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Exploratory Activity in Drosophila Requires the *kurtz* Nonvisual Arrestin

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ABSTRACT

When Drosophila adults are placed into an open field arena, they initially exhibit an elevated level of activity followed by a reduced stable level of spontaneous activity. We have found that the initial elevated component arises from the fly's interaction with the novel arena since: (1) the increased activity is independent of handling prior to placement within the arena, (2) the fly's elevated activity is proportional to the size of the arena, and (3) the decay in activity to spontaneous levels requires both visual and olfactory input. These data indicate that active exploration is the major component of elevated initial activity. There is a specific requirement for the *kurtz* nonvisual arrestin in the nervous system for both the exploration stimulated by the novel arena and the mechanically stimulated activity. *kurtz* is not required for spontaneous activity; *kurtz* mutants display normal levels of spontaneous activity and average the same velocities as wild-type controls. Inhibition of dopamine signaling has no effect on the elevated initial activity phase in either wild-type or krz^1 mutants. Therefore, the exploratory phase of open field activity requires *kurtz* in the nervous system, but is independent of dopamine's stimulation of activity.

LOCOMOTION is a fundamental and vital behavior. Through movement, animals can exert control over their surroundings to locate essential resources and avoid hazards. Without efficient and productive movement, animals can be easily preyed upon. Experimentally, we rely on an animal's movement to instruct us on the acuity of sensory systems, the vigor of courtship, and even the strength of learned associations. Locomotion, however, is a complex biological response encompassing sensory processing, integration of stimuli, executive functions, and motor response pathways. By understanding the genetic determinants underlying an animal's decision to move, we may gain significant insights into the molecular mechanisms involved in all of these levels of processing.

Activity in an open field arena is one of the oldest and most widespread experimental behavioral tasks (Hall 1936; Walsh and Cummins 1976). This task is typically used as a simple measure of general activity. Many species, however, display nonlinear activity profiles when first placed into an open field, indicating a complexity in this behavior. *Drosophila melanogaster*, several species of rodents, chickens, domestic cats, and dogs all demonstrate an elevated level of initial activity, followed by a rapid decline to a lower steady-state level of ambulation when assayed in an open field arena (Glickman and Hartz 1964; Connolly 1967; Candland and Nagy 1969; Lát and Gollová-Hemon 1969). Some insight into the initial elevated activity component came when Ewing (1963) and Connolly (1967) selectively bred Drosophila for differences in locomotor activity. The selected genotypes were actually found to have differences in how they react to stimulation, including the presence of other flies. The highly reactive flies had more activity because they were more vigorously running away from the other flies present in the apparatus (Ewing 1963). These flies also displayed differences in initial activity when individually placed into an open field arena, but not in the steady-state level of spontaneous activity (Ewing 1963; Connolly 1967). This early, elevated component of activity has been consequently termed reactivity or stimulated activity (Meehan and Wilson 1987). Surprisingly, very little is known about why animals increase their activity when placed into the open field, what the proximal causes for this stimulated activity are, or what the genetic determinants are that govern this response.

Herein we demonstrate that the *kurtz* (*krz*) gene of *D. melanogaster* is required within the nervous system for the elevated initial activity phase in an open field arena, but is not required for spontaneous activity. The *krz* gene encodes the only nonvisual arrestin in Drosophila (Roman *et al.* 2000). Nonvisual arrestins are important scaffolding proteins that regulate the activity of several families of cell-surface receptors, including G-protein-coupled receptors (GPCRs) (Lefkowitz and Whalen 2004). The arrestins are indispensable players in the agonist-dependent desensitization of GPCRs and are required to keep low levels of ligand from saturating cellular responses.

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Since the nature of the elevated initial activity phase in Drosophila was unknown, we further explored this behavior to better understand the *krz* phenotype. We show here that the elevated initial activity phase is an evolutionarily conserved response to the arena and is principally composed of exploration, defined as behavioral acts that are evoked by novelty, and we provide information about the surrounding environment (Crusio and van Abeelen 1986). A role for dopamine in initial activity had been previously suggested (Meehan and Wilson 1987). We show that the requirement for *krz* in elevated initial activity is independent of dopamine signaling and that dopamine is most likely dispensable for the response evoked by the novel arena.

MATERIALS AND METHODS

Fly stocks and genetics: All stocks were raised and maintained on standard yeast–cornmeal agar food at room temperature. Flies that were used in behavioral assays, unless otherwise noted, were raised on standard food at 25°, 60% humidity, with 12 hr of light/day. The krz^1 allele, the b5.8T4 genomic *krz* transgene, and the UAS*krz*T5 and UAS*krz*T12 cDNA transgenes were originally described in Roman *et al.* (2000). The following fly stocks were all obtained from the Bloomington Stock Center: P{hspGal4$^{89\text{-}2\text{-}1}$}, c155^{elavGal4}, P{UAS-LacZ}$^{Bg4\text{-}1\text{-}2}$, $norpA^7$, gl^2, $Antp^{NS}$/+, and Dr^1/TM3, P{hs-hid}14, Sb^1. The *Drosophila virilis* and *Drosophila simulans* stocks were obtained from the Tucson Stock Center. The TH-Gal4 driver was as described in Friggi-Grelin *et al.* (2003). The $or83b^2$-bearing line was the generous gift of Leslie Vosshall (Rockefeller University). The $Antp^{NS}$, Dr^1, and $or83b^2$ mutations were all outcrossed into Canton-S for a minimum of six generations before behavioral testing. The gl^2 mutation was outcrossed to Canton-S for three generations prior to behavioral testing. Additionally, all transgenes were crossed into w^{1118}[CS10] for a minimum of six generations; the Canton-S X chromosome was subsequently used to replace the w^{1118}[CS10] X chromosome where noted. To generate inducible tetanus toxin light-chain expression, we recombined onto the same second chromosome the Gal80^{ts}20 transgene (McGuire *et al.* 2003) and the UASTNT-H transgene (P{UAS-TeTxLC.tnt}H2) (Sweeney *et al.* 1995). The cross of TH-Gal4 × Gal80ts20, UASTNT-H was incubated at 18° for 1 week before parents were cleared. The progeny were raised at 18°; under these conditions, Gal80^{ts}20 effectively represses Gal4 (McGuire *et al.* 2003). The day before the experiment, males were selected randomly and placed in normal food vials either in the Gal80^{ts} nonpermissive 32° for 14 hr or back into the permissive 18°. The flies were then removed to room temperature and used for open field experiments. A 14-hr induction at 32° was chosen for the Gal80^{ts20}-containing genotypes since it is sufficient to induce lethality with several different Gal4 drivers and the UASTNT-H transgene and to induce UAS reporter expression to levels approximating the Gal4 expression in the absence of the Gal80^{ts20} repressor (data not shown).

To generate adult flies that are deficient in *krz* expression, we used heat-shock Gal4-induced *krz* activity to rescue the developmental lethality of the krz^1 allele (Roman *et al.* 2000). The following cross was used to generate the krz^1 homozygotes: krz^1, P{hspGal4$^{89\text{-}2\text{-}1}$}/TM3, P{hs-hid}14 × P{UAS*krz*T5}; krz^1/TM3, P{hs-hid}14. The embryo and first instar larva progeny from this cross were placed in a cycling incubator for 10 days. This incubator provided 1.5 hr of 37° heat shocks every 12 hr. After the 10th day in the cycling chamber, pupa and late third instar larvae were removed to room temperature until eclosion. The homozygous adults were selected and then placed at 18° for 4 days immediately after eclosion. These same conditions were used to raise all control flies for experiments that included the krz^1 homozygotes. The OR83bGal4; krz^1 rescued flies were generated as stated in Ge *et al.* (2006), using the second chromosome OR83bGal4 driver (Kalidas and Smith 2002).

The pharmacological manipulation of dopamine synthesis was accomplished by feeding the flies 3-iodatyrosine (3-IY) or L-dopa (Sigma, St. Louis) according to the methods of Bainton *et al.* (2000).

Immunohistochemistry and *in situ* hybridization: The anti-*krz* polyclonal antibodies were prepared as described in Ge *et al.* (2006). The immunohistochemistry on paraffin sections and *in situ* hybridization on cryosection experiments were performed largely as previously described with a few modifications (Roman *et al.* 1998). A sense digoxigenin–UTP-labeled riboprobe for *in situ* hybridization was made by digesting the p478d cDNA (pSK vector) with *Xho*I and utilizing T3 RNA polymerase according to the manufacturer's protocols (Boehringer Mannheim, Indianapolis). The antisense riboprobe was made using the p478d cDNA, digesting with *Xba*I, and the transcription with T7 RNA polymerase. For immunohistochemistry, paraffin was removed from the sections with 2-min incubations in xylenes (Fisher Scientific, Hampton, NH). The sections were next hydrated by successive 5-min incubations in a series of decreasing ethanol concentrations (100, 80, 60, 40, 20, and 0% ethanol). It was also necessary to perform antigen retrieval to detect KRZ. To uncover the KRZ antigen, the hydrated slides were placed in 1× PBS heated to 95° for 5 min. The slides were then removed and placed into 1× PBS at room temperature for 10 min prior to immunoblocking. Nonspecific antibody binding was blocked with a 4-hr incubation at room temperature in 5% normal goat sera (Sigma Chemicals, St. Louis) in 1× PBS. For these experiments, affinity-purified antibody was used at a concentration of 1:100. Antibody was detected using the Vectastain ABC kit (Vector Labs, Burlingame, CA).

Behavioral analysis: The base of the open field arena was the bottom a 9.1-cm polystyrene petri plate and the top of the arena was made from the lid of a 15-cm petri plate (Fisher Scientific). A 2-mm hole was drilled in the top of the arena, near the side to allow for the aspiration of a fly into the arena. Since the top of the arena was larger than the bottom, the hole could be shifted out of the active arena area after the fly was added. The arena was illuminated by two 120-W flood lights. The output of these flood lights was controlled by rheostats and set to 4000 lx on the arena unless otherwise noted. The movement of the fly within the arena was tracked with either the HVS Image automated tracker or the Ethovision Tracking system (Noldus Information Technology, Leesburg VA). The trackers record the position of the fly in the X–Y plane every 100 msec. The collected data were then analyzed with Water2020 software (HVS Image), Ethovision Pro (Information Technology, Leesburg VA) or with the Wintrack software (Wolfer *et al.* 2001). Before beginning the experiments, it was determined that Canton-S had no preferences for individual arena quadrants. The measured variables included total path length, distance from center, the percentage of time spent in outermost one-third of arena, the percentage of time active, the number of stops (a stop is a pause lasting <5 sec), the number of rests (a rest is defined as a pause lasting >5 sec), and the average duration of a rest. The average velocity when moving was computed as path length/(percentage of time active × 60 sec). Each measure was determined for each successive minute in the apparatus. The percentage of time spent

in each section of the 5.5-cm^2 arena was determined using the HVS field data acquisition and analysis software (HVS Image). The automated video trackers were able to follow the flies for a minimum of 97.1% of the time. The analyzed data were imported into StatView v5.0.1 (SAS Institute, Cary NC) for statistical analysis. The activity in the Trikinetics (Waltham, MA) activity monitor was determined according to the manufacturer's recommendations. Flies that died during the experiment were removed from the subsequent analyses.

