, and serotonin in the bees' haemolymph (Bateson et al., 2011; Chen, Hung, & Yang, 2008). We used the PER paradigm as this method allows precise control of conditioning. We carried out three experiments to ask the following questions: (1) what effect does 24 h of stress have on an individual's ability to recall a previously learned association?; (2) what effect does 30 min of stress have on an individual's ability to recall a previously learned association?; and (3) how does 30 min of stress preceding conditioning affect bees' ability to learn and remember an association?

Methods

General methods

Subjects and maintenance

We used colonies of *Bombus impatiens* (Koppert Biological Systems, MI, USA) which were connected via plastic tubes to a central arena sized 98 cm × 96 cm × 91 cm (length × width × height) containing unrewarding artificial flowers (for enrichment). We provided bees with *ad libitum* pollen placed directly into their colonies, and allowed them to free-forage in the central arena on a white cotton-wicked artificial feeder containing 15% (w/w) sucrose solution. While the communal