Cystine-glutamate transporters import cystine into cells for glutathione synthesis and protection from oxidative stress, but also export significant amounts of glutamate. Increasing evidence suggests that 'ambient extracellular glutamate' secreted by cystine-glutamate transporters in the nervous system modulates glutamatergic synapse strength and behavior. To date, the only cystine-glutamate transporter mutants examined behaviorally are Drosophila genderblind mutants. These animals contain loss-of-function mutations in the genderblind gene, which encodes an xCT subunit essential for cystine-glutamate transporter function. Genderblind was named based on a mutant courtship phenotype: male genderblind mutants are attracted to normally aversive male pheromones and thus court and attempt to copulate with both male and female partners equally. However, genderblind protein is expressed in many parts of the fly brain and thus might be expected to also regulate other behaviors, including behaviors not related to male courtship or chemosensation. Here, we show that genderblind mutants display faster recovery and increased negative geotaxis after strong mechanical stimuli (e.g., they climb faster and farther after vial banging). This phenotype is displayed by both males and females, consistent with strong genderblind expression in both sexes.

Cystine-glutamate transporters are transmembrane proteins that bring cystine into cells and secrete glutamate. They are heterodimeric proteins composed of 'heavy' 4F2hc subunits, and 'light' xCT subunits. 4F2hc subunits are thought to regulate protein trafficking, and are utilized by several different types of amino acid transporter. xCT subunits, in contrast, are required for amino acid selectivity and transport, and are found only in cystine-glutamate transporters. Cystine uptake by xCT is important for glutathione synthesis and protection from oxidative stress. Glutamate export by xCT increases ambient extracellular glutamate. In the nervous system, where xCT proteins are most highly expressed, altered ambient extracellular glutamate has important functional consequences. For example, cystine-glutamate transport is strongly upregulated in gliomas. This causes glutamate excitotoxicity and neurodegeneration of surrounding brain tissue, which provides room for tumor growth. In nonpathological conditions, ambient extracellular glutamate is sufficient to alter glutamate receptor function. Pharmacological inhibition of cystine-glutamate transport in rats decreases tonic activation of metabotropic glutamate receptors and alters drug behavior. Ambient extracellular glutamate also causes constitutive desensitization of ionotropic glutamate receptors, which suppresses glutamatergic neurotransmission. This latter role for xCT is exemplified by Drosophila genderblind.

Genderblind (GB) is a Drosophila xCT protein expressed in glia throughout the peripheral and central nervous system. Ambient extracellular glutamate in Drosophila genderblind mutants is reduced to approximately one-half normal, and the number of functional synaptic glutamate receptors increases 200–300%. Genderblind was named based on the observation that mutant males are attracted to normally aversive male pheromones, and thus court and attempt to copulate with both females and other males equally. Consistent with the idea that genderblind regulates behavior by suppressing glutamatergic transmission, the homosexual courtship phenotype could be replicated and/or reversed by genetic and pharmacological manipulation of glutamatergic synaptic strength independent of genderblind. For example, genderblind mutant males fed apple juice laced with gamma-D-glutamylglycine (γ-DGG), a competitive glutamate receptor antagonist, reverted to normal courtship within 24 hours. Wildtype males fed concanavalin A (a glutamate receptor desensitization inhibitor), on the other hand, began courting other males. Similarly, overexpression of the Drosophila vesicular glutamate transporter, which overloads synaptic vesicles with glutamate and increases synaptic strength, replicated the genderblind mutant phenotype.

Neither genderblind nor glutamate receptors are restricted to regions of the fly brain controlling pheromone processing or courtship. Furthermore, both male and female Drosophila express genderblind equally. Therefore, it seems likely that genderblind mutants might show additional behavioral phenotypes that are not associated with courtship or gender.
Because genderblind suppresses glutamatergic transmission, we reasoned that genderblind mutant behaviors might most often represent an overreaction to sensory stimuli. *Drosophila* display prominent negative geotaxis, and prefer residing on vertical surfaces. When placed in vials, *Drosophila* quickly climb the sides. If the bottom of a vial containing flies is brought forcefully into contact with a hard surface, flies are dislodged from the sides and fall to the bottom of the vial. Normally, flies quickly right themselves after being knocked down and immediately begin climbing the sides of the vial, where they eventually distribute themselves. Flies that do not recover well after being knocked to the bottom of the vial are typically referred to as ‘bang-sensitive’ or ‘stress sensitive’. Bang sensitive mutations typically disrupt neuronal excitability or mitochondrial function.29-34