To measure activity after mechanical stimulation, the 9.1-cm circular arena was mounted on a Vortex Genie II (VWR Scientific). The arena's distance from the lighting and camera were kept the same as in previous experiments. Immediately after the end of the 5 min within the arena, the vortex (set at level 2.5) was pulsed for 1 sec. This pulse generated a moderate shaking of the arena, without dislodging the fly from its position. After the pulse, tracking was again initiated for another 5 min.

RESULTS

Rescued *krz^1* homozygotes have undetectable levels of *krz* immunoreactivity within the central nervous system: We examined the sites of *krz* expression using both immunohistochemistry and *in situ* hybridization (Figure 1). In paraffin sections, KRZ was detected in almost all tissues of the adult, in both males and females. However, the highest level of KRZ expression was within the neuropil of the central nervous system (CNS) (Figure 1A). Previously, KRZ was also detected at high levels in the sensory systems of the second and third antennal segments (Ge *et al.* 2006). The second antennal segment contains Johnston's organ, the primary auditory organ for adult Drosophila, while the third antennal segment is the primary olfactory organ of the fly (Miller 1950). Within the CNS, high levels of expression were found in segments of the olfactory system, including the antennal lobe, and the calyces of the mushroom bodies (Figure 1A). Relatively lower levels of KRZ were found in the lobes of the mushroom bodies and in the ellipsoid body. KRZ levels were essentially undetectable within the cell body layer of the central cortex and the photoreceptor neurons (Figure 1A; data not shown). The *krz* mRNA was also more highly expressed within the nervous system than the surrounding tissues (Figure 1B). The expression of *krz* message within the CNS was both widespread and uniform. Hybridization was not detected in the sense probe control (data not shown). The expression of the *krz* mRNA appears to be ubiquitous within the CNS, consistent with the expression of KRZ protein. This general expression of *krz*, a modulator of G-protein signaling, within the nervous system suggests a broad role for this gene in behavioral plasticity.

To investigate a role for *krz* in regulating behavior, it was necessary to rescue the *krz^1* loss-of-function allele through development. The *krz^1* allele has a lethal phenotype that results from a *P*-element insertion within the only intron of this gene. In *krz^1* homozygous larvae, *krz* mRNA is undetectable by RT–PCR, indicating a loss of function (Roman *et al.* 2000). The *krz^1* lethal phase is protracted, occurring throughout embryonic and larval development. To surmount this problem, *krz^1* homozygotes were rescued to adulthood by using heat-induced Gal4 to drive the expression of a *krz* cDNA ubiquitously during development (Ge *et al.* 2006). The genotypes of

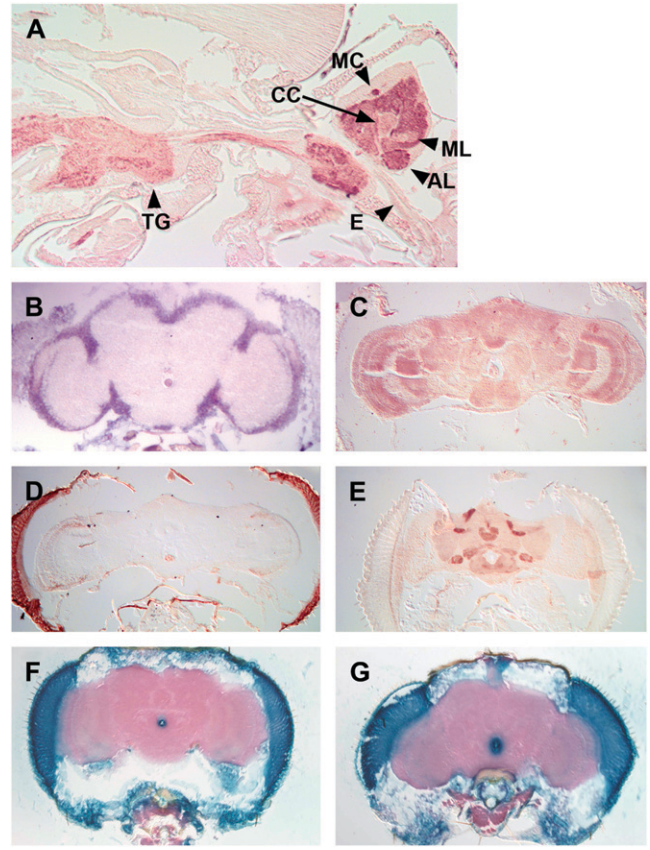

Figure 1.—*kurtz* expression in the CNS of wild-type and mutant animals. KURTZ was detected in paraffin sections using immunohistochemistry (A, C, D, and E). The frontal sections were performed and developed in parallel; sections shown in C and D are from the same slide. The *krz* message was detected by *in situ* hybridization on cryosections (B). (A) A sagittal section of a *w^{1118}* adult female. The head is slightly tilted in this section. KURTZ is seen as reddish-brown staining throughout the neuropil of the CNS, including in the thoracic ganglia (TG). The esophagus (E) divides the brain in this section. The edge of the mushroom body's calyx (MC) is seen as the dark reddish-brown spot on the posterior slope of the brain. The antennal lobe (AL) is also heavily stained. The mushroom body horizontal lobes (ML) and the central complex (CC) are more lightly stained. (B) *krz* message is detected by *in situ* hybridization on a *w^{1118}* female head. Abundant and widespread expression is detected throughout the CNS cell-body layer. (C) KURTZ expression in a wild-type female head, frontal section. (D) KURTZ expression in the CNS of a female P{UAS*krz*T5}/+; *krz^1*/*krz^1*, PhspGal4 fly, frontal section. (E) KURTZ expression in the CNS of a female c155/+; P{UAS*krz*T5}/+; *krz^1* fly. (F) P{UASLacZ}/+; PhspGal4/+, raised at 18°. β-Galactosidase activity is shown in blue. (G) P{UASLacZ}/+; PhspGal4/+, 3 hr after a 30-min, 37° heat shock. β-Galactosidase expression is shown in blue.

TABLE 1

Genotypes of the flies examined in the open field assays

Flies	Expanded genotype	Description	References
Canton-S	+; +; +	Wild-type *D. melanogaster*	
w^{1118}	w^{1118}; +; +	Background *white* allele outcrossed to the Canton-S for 10 generations	
krz^1	w^{1118}; P{UAS*krz*T5}/+; krz^1/krz^1, P{hspGal4$^{89\text{-}2\text{-}1}$}[a]	krz^1 heat-shocked rescued genotype; missing *krz* in the adult CNS	Roman *et al.* (2000); this study
krz^1/+	w^{1118}; P{UAS*krz*T5}/+; krz^1/ P{hspGal4$^{89\text{-}2\text{-}1}$}	Heterozygous control for krz^1	Roman *et al.* (2000)
PhspGal4/+	w^{1118}; +; P{hspGal4$^{89\text{-}2\text{-}1}$}/+	Heat-inducible Gal4 driver	Brand and Perrimon (1993)
c155; krz^1	c155, w^{1118}; P{UAS*krz*T5}/+; krz^1	elavGal4; krz^1 rescued genotype, expressing a *krz* cDNA throughout the nervous system	Roman *et al.* (2000)
c155; krz^1/ +	c155, w^{1118}; P{UASkrzT5}/+; krz^1/+	Heterozygous control for c155; krz^1	Roman *et al.* (2000)
b5.8T4; krz^1	w^{1118}; P{krzb5.8T4}; krz^1	krz^1 genomic transgene rescued genotype	Roman *et al.* (2000)
w^+; krz^1	+; P{UAS*krz*T12}/+; krz^1/krz^1, P{hspGal4$^{89\text{-}2\text{-}1}$}	krz^1 heat-shocked rescued genotype containing wild-type first chromosome	This study
$norpA^7$	+; +; $norpA^7$	Phospoholipase C mutation; blind	Harris and Stark (1977)
gl^2	+; +; gl^2	Missing photoreceptor cells; blind	Moses *et al.* (1989)
Dr^1/+	+; +; Dr^1/+	Severely reduced number of ommatidia	Krivshenko (1954)
THGal4/TNT	+; THGal4/P{UASTNT-H}, Gal80^{ts20}; +	Inducible tetanus toxin light-chain expression in most dopaminergic neurons	Friggi-Grelin *et al.* (2003)
$or83b^2$	+; +; $or83b^2$	*Or83b* mutation; anosmic	Larsson *et al.* (2004)
OR83bGal4; krz^1	w^{1118}; P{UAS*krz*T12}/OR83bGal4; krz^1/krz^1, P{hspGal4$^{89\text{-}2\text{-}1}$}	krz^1 heat-shocked rescued genotype with *krz* expression in olfactory receptor neurons	Ge *et al.* (2006)
OR83bGal4; krz^1/+	w^{1118}; P{UAS*krz*T12}/OR83Gal4; krz^1, P{hspGal4$^{89\text{-}2\text{-}1}$}/+	Heterozygous control for *ORGal4;* krz^1	Ge *et al.* (2006)
$Antp^{NS}$/+	+; +; $Antp^{NS}$/+	Antenna is converted to mesothoracic leg, missing arista	Scott *et al.* (1983)

[a] In Figure 2D, krz^1 homozygotes were generated with the P{UASkrzT12} transgene in place of P{UASkrzT5}.

