

Mechanisms of D2R signaling in the blood brain barrier that regulates courtship in *Drosophila melanogaster*

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Abstract

Flies have an open circulatory system and their Blood Brain Barrier (BBB) surrounds the brain like a tight cap. The fly BBB consists of two layers of glial cells. The outer layer is formed by the Perineurial Glia (PG). The inner BBB layer consists of the Subperineurial Glia (SPG) that form the tight barrier that, like its mammalian counterpart, acts both as a diffusion and a xenobiotic transport barrier¹. Underneath the SPG lie the neuronal cell bodies. The *Drosophila* BBB shows the same barrier properties as the mammalian barrier¹ and profiling of *Drosophila* BBB cells has shown a high degree of molecular conservation^{2,3}.

We have previously shown that the *Drosophila* BBB plays a sex-specific role in regulating behavior. Conditional adult feminization of SPG cells in otherwise normal males leads to significantly reduced courtship¹. In agreement with this, in a microarray screen of isolated SPG cells, we identified a number of male-enriched transcripts.² One of them encodes the *dopamine-2 like receptor (D2R)*. We have found that conditional knockdown of *D2R* in adult male *Drosophila* SPG decreases courtship. Likewise, *D2R* mutant males have courtship defects. They can be rescued by expression of wildtype *D2R* in the SPG cells of mature adult males, demonstrating a physiological requirement for the receptor in these cells for courtship control⁴. The *D2R* receptor is highly conserved. It has been found that it can act via biased signaling (through G protein or beta-arrestin) in mammals.³ We have previously found that signaling through Gao and arrestin in the BBB is required for proper male courtship. Although *D2R* is best known for signaling through cAMP, we have not found a requirement for cAMP/PKA signaling for courtship. To investigate the signaling pathways downstream of *D2R* that are responsible for courtship control we have mutagenized *D2R* proteins and examined their ability to rescue *D2R* mutants. Based on Peterson et al³ we designed proteins capable of G-protein or arrestin biased signaling, respectively, and tested their ability to rescue the courtship defects of *D2R* mutants. Our data suggest that *D2R* signaling through beta-arrestin is a major mediator of BBB courtship control.

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