We examined genderblind mutants for bang-sensitive phenotypes. Specifically, we compared homozygous gb[KG07905] flies to precise excision and ‘Oregon R’ wildtype controls. gb[KG07905] mutants carry a transposon insertion in the 5’ end of the genderblind gene, which reduces genderblind transcript and protein levels to 53% and 35% of normal, respectively.25 In ‘precise excision flies’, the gb[KG07905] transposon insertion has been cleanly excised (verified by sequencing).16,25 Precise excision flies therefore control for genetic background.

To test bang recovery, four flies of the same gender were placed in an empty polystyrene *Drosophila* vial (95 mm tall x 24 mm diameter), upon which was marked a line 7 cm from the bottom. The vial was manually banged twice, quickly and firmly, on a slightly padded surface (computer mouse mat). This caused all flies to fall to the bottom of the vial, after which they quickly righted themselves and climbed toward the top. Neither genderblind mutants nor control flies showed any apparent seizures or immobilization characteristic of bang-sensitive mutants. We recorded the number of flies that climbed past the 7 cm ‘finish line’ before stopping, along with the speed of each fly that crossed the line.

As shown in Figure 1, males and females (2–6 days after eclosion) climbed vials at the same speed. However, gb[KG07905] mutant flies climbed the vials approximately three times faster than either wildtype or precise excision controls (Fig. 1). When the percentage of flies crossing the finish line was compared, we noted that a higher percentage of wildtype females crossed the line compared to wildtype males, suggesting some natural gender difference in geotactic behavior. However, precise excision controls did not show this gender difference, and the largest difference was attributable to loss of genderblind, as significantly more gb[KG07905] flies crossed the finish line compared to wildtype or precise excision controls, regardless of gender (Fig. 1). Faster climbing by gb[KG07905] suggests that genderblind mutants might locomote faster. But gb[KG07905] mutants do not show faster locomotion, compared to controls.25 Consistent with this, we noted that the upward trajectory of gb[KG07905] flies was straighter than that of control flies, which tended to ‘wander’ slightly more on their way to the finish line. Genderblind mutants in vials at rest also do not obviously distribute themselves differently on the sides of the vial, suggesting that these climbing differences do not represent alterations in negative geotaxis. The accelerated bang recovery in genderblind mutants may therefore represent enhanced goal-directed activity (assuming climbing back up the vial after being knocked to the bottom represents a ‘goal’).

To determine whether repeated banging might amplify differences between genderblind mutants and control flies, we tested groups of flies three consecutive times in three minutes (Fig. 2). Males and females were tested separately, but measurements were combined for graphing (Fig. 2) and statistical comparisons. As shown (Fig. 2), there was no obvious trend in either climbing speed or the fraction of flies climbing past the 7 cm line during the three tests.

Age-related changes in geotaxis have been observed in *Drosophila*.35 To test whether changes in gb[KG07905] climbing behavior might be at least partially attributable to or enhanced by age-related geotaxis differences, we measured the number of flies crossing the finish line in each genotype 2, 4 or 6 days after eclosion (Fig. 3). As for Figure 2, males and females were tested separately, but measurements were combined for presentation and statistical comparison. We observed no effect of age on the behavior within this range; gb[KG07905] mutants always crossed the line with higher frequency compared to controls (Fig. 3).
The accelerated bang recovery and climbing shown here for \textit{gb[KG07905]} mutants is likely attributable to stronger glutamatergic transmission, as demonstrated for \textit{gb[KG07905]} male homosexual behavior.\textsuperscript{25} The glutamatergic circuits that control courtship in flies have yet to be worked out, and developmental alterations associated with mutant courtship remain controversial.\textsuperscript{36} The accelerated bang recovery and climbing described here are therefore very useful in that they increase the number of specific circuits available for analysis.

Our findings also support the idea that both male and female genderblind mutants have altered brain function, consistent with the fact that genderblind is expressed in both sexes.

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