these rescued homozygotes are listed in Table 1. The expression of KRZ in wild-type control and krz^1 rescued homozygotes were compared in parallel by immunohistochemistry (Figure 1, C and D). Under these conditions, KRZ immunoreactivity is prominent in the CNS of wild-type flies (Figure 1C). In the developmentally rescued krz^1 homozygotes, we detected strong immunoreactivity in eyes and slight immunoreactivity in the optic lobes (Figure 1D). No immunoreactivity was ever detected in the central brain neuropil of the krz^1 developmentally rescued homozygotes during seven independent experiments. In contrast to these krz^1 rescued homozygotes, the c155 pan-neuronal rescued krz^1 homozygotes expressed *krz* in all areas of the nervous system (Figure 1E). In these animals, however, *krz* expression is elevated in the central complex, antennal lobes, and mushroom bodies as compared to wild type (Figure 1E). The absence of *krz* expression in the CNS of adult krz^1 rescued homozygotes is facilitated by a property of the PhspGal4 driver. This driver is very poor at inducing the LacZ reporter gene expression in the adult CNS (Figure 1, F and G). In the uninduced state, β-galactosidase activity was detected in a manner very similar to that of *krz* in the krz^1 developmentally rescued homozygotes (Figure 1, D and F). This driver therefore provides *krz* expression during development to rescue lethality, but then turns off in the adult nervous system. The KRZ protein found in the optic lobe neuropil may be located in the axons of the photoreceptor neurons that innervate both the lamina (R1-6) and the medulla (R7 and R8), and not within the neurons of the CNS. The P{UASLacZ}/+; PhspGal4/+ flies have abundant β-galactosidase activity within these pho-toreceptor cells, but not within the neurons of the optic lobes (Figure 1, F and G). KRZ protein was detected in krz^1/+ flies at levels similar to the those of control wild-type flies (data not shown). These data indicate that KRZ levels are very low in the central brain of the developmentally rescued krz^1 homozygotes. The absence of *krz* expression within the CNS and antenna indicates that these krz^1 rescued homozygous adults, which have been rescued through development by the induced expression of a *krz* cDNA, can be used as strong reduction-of-function mutants for behavioral analysis.

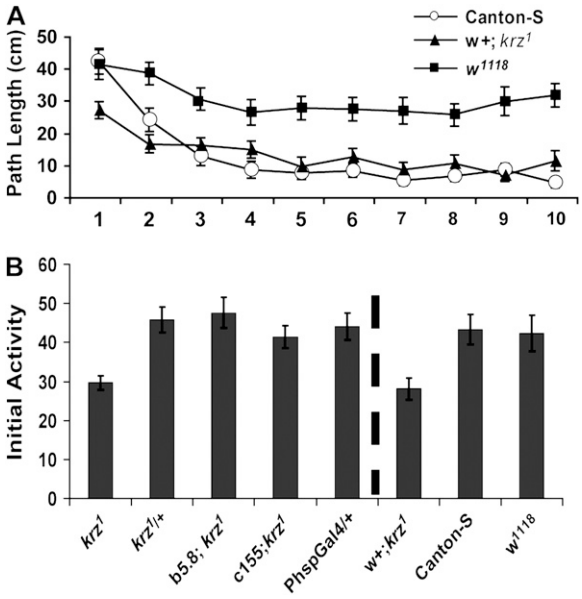

FIGURE 2.—*krz* is required for the initial activity phase in an open field arena. (A) Activity was measured in the circular open field arena. $n = 24$ for each genotype. There is a significant effect of both time ($F = 9.02$; $P < 0.001$) and genotype ($F = 72.44$; $P < 0.001$) on activity. The w^+; krz^1 homozygotes were significantly different from Canton-S control flies only during the first minute (Bonferroni–Dunn, $P = 0.005$). (B) The path length during the first minute in the open field arena ("Initial Activity") for male krz^1 and control genotypes (Table 1) is shown. $n = 24$ for each genotype. The initial activity from the first experiment is also shown for comparison, separated by a vertical dashed line. The krz^1 homozygotes are significantly less active than PhspGal4/+ control flies (Bonferroni–Dunn, $P < 0.002$). There were no significant differences between the krz^1 heterozygotes, c155; krz^1 and the b5.8; krz^1, and the PhspGal4/+ controls.

***krz* is specifically required for the elevated initial activity phase in Drosophila:** As a first step in defining the potential functions for *krz* in behavioral plasticity, we examined the role for this gene in regulating locomotor activity in adults. Locomotor activity of the developmentally rescued krz^1 homozygous adults was measured in an open field circular arena, 0.7 cm high and 9.1 cm in diameter and constructed of clear polystyrene. The limited height restricted most movement to the X–Y plane. The data were collected with an automated video tracking system, and activity was measured as the path length per minute. The krz^1 developmentally rescued adult males were examined in this open field assay for 10 min and compared to both wild-type Canton-S and a w^{1118} genetic background control group (Figure 2A). The w^{1118} allele used in these experiments had previously been outcrossed into Canton-S for 10 generations [*w*(CS10)]. Furthermore, we tested the rescued krz^1 homozygotes with a wild-type X chromosome, providing the mutants with wild-type levels of eye pigments and a Canton-S genetic background.

The initial activities of the w^{1118} and Canton-S flies were virtually the same (Figure 2A; $P = 0.866$). Canton-S flies then demonstrated a decline in activity during the next few minutes. This activity profile of Canton-S is qualitatively equivalent to the previously reported stimulated activity and the decay in stimulated activity to a steady-state level of spontaneous activity seen in *D. melanogaster* (CONNOLLY 1967; MEEHAN and WILSON 1987; MARTIN *et al.* 1999). Yet, the w^{1118} flies did not reduce their activity to the same extent as Canton-S males did during the first 10 min, suggesting a role for visual acuity in the activity decay or in establishing the baseline of spontaneous activity (KALMUS 1943). The w^{1118} flies containing mini-*white* transgenes, such as c155, show partial-to-full rescue of the decay from elevated initial activity (data not shown).

During the first minute in the arena, the developmentally rescued w^+; krz^1 homozygotes had significantly lower levels of activity than the control genotypes. This initial difference in path length between Canton-S and w^+; krz^1 is primarily due to the percentage of time that the flies were moving (Canton-S, 82.0 ± 0.3%, w^+; krz^1, 67.0 ± 4.3%; $P = 0.007$) and not to the speed at which the flies were moving (Canton-S, 0.92 ± 0.09 cm/sec, w^+; krz^1, 0.81 ± 0.11 cm/sec; $P = 0.464$). During the first minute, the w^+; krz^1 homozygotes had the same number of stops as Canton-S (1.54 ± 0.23 and 1.63 ± 0.23, respectively; $P = 0.989$). However, the w^+; krz^1 homozygotes had longer periods of immobility as seen by a significant increase in the time spent resting compared to Canton-S flies (Canton-S, 6.65 ± 0.1.9 sec, w^+; krz^1, 16.0 ± 2.7 sec; $P = 0.007$). Rest is defined as a pause in movement lasting at least 5 sec.

The spontaneous activity of the developmentally rescued w^+; krz^1 mutants was not significantly different from Canton-S starting at the second minute ($P = 0.114$, Bonferroni–Dunn) and continuing through the tenth minute (Figure 2A; $P = 0.249$, Bonferroni–Dunn). By the second minute in the open field arena, the difference in the percentage of time spent in movement between these two genotypes had decreased (Canton-S, 65.2 ± 5.4%, w^+; krz^1, 50.7 ± 5.4%; $P = 0.064$), and the average speed stayed about the same (Canton-S, 0.81 ± 0.20 cm/sec, w^+; krz^1, 0.73 ± 0.22 cm/sec; $P = 0.795$). During the third minute, the differences between these genotypes in the percentage of time spent in movement had disappeared (Canton-S, 50.7 ± 5.4%, w^+; krz^1, 51.0 ± 4.4%; $P = 0.957$) and the average speed of these genotypes remained the same (Canton-S, 0.88 ± 0.34 cm/sec, w^+; krz^1, 0.84 ± 0.24 cm/sec; $P = 0.919$). Furthermore, there were no significant differences in spontaneous activity between the developmentally rescued krz^1 homozygotes and w^{1118} measured in the dark over a period of days using a Trikinetics activity monitor (data not shown). In summary, the developmentally rescued krz^1 homozygous flies are capable of moving as fast as wild-type flies and do not differ from wild type in spontaneous activity levels, indicating that the these krz^1 homozygotes do not have a problem with coordinated

movement. The data therefore suggest that *krz* is specifically required for the elevated initial activity phase in an open field arena.

We examined several control genotypes to verify a role for krz^1 in elevated initial activity (Figure 2B). Heterozygous flies (w^{1118}; P{UAS*krz*T5}/+; krz^1/P{hspGal4$^{89\text{-}2\text{-}1}$}; Table 1) were used as controls for possible dominant effects of any of the transgenes found within the developmentally rescued homozygous krz^1 adults. Additionally, we examined krz^1 homozygotes rescued by a genomic transgene and the c155 rescued flies, which have *krz* expression throughout the nervous system in a krz^1 background (c155, w^{1118};P{UAS*krz*T5}/+; krz^1; Table 1; Figure 1E). In this experiment, the krz^1 homozygous flies demonstrated a severe reduction in path length traveled during the first minute compared to both wild-type and heterozygous controls (Figure 2B). The heterozygous controls in these experiments demonstrate that the krz^1 activity deficit in the initial minute is recessive, which rules out the possibility that it is caused by the P{UAS*krz*}, PhspGal4, or a combination of both *P*-element insertions. Furthermore, this initial activity deficit was fully rescued both by a genomic *krz* transgene and by the directed expression of a full-length *krz* cDNA throughout the nervous system (Figure 2A). Hence, the activity during the first minute within the open field arena is dependent on *krz* activity within the nervous system. Since the developmentally rescued krz^1 mutant flies display structural antenna defects with variable expressivity and penetrance (Ge *et al.* 2006), we also separately examined krz^1 homozygotes with either severe antennal defects or normal antennal structures, but failed to find any significant differences between these phenotypic classes in open field arena activity (data not shown).

The high level of initial activity is a conserved response to the arena: Since *krz* is the first gene identified with a defect in the elevated initial activity phase, and there is a paucity of data defining this particular behavior, we sought to learn more about this activity phase by investigating the proximal and ultimate causes for this behavior (Tinbergen 1963). If the elevation of initial activity has a general survival or reproductive value, then it should be conserved through evolution. When wild-type members of *D. melanogaster*, *D. simulans*, and *D. virilis* were placed into the open field arena, the stimulated activity, the decline in stimulated activity, and the spontaneous activity phases all were remarkably well conserved (Figure 3A). The maintenance of these components through evolution suggests that these behaviors confer a significant survival or reproductive advantage.

