First evidence of neurons in the male copulatory organ of a spider (Arachnida, Araneae)

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Spider males have evolved a remarkable way of transferring sperm by using a modified part of their pedipalps, the so-called palpal organ. The palpal organ is ontogenetically derived from tarsal claws; however, no nerves, sensory organs or muscles have been detected in the palpal bulb so far, suggesting that the spider male copulatory organ is numb and sensorily blind. Here, we document the presence of neurons and a nerve inside the male palpal organ of a spider for the first time. Several neurons that are located in the embolus are attached to the surrounding cuticle where stresses and strains lead to a deformation (stretching) of the palpal cuticle on a local scale, suggesting a putative proprio-receptive function. Consequently, the male copulatory organ of this species is not just a numb structure but likely able to directly perceive sensory input during sperm transfer. In addition, we identified two glands in the palpal organ, one of which is located in the embolus (embolus gland). The embolus gland appears to be directly innervated, which could allow for rapid modulation of secretory activity. Thus, we hypothesize that the transferred seminal fluid can be modulated to influence female processes.

1. Introduction

Male fertilizing ability is strongly influenced by postcopulatory sexual selection, i.e. sperm competition and cryptic female choice [1]. However, whenever females mate with multiple males the ability of males to detect females as potential mating partners with respect to e.g. age or mating status is of particular relevance [2]. Consequently, if resources are limited, or the mating effort is high, mate choice and mating investment play a crucial role and males not only discriminate between potential mates, but also are able to adjust their investment, e.g. quality of ejaculates [3].

Males, however, initially need to identify and distinguish the quality of a female, at the latest while mating. During copulation, spider males use a modified part of their pedipalps, the so-called palpal organ, to transfer seminal fluid (sperm and secretions). One major finding from several histological studies of the last decades—including a considerable number of spider taxa—is the lack of nervous tissue in the male palpal organ [4,5]. For this reason, it has been assumed that spider males are not able to receive sensory input through their copulatory organs while mating, despite the fact that males need to manoeuvre these 'numb' structures precisely into the female genitalia [4]. Although many spiders, for example the Entelegynae, have evolved highly complex male pedipalps that...
are equipped with prelocking structures that may aid in mating, many other spiders are equipped only with simple pedipalps that lack such structures and could certainly profit from sensory input. Here, we demonstrate the presence of neurons in the simple male palpal organ of such a spider, the Tasmanian cave spider *Hickmania troglodytes* (Higgins & Petterd, 1883), for the first time (we believe). We discuss the potential role of these neurons in processing sensory information during copulation and in mediating the interaction between male and female genitalia. In addition, we provide evidence for a directly innervated gland in the furthermost part of the palpal organ, the embolus, which releases its secretions into a distinct glandular duct. This configuration might enable the male to directly adjust the transferred ejaculate during copulation.

2. Material and methods
We investigated adult males of the Tasmanian cave spider by means of histological and ultrastructural analyses, as well as X-ray
perceive sensory input with respect to stress and strain. Proprioceptive function, enabling males of *H. troglodytes* (figure 2). Thus, neurons located in the embolus might have a wall in the area next to the tip of one of the neuron branches (figure 2). We found a pronounced stretching in the cuticle pressed in certain areas depending on the acting forces (figure 1). As a result of the geometry, materials properties and wall thickness of the cuticle the embolus gets stretched or compressed in certain areas depending on the acting forces (figure 2). Thus, neurons located in the embolus might have a proprioceptive function, enabling males of *H. troglodytes* to perceive sensory input with respect to stress and strain.

3. Results

The major findings of this study are (i) the presence of a nerve, projecting as far as the embolus and (ii) the presence of two clusters of neurons within the male palpal bulb (figure 1). A first cluster of neurons is located near the blind end of the internal cuticular tube containing the seminal fluid, the spermophor (figure 1a, asterisk, b), while a second one is situated in the intromitting part of the organ, the embolus (figure 1a). In addition, transmission electron microscopy analysis revealed the presence of large neurites, forming a small nerve that projects through the palpal organ (figure 1b,c), as well as putative neurotransmitter (clear vesicles) inside some of these neurites (figure 1d).

We furthermore revealed the presence of two glands inside the male palpal organ. A prominent gland, which occupies the main part of the bulb, is associated and likely connected to the spermophor, through a porous portion at the base of the latter (data not shown). A second, smaller gland is located in the embolus and is provided with its own glandular duct. The spermophor runs through this duct, which opens in combination with the spermophor at the tip of the embolus (figure 1a).

Although only the tip of the embolus is inserted in the female genitalia during mating, the stresses and strains in the embolus expanding from the contact area of the tip and the female genitalia are distributed along the entire structure. As a result of the geometry, materials properties and wall thickness of the cuticle the embolus gets stretched or compressed in certain areas depending on the acting forces (figure 2). We found a pronounced stretching in the cuticle wall in the area next to the tip of one of the neuron branches (figure 2). Thus, neurons located in the embolus might have a proprioceptive function, enabling males of *H. troglodytes* to perceive sensory input with respect to stress and strain.

4. Discussion

Our data provide the first evidence of neuronal tissue in the spider male copulatory organ. Thus, this spider’s male genitalia are likely not numb and sensory input could play an important role in copulation and consequently in male mating investment and/or securing paternity. The majority of comparative studies suggest that female spiders are in control of processes regarding sperm utilization [6]. A putative sensitive palpal organ might be an advantage as it could be used to alter the male’s copulatory behaviour in order to stimulate the female for the male’s own benefit (e.g. [7]). Alternatively, a sensory copulatory organ might enable the male to assess female parameters and adjust his investment accordingly. However, this remains speculative at present, as there are no data relating male copulatory behaviour to paternity success in *H. troglodytes*.

Our study revealed the presence of two glands inside the palpal organ, the spermophor gland and a gland that we called the embolus gland. Based on the presence of neurotransmitter our data suggest that at least the embolus gland is likely directly innervated. The spermophor gland of *H. troglodytes* is associated with the base of the spermophor and therefore similar to the organization described for other spider taxa. Here, the glandular tissue is expected to play an important role during sperm uptake and transfer into the female during copulation [4].

Glands accessory to copulation, such as the embolus gland we have described here, are usually thought to play a role in assuring paternity [8–10]. For example, secretions from such glands are often associated with the production of mating plugs [11]. However, there is no evidence for mating plugs in *H. troglodytes* [12] and thus the gland present in the embolus would appear to serve other function(s). In theory, males should invest more sperm in high-quality females, but take advantage of any further mating. Thus, males of *H. troglodytes* might be able to attenuate the seminal fluid with additional secretions as shown for the accessory glands of other animal taxa. Such secretions can have a variety of functions influencing female processes to secure paternity. For example, male accessory gland secretions in insects are known to affect the receptivity to remate, to modulate oviposition behaviour and to influence egg development [13]. So far, in spiders, only indirect evidence from the wolf spider *Schizocosa mutiliosa* suggests a positive correlation.
of male transferred substances and female reluctance to remate after a successful mating [14].

In conclusion, the possibility for sensory reception in males of *H. troglodytes*, together with the directly innervated embolus gland, suggests the presence of alternative mechanisms for securing paternity unknown for spiders. Future studies will reveal if similar mechanisms are also present in other spider species.

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**References**