A distinctive ontogeny or sex specificity of elevated initial activity could suggest potential developmental or reproductive functions for the behavior. The elevated initial activity phase of both males and females is stable for the first 10 days posteclosion (Figure 3B; supplemental Figure 1 at http://www.genetics.org/supplemental/). However, at 14 days of age, females, but not males, show a significant decline in this stimulated activity phase (Bonferroni–Dunn, $P < 0.001$ for each comparison). The spontaneous activity phase also appeared to show distinct sexual dimorphism. Although path length was similar between males and females, males spent less time moving and had a longer duration of rests (supplemental Figure 1 at http://www.genetics.org/supplemental/). These data are consistent with males taking longer pauses, but compensating with faster movement, as was seen previously with a dissimilar apparatus (Martin *et al.* 1999). Thus, although spontaneous activity is modified by both age and sex, the elevated initial activity phase is present in both genders and in flies up to 10 days old, suggesting that this behavior has general utility for survival or reproduction.

We hypothesized that the elevated initial activity is likely a conserved response to novel stimuli. A source of these stimuli may be the experimenters handling of the fly—the fly may be agitated from being aspirated into the arena—or it may be due to a reaction to stimuli provided by the arena, such as novelty, open space, the absence of food, or bright lights. To address these hypotheses, we initially manipulated the amount of handling the flies received prior to being placed into the arena. In this experiment, posteclosion flies were housed individually for 3 days. They were then allowed to passively crawl from the food vial into the arena without aspiration, placed into the arena as before with a single aspiration, or aspirated into and out of a food vial 10 times within a span of 30 sec immediately before being placed into the arena (Figure 3C). Surprisingly, neither hyperagitation nor the lack of agitation had a measurable effect on the magnitude of the stimulated activity. The decline in initial activity and the amount of spontaneous activity also were not changed by these conditions (data not shown). We also found that posteclosion social isolation also did not affect open field behavior when compared to group-housed animals (data not shown).

If stimulated activity is not due to handling, then it may be a response to the arena itself. To test this hypothesis, we examined Canton-S flies for activity in arenas of different sizes and shapes (Figure 3, D–F). When the size of a circular arena is decreased, there is a coincident and linear decrease in the initial activity (Figure 3, D and F). In fact, in the smallest circular arena, the stimulated activity phase appeared to be completely absent. By the fifth minute, the activities in these circular arenas were essentially identical, suggesting that arena size does not alter spontaneous activity. In square arenas, there was a decrease in initial activity when the distance between corners was increased (Figure 3F). The stimulated activity changes in response to arena size and the interaction of this change with arena shape indicate that the flies respond to stimuli intrinsic to the different arenas.

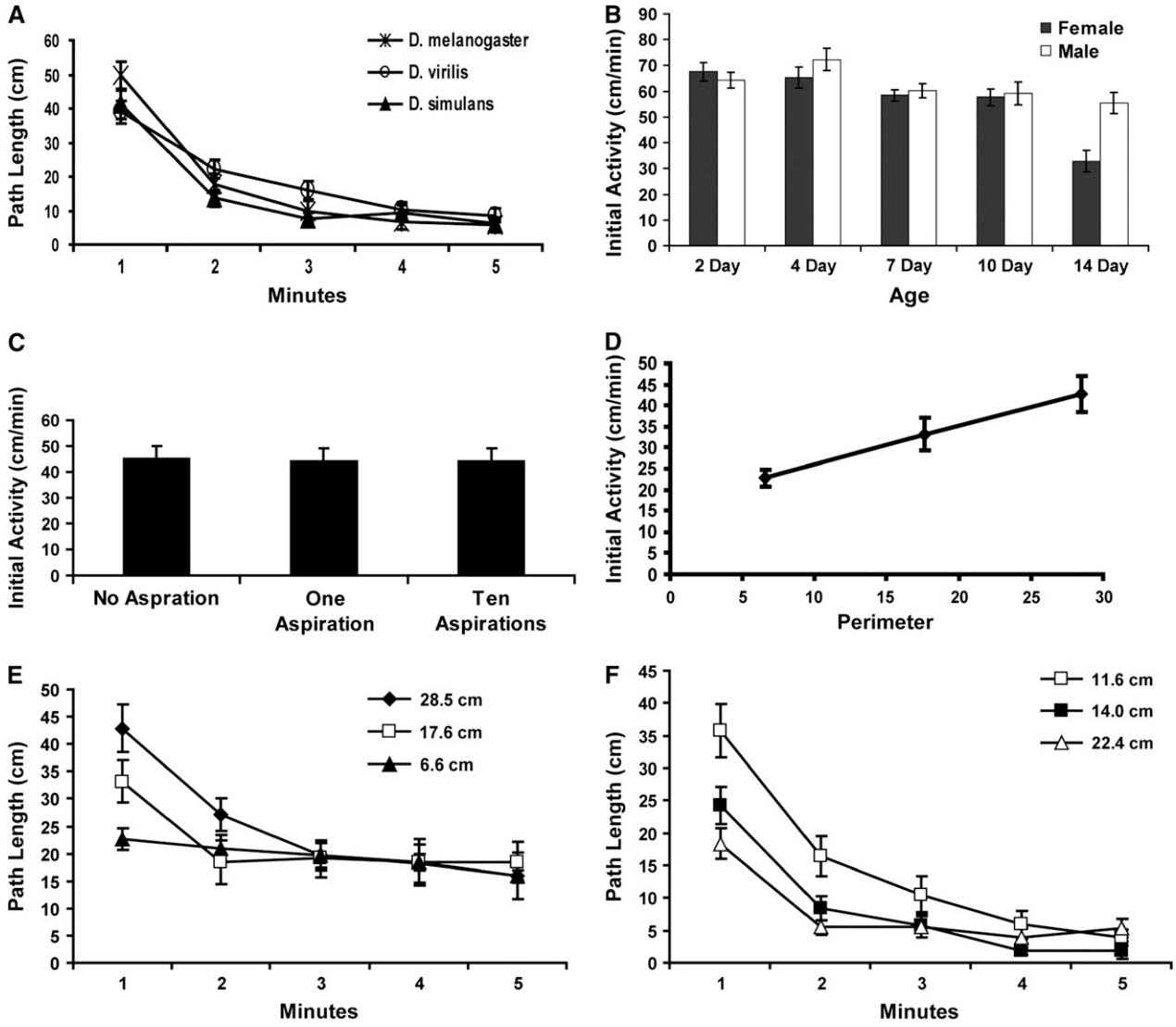

FIGURE 3.—Elevated initial activity is a conserved response to the arena. (A) The open field activity is conserved in three species: *D. melanogaster* (Canton-S), *D. simulans*, and *D. virilis*. (B) The elevated initial activity phase is present in both male and female flies and in both young and older flies. Fourteen-day-old females displayed significantly reduced path lengths during the first minute in the open field arena (Bonferroni–Dunn, $P < 0.001$ for each comparison). There were no other significant differences in path length within the first minute. The extended data set can be found in supplemental Figure 1 at http://www.genetics.org/supplemental/. (C) The elevated initial activity phase is not affected by handling. The activity during the first minute within the circular arena is shown. $n = 24$ for each treatment. (D) The activity of Canton-S flies was measured in circular arenas of three sizes. $n = 24$ for each arena size. The elevated initial activity increased linearly with the perimeter size of these circular arenas ($r^2 = 0.999$). (E) The path length of Canton-S was measured in circular arenas of three sizes. The circumferences of these arenas are shown. (F) The path length of Canton-S flies was measured in square arenas of three sizes. The perimeter lengths of these arenas are shown. Since in either square or circular arenas the flies spend the majority of time near the edge, the perimeter is the more relevant measure for arena size.

Although the spontaneous activity in square arenas was not affected by arena size, it was significantly lower than the spontaneous activity in circular arenas (Figure 3, E and F). This result suggested that a component of the square arena, most probably the corners, may inhibit spontaneous activity relative to the circular arenas. To test this hypothesis, we reexamined wild type and the developmentally rescued w^+; krz^1 homozygote flies in a square arena (Figure 4). Both w^+; krz^1 and wild-type Canton-S males spend significantly more time in the corners than in other areas of the arena (Figure 4B). Loitering in the corners would explain the decreased spontaneous activity in square arenas as compared to the circular arenas. The sensory systems and executive functions that control the corner preference are not significantly affected in the rescued krz^1 mutant flies, indicating that the activity of this gene is not required for these functions. Intriguingly, the two activity phases in the 22.4-cm^2 arena of the developmentally rescued w^+; krz^1 homozygotes were also not significantly different

A

Corner	Flank	Mid Pt.	Flank	Corner
Flank	Center	Center	Center	Flank
Mid Pt.	Center	Center	Center	Mid Pt.
Flank	Center	Center	Center	Flank
Corner	Flank	Mid Pt.	Flank	Corner

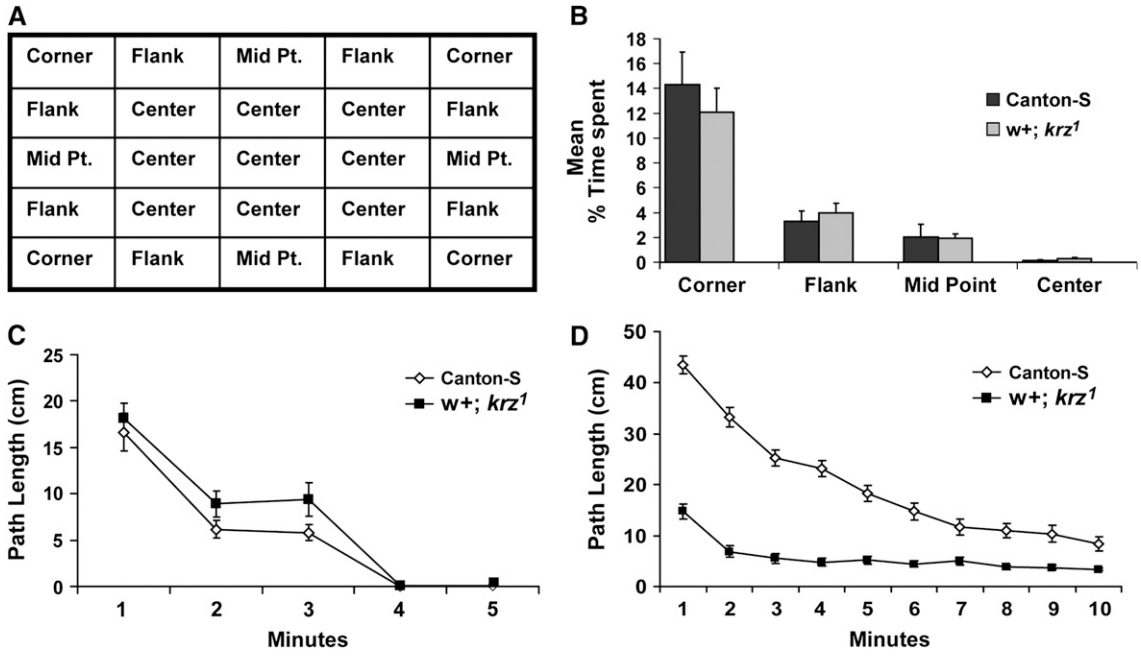

FIGURE 4.—Wild-type and *krz*^{1} mutant flies prefer corners when in a square arena. Open field assays were performed with both male Canton-S and w^{+}; *krz*^{1} homozygous flies within square arenas. (A) For this analysis, the 22.4-cm^{2} arena was divided into 25 sectors. These sectors were characterized as a corner, a flank located adjacent to a corner, a perimeter midpoint, and a center. The position of these sectors within the arena is shown. (B) The mean percentage of time that flies spent in each sector category ±SEM is shown. The time spent in a corner sector was significantly greater than the mean time spent in any of the other sector categories (Bonferroni–Dunn, $P < 0.001$). There were no significant differences in time spent in each sector between Canton-S and the w^{+}; *krz*^{1} homozygous flies. The males spent a total of ~54% of the time in the four corner sectors, 32% of the time in the eight flanking sectors, and 10% of the time in the midpoint sectors. These flies were also centrophobic, spending only 3% of the time in the nine central sectors. (C) The average path lengths ±SEM in w^{+}; *krz*^{1} and Canton-S of the 22.4-cm^{2} arena are shown. There were no significant differences in activity in path length between these two genotypes in the square arena ($F = 2.02$, $P = 0.156$). $n = 24$ for each genotype. (D) The average path lengths ±SEM of w^{+}; *krz*^{1} and Canton-S in the 11.6 cm^{2} arena are shown. There were significant differences in path length between the two genotypes ($F = 617.6$, $P < 0.0001$). These differences in path length were significant at each time point (Bonferroni–Dunn).

from Canton-S (Figure 4C). There were, however, significant differences between the developmentally rescued w^{+}; *krz*^{1} homozygotes and Canton-S in the smaller square arena (Figure 4D). Thus, the apparent inhibition of the elevated initial activity phase in the larger square arenas is capable of masking the *krz*^{1} phenotype.

Dopamine is not required for exploratory activity: Previous experiments have suggested a role for dopamine in both ethanol-induced hyperactivity and mechanically stimulated activity (BAINTON *et al.* 2000; FRIGGI-GRELIN *et al.* 2003; KUME *et al.* 2005). It has been postulated that dopamine is a general regulator of arousal in Drosophila (ANDRETIC *et al.* 2005; KUME *et al.* 2005). Since the elevated initial activity phase may represent stimulation by the novel arena, which should in principle be effected by arousal, we have examined the effects of dopamine on both initial activity and after mechanical stimulation using both the transgenic inhibition of dopaminergic neurotransmission and the reduction of dopamine levels pharmacologically (Figure 5). In our first experiment, we used a tyrosine hydroxylase-Gal4 line (TH-Gal4) to drive tetanus toxin light chain (TNT) in dopaminergic neurons (FRIGGI-GRELIN *et al.* 2003). In this experiment we also utilized a temperature-sensitive Gal80 transgene to repress TNT expression during development [temporal and regional gene expression targeting (TARGET) system; MCGUIRE *et al.* 2003]. In the TARGET system, Gal80^{ts} is ubiquitously expressed and can conditionally repress Gal4. This Gal80^{ts} transgene allowed us to examine uninduced flies, raised at 18°, as a within-genotype control. Interestingly, the induction of TNT in the dopaminergic neurons with a 14-hr incubation at the restrictive 32° had no apparent effect on either elevated initial activity phase or spontaneous activity phase (Figure 5A). However, after mechanical stimulation following the fifth minute, the induced flies exhibited significantly greater levels of activity, consistent with the results of FRIGGI-GRELIN *et al.* (2003). These data suggest that neurotransmission from the neurons defined by the TH-Gal4 inhibits mechanically stimulated activity, but does not play a role either in the elevation of initial activity or in spontaneous activity within a circular arena.

In a second experiment examining the role of dopamine in open field behavior, the synthesis of this neurotransmitter was pharmacologically inhibited with

3-IY using the protocols of Bainton *et al.* (2000; Figure 5B). This treatment has been shown to reduce total dopamine content in the whole fly to <10% of wild-type levels (Bainton *et al.* 2000). In the circular open field arena, the depletion of dopamine failed to alter the level of initial activity, consistent with the previous transgenic approach (Figure 5A). However, a significant reduction in the level of spontaneous activity was found after dopamine depletion (Bonferroni–Dunn, $P < 0.0001$). This inhibition of spontaneous activity was partially rescued by feeding the flies L-dopa, the product of tyrosine hydroxylase, suggesting that it is the inhibition of this enzyme that causes the reduction in spontaneous activity. Mechanical stimulation increased the activity of each treatment by ~15 cm during the first minute. The resulting activity after mechanical stimulation closely matched the spontaneous activity prior to mechanical stimulation (Figure 5B).

We next utilized 3-IY to inhibit tyrosine hydroxylase in Canton-S and the developmentally rescued w^+; krz^1 homozygous flies (Figure 5C). The pharmacological reduction in dopamine levels in either of these two genotypes did not have a significant effect on initial activity. Interestingly, the reduction of spontaneous activity in 3-IY-treated wild-type Canton-S flies was not found in the developmentally rescued w^+; krz^1 homozygotes, suggesting that *krz* is required for the inhibition of spontaneous activity by lowered dopamine levels (Figure 5C). After mechanical stimulation, both treated and untreated Canton-S flies increased their activity, an increase over spontaneous activity of ~15 cm for the 3-IY-treated wild-type flies and of 25 cm for the untreated wild-type flies. It is not clear why the untreated Canton-S males were more responsive to mechanical stimulation in this experiment than in the previous experiment, but these activity levels were significantly higher than the 3-IY treated flies (Bonferroni–Dunn, $P < 0.0001$). Both the 3-IY-treated and -untreated developmentally rescued w^+; krz^1 homozygotes in this experiment failed to show a robust increase in activity after mechanical stimulation (Figure 5C). The results from this experiment are consistent with a positive role for dopamine in regulating spontaneous activity and mechanically stimulated activity, but not elevated initial activity. *krz* activity is required for both elevated initial activity

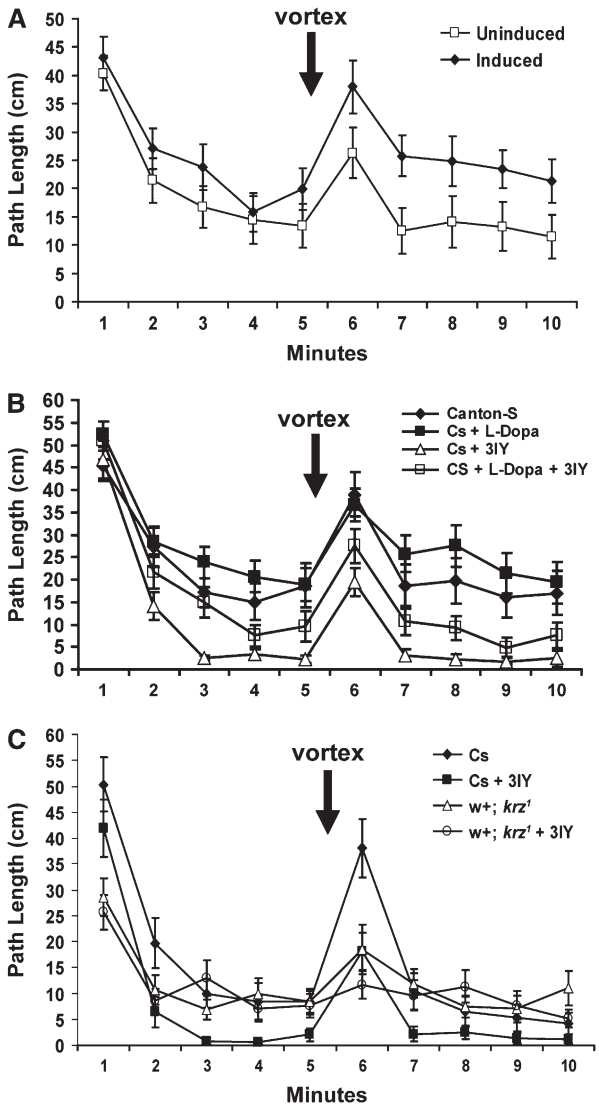

Figure 5.—Dopamine signaling is dispensable for the elevated initial activity within the open field arena. All flies were tested for 5 min in the circular open field arena. Immediately after the initial 5 min, the flies were mechanically agitated by moderate vortexing of the arena. The locomotion during the subsequent 5 min was then also recorded. Path lengths ±SEM are shown for each experiment. $n = 24$ for all groups. (A) Flies of the genotype UASTNT-H, Gal80^{ts}20/+; TH-Gal4/+ were tested in the circular open field arena either after a 14-hr 32° induction of tetanus toxin light chain or without induction. The induction of tetanus toxin in the TH neurons had a significant effect on activity ($F = 17.57$, $P < 0.0001$). There were no significant differences in path length between the induced and uninduced flies during first 5 min ($P = 0.078$), but after mechanical stimulation the induced flies had significantly higher levels of activity ($P < 0.0001$). (B) Wild-type Canton-S males were tested in the open field after treatment with 3-IY, L-dopa, both, or neither. During the first 5 min, 3-IY significantly inhibited spontaneous activity as measured during the fifth minute ($P < 0.0001$). This affect of 3-IY was partially reversed by L-dopa. (C) Wild-type Canton-S and w^+; krz^1 homozygous males were fed either 3-IY or a vehicle. These flies were then examined for activity in the open field arena. The 3-IY had a significant effect on the path length of Canton-S ($P = 0.0029$), but not of w^+; krz^1 ($P = 0.800$). The 3-IY did not have a significant effect on initial activity in either Canton-S ($P = 0.194$) or w^+; krz^1 ($P = 0.651$) males. However, during minutes 3, 4, and 5, 3-IY-treated Canton-S flies were significantly different from vehicle-treated Canton-S ($P < 0.0001$) and the 3-IY and vehicle-treated w^+; krz^1 ($P < 0.0001$ and $P = 0.0002$, respectively). Immediately after mechanical stimulation, the only significant comparisons were vehicle-fed Canton-S and either vehicle or 3-IY-treated w^+; krz^1 ($P < 0.0011$ and $P = 0.0002$, respectively).

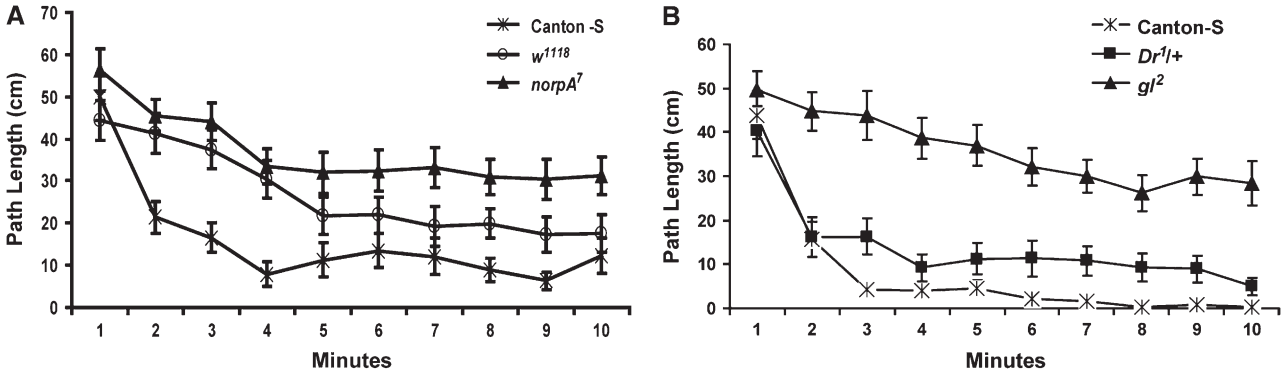

FIGURE 6.—The decay of the elevated initial activity to spontaneous activity depends on visual acuity. (A) The average path length ±SEM in a circular arena for male Canton-S, w^{1118}, and $norpA^7$ flies. $n = 24$ for each genotype. There were no significant differences in path length between the three genotypes during the first minute. At 10 min, the path length of Canton-S was significantly less than $norpA^7$ (Bonferroni–Dunn; $P = 0.0065$). This experiment was performed at 6000 lx. (B) Path length ±SEM in a circular arena for male Canton-S, gl^2, and $Dr^1/+$. There were no significant differences in path length between the genotypes during the first minute. Although Canton-S males were moving less than $Dr^1/+$ during the third minute, the difference was not significant ($P = 0.0446$; Bonferroni–Dunn). The difference between gl^2 males and either Canton-S or $Dr^1/+$ flies during the third minute was significant ($P < 0.0001$ for both comparisons). During the tenth minute, the gl^2 males were moving significantly more than either the $Dr^1/+$ or the Canton-S males ($P < 0.0001$ for both comparisons), and the path lengths of the $Dr^1/+$ and Canton-S males were not significantly different ($P = 0.288$). In both experiments, vision is required for the wild-type decay from stimulated activity. $n = 24$ for each genotype.

and mechanically stimulated activity. Furthermore, *krz* activity is required for the lowered spontaneous activity brought about by 3-IY-mediated inhibition of dopamine synthesis. Finally, we have found that the two independent techniques for inhibiting dopamine signaling produced markedly different effects on activity within the circular arena.

Visual acuity is required for the decay of initial activity: The reduced decay of initial activity in the w^{1118} flies suggests that visual acuity is required for the decay from the elevated initial activity phase and is not required for the initiation of the elevated activity phase (KALMUS 1943; Figure 2A). The compound Drosophila eye is appositional, with each ommatidium being visually insulated by pigment cells. In the w^{1118} mutants, the screening pigments within these cells are not made and therefore neighboring ommatidia receive a superimposed image, resulting in poor visual acuity. These flies are positively phototactic, but perform some optimotor responses very poorly (KALMUS 1943). If poor visual acuity is responsible for the maintained high level of activity of w^{1118}, then blind flies should also have similar defects in this assay. The $norpA^7$ mutant flies lack phospholipase C activity, are physiologically blind, and maintain high levels of activity in the open field arena over the full 10-min assay (Table 1; Figure 6A). The gl^2 mutant flies are also blind (they lack ommatidia) and they too maintain a high level of activity during the entire trial of 10 min (Table 1; Figure 6B). Moreover, we have found that the $bw^1; st^1$ double mutants, which also lack all screening pigments in the eye, phenocopy the w^{1118} activity phenotype (data not shown). These data show that flies with poor visual acuity maintain a high level of activity in the open field far longer than normally sighted flies. Remarkably, however, the $Dr^1/+$ heterozygotes demonstrate a strong decline in activity after the first minute (Figure 6B). The Dr^1 allele is a dominant gain-of-function mutation that results in a reduction from 700 to ~10 ommatidia/eye. The activity profile of the $Dr^1/+$ flies suggests that even a small number of functional ommatidia can lead to nearly normal activity decay, but the total lack of vision, or the poor acuity brought about by reduced screening pigments, leads to prolonged stimulated activity.

Olfaction is dispensable for elevated initial activity: In addition to the elevated initial activity defect, the developmentally rescued krz^1 homozygous adults also have blunted electrophysiological and behavioral responses to odorants (GE *et al.* 2006). If the initial elevation in activity arises from a new odor in the open field, then the krz^1 phenotype may be explained by the poor perception of this novel odor. We examined this possibility with three experiments. Both the physiological and behavioral olfactory defects of krz^1 homozygotes can be rescued by the expression of *krz* in the olfactory receptor neurons (GE *et al.* 2006). An OR83bGal4 driver that rescues the krz^1 olfactory defect did not rescue the initial activity defect of developmentally rescued krz^1 homozygotes, indicating that the olfactory phenotype of krz^1 was not the source of the initial activity deficit (Figure 7A).

We also examined flies homozygous for the broadly anosmic $or83b^2$ mutation in the open field arena (LARSSON *et al.* 2004; Figure 7B). Interestingly, the $or83b^2$homozygotes did not move significantly more or less than the control genotypes during the first minute in the open field arena, but by the fifth minute their activity was intermediate between wild-type Canton-S

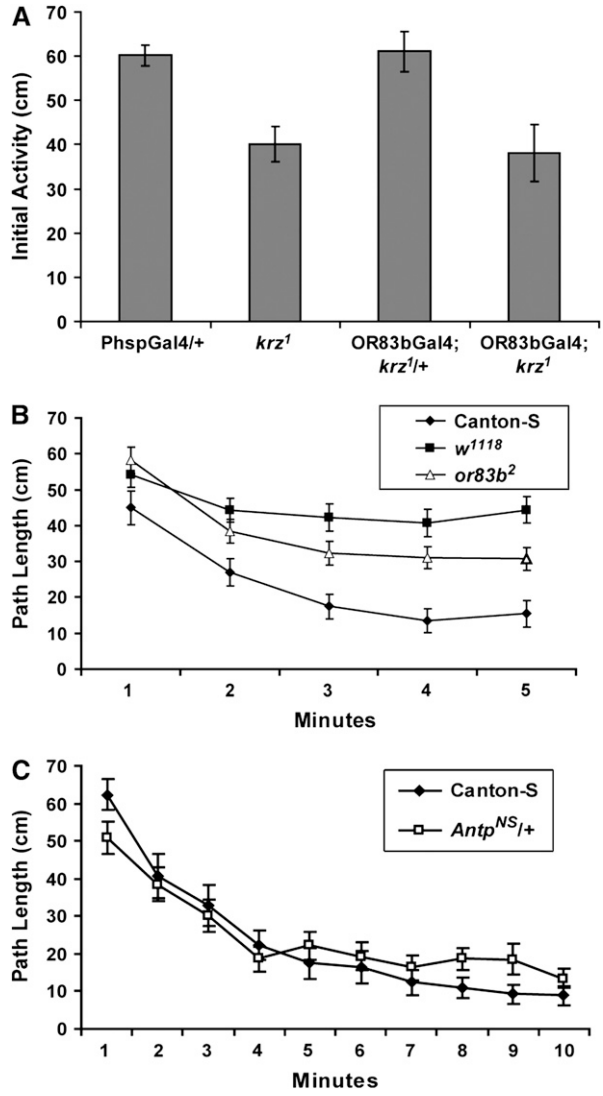

FIGURE 7.—Olfaction is not required for elevated initial activity. (A) The rescue of the olfactory deficit in *krz*^{1} homozygotes does not rescue the elevated initial activity phenotype in the open field arena. The path length during the first minute in the circular open field arena is shown for the four genotypes. The full genotypes are as listed in Table 1. The OR83b; *krz*^{1} flies, which have normal levels of olfactory behavior and odorant receptor potentials (GE *et al.* 2006), have significantly less initial activity than either the OR83bGal4; *krz*^{1}/+ or the PhspGal4/+ control genotypes ($P < 0.0015$), but not significantly different from *krz*^{1} homozygotes ($P = 0.754$). (B) The anosmic *or83b*^{2} mutants display normal levels of initial activity in the open field arena. During the first minute, in the open field arena, there were no significant differences in path length between *or83b*^{2} and Canton-S ($P = 0.023$; significance with the Bonferroni–Dunn posthoc test requires a *P*-value of <0.0167) or *w*^{1118} ($P = 0.47$). However, during the fifth minute, the path length of the *or83b*^{2} mutants was greater than that of Canton-S ($P = 0.003$) and less than that of *w*^{1118} ($P = 0.008$). (C) The homeotic *Antp*^{NS}/+ mutants do not show a significant difference from Canton-S in open field activity ($F = 0.571$, $P = 0.45$). $n = 24$ for each genotype.

and the visually defective *w*^{1118} (Figure 7B). This result suggested that olfactory input was an important component, albeit a lesser component than visual input, in the decay from initial activity to spontaneous activity.

In our third experiment to examine the role of the olfactory system in the elevated initial activity phase, we examined the homeotic *Antennapedia*^{NS} mutation for activity defects (Figure 7C; Table 1). *Antp*^{NS} is a dominant neomorphic allele that results in the transformation of the antenna into a mesothoracic leg with a high frequency of mutants lacking aristae (JORGENSEN and GARBER 1987; KANKEL *et al.* 2004). The *Antp*^{NS}/+ flies also demonstrate severe defects in the olfactory startle reflex, indicating a lack of olfactory acuity expected from the loss of the third antennal segment (MCKENNA *et al.* 1989). Prior to this experiment, the *Antp*^{NS} mutation was outcrossed to Canton-S for 10 generations. The subsequently selected *Antp*^{NS}/+ heterozygotes used for activity measures lacked arista, but appear to have normal maxillary palps (data not shown). Although the *Antp*^{NS}/+ flies displayed lower levels of ambulation during the first minute, this difference was not significant ($P = 0.0562$). The overall level of activity between these flies without normal antenna sensory input and the control Canton-S genotype was also not significant ($P = 0.45$). The failure to see significant differences in activity suggests that the antenna plays a minor role, if any, in the expression of the elevated initial activity phase.

Thigmotaxis: When placed into a well-lit open field arena, adult Drosophila avoid the center and mostly stay near the edge; a behavior most often referred to as centrophobicity (supplemental Table 2 at http://www.genetics.org/supplemental/; GÖTZ and BIESINGER 1985; MARTIN 2004). We quantified centrophobicity in the open field by measuring the fly's average distance from the center of the arena and the percentage of time that the fly spends in the outermost one-third of the arena. A fly moving about at random may be expected to spend ~33% of the time within this outer region. Wild-type Canton-S flies spend ~90% of the time within the outermost zone of the arena and average ~4 cm from the center. By either measure, *krz*^{1} homozygotes displayed normal levels of centrophobicity (supplemental Table 2). Canton-S males were significantly less centrophobic than females. A similar sex difference in centrophobicity within square arenas has been previously reported (MARTIN 2004). The *w*^{1118} flies showed significantly less centrophobicity in two experiments, but the differences did not reach the level of significance in a third experiment (supplementary Table 2). The *norpA*^{7} flies also displayed less centrophobicity, although it was significant only with the percentage of time spent in the outermost one-third of the arena and not with the mean distance from the center. Nevertheless, both *w*^{1118} and *norpA*^{7} are quite centrophobic, spending more than twice as much time in the outer zone than the other two

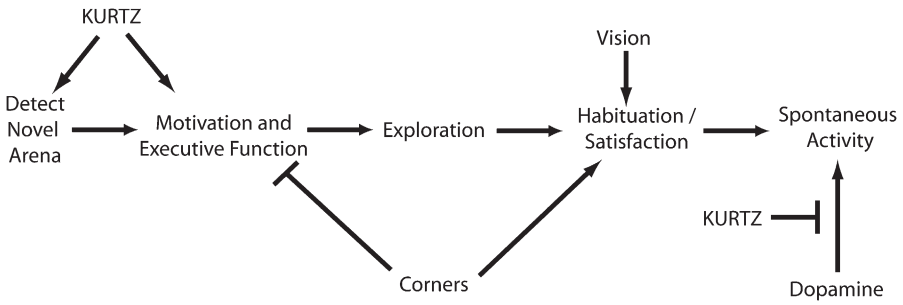

FIGURE 8.—A model for the exploration of an open field arena. In this model, a fly recognizes the novelty through multiple and redundant sensory inputs. The decision to explore the novel arena is then made through executive function centers and modified by motivational states. The KURTZ nonvisual arrestin is required in these early steps leading to exploration. The process of exploration can lead either to habituation of the novelty or to satisfaction if the fly has found what it sought. The result of either habituation or satisfaction is the much lower level of spontaneous activity. Poor visual and, to a lesser extent, olfactory acuity leads to prolonged exploration since the fly takes longer to habituate to the novelty. The presence of dispersed corners in a square arena leads to an overall reduced activity. The corners may provide a sense of shelter, which could satisfy the exploration drive. Alternatively, corners may generally affect activity through a different motivational or executive decision process. For example, a strong thigmotactic drive could supersede exploration and keep flies in corners.

zones together. These data are consistent with a report in which a transgenic approach was used to inhibit visual perception in square arenas (BESSON and MARTIN 2004). Our results strongly support a limited role for visual processing in the expression of centrophobicity. We have also found no role for olfaction in centrophobicity since the *or83b*^{2} mutant flies are as centrophobic as Canton-S (supplemental Table 2 at http://www.genetics.org/supplemental/). It is likely, therefore, that other factors, such as tactile stimulation or programmed radial search strategies, play a much larger role in the avoidance of the center of either square or circular arenas (supplemental Table 2 at http://www.genetics.org/supplemental/; GÖTZ and BIESINGER 1985; BESSON and MARTIN 2004).

DISCUSSION

An understanding of the significance of the elevated initial activity brought about by the open field arena requires better insight into the relevance of this behavior for the fly. When trying to decipher the ethological basis of a specific behavior, one should consider the proximal causes of a behavior, including the inducing stimuli and the neurobiological requirements for the expression of that behavior, an evolutionary relationship for the behavior, and whether the behavior has any survival value (TINBERGEN 1963). In dissecting the elevated initial activity, we have shown that the magnitude of the effect is dependent on the properties of the arena, independent of experimenter handling, and that the cessation of the elevated activity is dependent on vision and, to a lesser extent, on olfaction. We have also shown that elevated initial activity is present in diverged species, suggesting that it may underlie an important and general survival function. On the basis of these observations, we propose that the elevated initial activity constitutes exploration (Figure 8). Herein, we refer to the definition of exploration as stated by CRUSIO and VAN ABEELEN (1986, p. 55): "exploration is evoked by novel stimuli and consists of behavioral acts and postures that permit the collection of information about new objects and unfamiliar parts of the environment." A specific defect in exploration may arise either from a failure to detect or process the novel stimuli or from a failure in motivation and executive function.

Our hypothesis of Drosophila exploration occurring during the elevated initial activity phase in a circular open field arena is supported by the demonstration that the proximal causes of this stimulated activity come from properties of the arena and not from handling. The amount of activity during the first minute in a circular arena responded significantly to changes in arena size, indicating that some property of the arena itself is a proximal cause for this behavior: the greater the area to explore, the greater the amount of activity required to habituate the novelty stimulus. Sensory-deprived flies are deficient in the decay from elevated initial activity, which also supports the exploration hypothesis. The failure to visually observe the surroundings leads to an inability to recognize the surroundings and habituate to the stimulus, prolonging the exploration. The decline in exploration was also inhibited in the anosmic *or83b*^{2} mutants, suggesting that olfaction also has a role in the habituation to the novelty of the arena. The absence of an activity deficit in the antenna-transformed *Antp*^{NS}/+ flies suggests that this sensory organ is largely dispensable for activation of exploration. The gross structural changes in *Antp*^{NS}/+ flies lead to severe defects in olfactory jump responses (MCKENNA *et al.* 1989). Hence, it is apparent from *Antp*^{NS}/+ that the elevated initial activity found in the open field arena and the olfactory jump responses have different sensory requirements.

Dopamine and stimulated activity: Previous evidence has suggested that dopamine may generally regulate stimulated activity, including: initial activity within an open field, ethanol-vapor-stimulated activity, and mechanically stimulated activity (MEEHAN and WILSON 1987; BAINTON *et al.* 2000; FRIGGI-GRELIN *et al.* 2003). The only other mutation known in Drosophila to specifically affect initial activity in an open field arena

activity is a spontaneous allele of the *tyrosinase-1* gene; the *tyr1*^{1} allele results in flies with significantly higher levels of initial activity than wild-type flies, but no differences are found in the level of spontaneous activity (Meehan and Wilson 1987). The *tyr1*^{1} allele was identified as a spontaneous mutation having only 70% of the normal levels of dopamine, the molecular identity of this mutation (Lewis and Lewis 1963; Burnell and Daly 1982). In another study, Friggi-Grelin *et al.* (2003) selectively inhibited dopaminergic signaling with the targeted expression of tetanus toxin light chain in the majority of tyrosine-hydroxylase-expressing cells. The resulting flies were hyperactive after being banged to the bottom of a cylinder in a negative geotaxis assay but apparently have normal locomotion in the absence of this mechanical stimulation (Friggi-Grelin *et al.* 2003). In a third approach, Bainton *et al.* (2000) inhibited dopamine synthesis with 3-IY, an inhibitor of tyrosine hydroxylase that reduces total dopamine levels to ~10% of the wild-type levels. The 3-IY-treated flies initially displayed normal activity in a 6-cm^{2} arena; however, after stimulation with ethanol vapor, the activity of the 3-IY-treated flies was reduced relative to the untreated flies (Bainton *et al.* 2000). These studies suggest that dopamine has a role in stimulated activity, although the direction of the response may differ, depending on either the treatment or the stimulus. There are, however, clear differences in how these approaches disrupt the dopamine pathway. In the transgenic approach, most but not all dopaminergic neurons express the toxin, leaving a small number of dopamine-signaling pathways intact (Friggi-Grelin *et al.* 2003). In both the *tyr1*^{1} mutation and the pharmacologic inhibition of dopamine synthesis, it is not known if synthesis is globally inhibited (Burnell and Daly 1982; Bainton *et al.* 2000). It also remains possible that the residual dopamine levels in these flies may have enhanced effects due to a sensitization of their respective circuits.

More recently, several studies have highlighted a role for dopamine in regulating spontaneous activity and arousal in Drosophila. Lima and Miesenböck (2005) have found that activation of dopaminergic pathways results in state-dependent locomotor responses. In most flies, photostimulated dopamine release leads to an immediate increase in locomotor activity; however, in flies already expressing higher levels of ambulation, the forced release of dopamine leads to lower levels of activity (Lima and Miesenböck 2005). In two studies where synaptic levels of dopamine were likely to be increased, significant increases in locomotor activity were found (McClung and Hirsh 1998; Kume *et al.* 2005). The inhibition of dopamine reuptake with cocaine activates locomotor activity, whereas very high doses of this drug suppress ambulation (McClung and Hirsh 1998). Kume *et al.* (2005) identified a mutation in a dopamine transporter, *fumin*, which results in dramatically increased levels of spontaneous activity. The absence of *fumin* in neurons and glia may lead to higher synaptic levels of dopamine. These recent studies signify that dopamine probably has a dual role in regulating activity: an increase in locomotor activity occurs with lower levels of dopamine and higher levels suppress spontaneous activity.

It is possible that *krz* regulates elevated initial activity through an effect on dopamine signaling. If one presupposes that, in the developmentally rescued *krz*^{1} homozygotes, G-protein-coupled receptor signaling is generally extended and amplified due to the absence of agonist-dependent desensitization, then the reduction in the exploratory activity phase may result from an increased sensitivity to the effects of dopamine on activity. However, our data failed to show any effect of inhibition of dopamine signaling on the exploratory activity phase of wild-type flies or the developmentally rescued *krz*^{1} homozygotes, indicating that dopamine is not required in flies for the exploratory activity phase in an open field arena.

Spontaneous activity was dramatically reduced after feeding wild-type flies 3-IY, consistent with the increase in activity found with photostimulated dopamine release, with the increase in synaptic dopamine levels in the *fumin* mutant, or after cocaine treatment (McClung and Hirsh 1998; Kume *et al.* 2005; Lima and Miesenböck 2005). The affect of 3-IY on spontaneous activity was not found in the *krz*^{1} homozygous flies. Since the *krz*^{1} mutation suppresses the effect of 3-IY on spontaneous activity, *krz* most likely acts as a negative regulator of dopamine signaling during spontaneous activity. In the absence of *krz*, the dopamine receptors may perdure in an activated state, compensating for the shortfall of dopamine in the 3-IY-treated flies.

Conservation of exploration: We have found a considerable conservation of the open field behavior in three species of drosophilids. *D. simulans* and *D. melanogaster* are closely related members of the *melanogaster* species group, having diverged ~2.5–4 million years ago (MYA) (Ashburner *et al.* 1984; Cariou 1987). In contrast, the last shared ancestor for *D. virilis* and *D. melanogaster* is thought to have lived ~65–70 MYA during the late Cretaceous period (Beverley and Wilson 1984; Cariou 1987). Remarkably, however, a potentially homologous behavior is also present in many species of vertebrates (Glickman and Hartz 1964; Connolly 1967; Candland and Nagy 1969; Lát and Gollová-Hemon 1969). The presence of a conserved behavioral response in such divergent species strongly suggests that it provides general advantages, for example, leading the animal to new food sources, mating partners, or protective shelter.

The responses to an open field arena have been most thoroughly studied in rodents where the behavior in an open field arena is thought to be shaped by at least two conflicting internal drives: emotionality and curiosity

(Whimbey and Denenberg 1967; Walsh and Cummins 1976; Crusio 2001). The emotionality factor is thought to represent anxiety or fear and is typically measured by an inhibition of ambulation, an increased number of defecations, and decreased entries into the center of the open field (Hall 1936). These three ethological parameters in the open field arena are frequently reversible with anxiolytic drugs and enhanced by anxiogenic drugs, strengthening the association of these behaviors with anxiety (Prut and Belzung 2003). However, factor analysis has shown that anxiety in rodents is multidimensional, with decreases in locomotion and defecation influenced by different factors (Lister 1990; Ramos and Mormede 1998; Crusio 2001). In rodents, the inhibition of ambulation in the open field arena by an anxiety-like factor appears to be initially countered by a curiosity drive that leads to increased exploration (Walsh and Cummins 1976).

Outwardly, the behavior of the drosophilids in the open field resembles that of rats and mice: they all have an initially elevated period of activity, avoid the center of the arena, and prefer corners. It is not clear whether these behaviors of the drosophilids are functional analogs to the rodent behaviors since there is as yet much less known about the motivational factors that drive Drosophila behavior. The stressors of hyperagitation prior to placement in the arena or of social isolation do not affect the Drosophila exploratory activity phase, indicating that, if an emotionality factor exists in Drosophila, these potential stressors are insufficient to affect exploration (Figure 3C; G. Roman, unpublished result). We expect, however, that an exploratory drive similar to the one in rodents may exist in flies since it would serve the important survival functions of finding food, mating partners, or shelter. In fact, one explanation of the strong preference for corners and the striking thigmotactic responses in Drosophila is that the flies are seeking shelter. An increase in the size of a square arena resulted in a decrease in the amount of activity during the first minute—the opposite of the result in circular arenas. Although the corners inhibited the spontaneous activity phase independently of arena size, the exploration phase was most inhibited when the corners were farther apart: as the distance between shelters increases, the propensity to explore decreases. The phenomenon of staying in sheltered areas may also be associated with the avoidance of the arena's center and may represent the expression of fear or anxiety in Drosophila.

Alternatively, the centrophobicity and corner preference may be independent of any anxiety-like construct. Drosophila may have a powerful innate thigmotactic response that is independent of shelter seeking. In this case, the corners would offer more surface to rub against and would therefore become the preferred location. We do not favor this later explanation since the flies are, in general, not continuously rubbing against the arena's edge as much as maintaining proximity to it (L. Liu and G. Roman, unpublished observation). Drosophila may also have innate search strategies that drive them to the arena's edge through biased orientation and a persistence of direction during walking (Götz and Biesinger 1985).

The role of *krz* in exploration: The rescue of the krz^1 elevated initial activity deficit with both a genomic transgene and the pan-neural expression of a *krz* cDNA demonstrates a requirement for this gene in the nervous system for the expression of this activity phase. Since the krz^1 homozygotes can ambulate at speeds identical to those of wild-type flies during the first minute within the arena and have wild-type levels of spontaneous activity in either the open field or the Trikinetics activity monitor, the krz^1 phenotype in exploration is not due to a defect in motor function. Consequently, the requirement for *krz* in exploration more likely lies in either sensory- or executive-function-level processing. The developmentally rescued krz^1 homozygotes have reduced olfactory sensitivity and antennal structural defects, which exhibit variable penetrance and expressivity (Ge *et al.* 2006). However, the olfaction defect appears not to be the proximal cause of the activity deficit, since we can rescue the olfaction phenotype without altering the exploration phenotype, and olfaction is dispensable for the exploration phase. The selection of krz^1 homozygotes with or without antenna defects failed to affect the exploratory activity phase, suggesting that this exploration phenotype is independent of the antennal defect. Moreover, the more severe structure defects in $Antp^{NS}/+$ flies failed to produce an elevated initial activity phenotype, further suggesting that the antennal structural defect is not responsible for the exploration phenotype seen in the developmentally rescued krz^1 homozygotes. As the rescued krz^1 homozygotes are unresponsive to novelty and mechanical agitation, this gene may have a more central role in gating the responses to certain forms of stimulation.

In the large square arena, the krz^1 homozygotes behave indistinguishably from wild type: the same preference for corners, the same avoidance of the center of the arena, and the same activity profiles. In contrast, the rescued krz^1 homozygotes show an exploration deficit in the small square arena, where the corners do not seem to inhibit exploration to nearly the same extent as in the larger arena. The presence of an initial activity deficit in both the circular arena and the smaller square arena and the absence of the initial activity deficit in large square arenas would occur if either (1) the repression of activity in large square arenas indirectly masks the krz^1 deficit or (2) it more directly compensates for the absence of *krz*. An example of the former would be if the preference for corners is a general inhibitor of all locomotion, reducing the exploratory activity phase to a floor level commensurate with that of the developmentally rescued krz^1 homozygotes. In an example

of the later case, the effect of the corners may specifically reduce exploration by satiating a portion of the drive, which is reduced in *krz*^{1} homozygotes. In either case, the results do not argue against *krz* activity being required for exploration. The absence of this *krz* exploration phenotype also illustrates that tests of activity in some square arenas are restrictive—not all parameters of activity are identifiable in the 22.4-cm^2 arena.

Mice have two nonvisual arrestins, βarr1 and βarr2 (Conner *et al.* 1997; Bohn *et al.* 1999). These proteins are required for the agonist-dependent desensitization of GPCRs and for the regulation of a number of other cell-surface molecules (Lefkowitz and Whalen 2004). The mouse βarr2$^{-/-}$ mutation also results in reduced locomotor activity, although it appears to affect both the elevated initial activity and the plateau phases of activity (Bohn *et al.* 2003). The causes of the innate βarr2$^{-/-}$ defect in activity are not currently known; these mice may have problems with emotionality or perhaps they have minor motor defects. The murine βarr2 may also have different roles from those of *krz* in Drosophila in regulating locomotion; however, there are also significant experimental differences in how these experiments were performed. The βarr2 mutants developed without this gene's activity, whereas the *krz* mutant flies were periodically supplied with *krz* activity throughout development (Bohn *et al.* 2003). The two nonvisual arrestins in mice are also at least partially redundant (Kohout *et al.* 2001; Bohn *et al.* 2003), whereas *krz* is the only nonvisual arrestin in Drosophila (Adams *et al.* 2000; Roman *et al.* 2000).

In humans, subtle defects in the agonist-dependent desensitization of G-protein-coupled receptors may be a considerable contributing factor to the severity of affective disorders. A variant of the human G-protein-coupled receptor kinase 3 gene has been identified as a candidate locus for bipolar disorder (Barrett *et al.* 2003). The levels of GRK3 are lower in the leukocytes of patients with severe bipolar symptoms (Niculescu *et al.* 2000). In the postmortem brains of depressed patients, there is a significant increase in the level of membrane-bound GRK2, which is not found in the brains of antidepressant-treated patients; βARR2 may be coordinately regulated with GRK2 in the brains of these depressed patients (Grange-Midroit *et al.* 2003). Avissar *et al.* (2004) found a strong correlation between lower βARR1 levels in leukocytes and the severity of patients with major depression. A greater understanding of arrestin function within the nervous system is required to understand how agonist-dependent desensitization of protein-coupled receptors may lead to pathological emotional states. Although these states are probably controlled by different neurotransmitter systems in Drosophila, they still most likely involve G protein signaling. Drosophila, with a single nonvisual arrestin and a simpler behavioral repertoire, provides an excellent opportunity for examining the role of agonist-dependent desensitization in behavior. Understanding how *krz* is involved in the responses to novelty and mechanical stimulation will likely provide us with important insights into how these molecules regulate emotional responses in vertebrates.

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