We identified a unique type II cAMP-dependent protein kinase regulatory subunit (PKA-RII) gene in *Drosophila melanogaster* and a severely hypomorphic if not null mutation, *pka-RII<sup>EP(2)2162</sup>*. Extracts from *pka-RII<sup>EP(2)2162</sup>* flies selectively lack RII-specific autophosphorylation activity and show significantly reduced cAMP binding activity, attributable to the loss of functional PKA-RII. *pka-RII<sup>EP(2)2162</sup>* shows 2-fold increased basal PKA activity and ~40% of normal cAMP-inducible PKA activity. *pka-RII<sup>EP(2)2162</sup>* is fully viable but displays abnormalities of ovarian development and multiple behavioral phenotypes including arrhythmic circadian locomotor activity, decreased sensitivity to ethanol and cocaine, and a lack of sensitization to repeated cocaine exposures. These findings implicate type II PKA activity in these processes in *Drosophila* and imply a common role for PKA signaling in regulating responsiveness to cocaine and alcohol.

cAMP is a widely used second messenger that participates in a plethora of cellular, neural, and developmental processes. Neural functions of cAMP signaling include roles in a number of plastic responses, including learning and memory (1–6), synaptic transmission (7, 8), circadian rhythmicity (9), modulation of photoreceptor responses to light (10), and modulation of responses to alcohol (11, 12) and other drugs of abuse (13, 14). A key intermediate in cAMP signaling is cAMP-dependent protein kinase, PKA (15). PKA acts as a key sensor of cellular cAMP levels and mediates the flow of signals to downstream effector pathways by phosphorylating their targets. PKA is a heterotetrameric holoenzyme composed of two catalytic subunits (C<sub>2</sub>) and a homodimer of regulatory subunits (R<sub>2</sub>). The R<sub>2</sub> homodimer not only inhibits the catalytic activity of the C subunits but also stabilizes C subunits against proteolytic degradation in the PKA heterotetramer. Binding of cAMP to the R subunits of the holoenzyme results in dissociation of R and C subunits, which are then catalytically active. These general features of PKA signaling are shared by most eukaryotic organisms.

PKA can be formed from several types of C and R subunits that can endow the PKA heterodimer with different signaling and subcellular localization properties. The three subtypes of C subunits (Ca, Cβ, and Cγ) have virtually identical kinetic and physiological properties (15). By contrast, the different R subunit types, RI and RII, exhibit distinct cAMP binding affinities and are differentially localized in cells, leading to PKA holoenzymes termed PKAI or PKAII, respectively. PKAII holoenzyme is predominantly cytoplasmic, whereas the majority of PKAI associates with cellular structures and organelles (16). This subcellular localization is largely mediated by anchoring of RII subunits to protein kinase A-anchoring proteins (AKAPs). RII subunits bind to AKAPs with nanomolar affinity (17), whereas RI subunits only bind weakly to AKAPs (18). Adding to this complexity, higher vertebrates express two types of RI and RII genes that have different spatial patterns of expression (19).

In *Drosophila*, genetic and molecular approaches have led to the identification of a type I R subunit (*pka-RI*) and three genes for catalytic subunit isoforms, *pka-C1* (also known as *DCO*), *pka-C2*, and *pka-C3* (20). Both RI and RII PKA subunits have also been detected biochemically. PKA-RI is detectable during larval stages and early and mid-pupation (21), although its transcripts can be detected in the adult mushroom bodies of the brain (5). Consistent with this expression pattern, a *pka-RI* mutant shows learning defects (5). PKA-RII is expressed much more abundantly than PKA-RI in adult *Drosophila* (21), and its expression is largely restricted to the nervous system, where it is also preferentially expressed in mushroom bodies (22). The *Drosophila* gene encoding PKA-RII has not been previously identified.

From the analysis of *pka-C1* and other cAMP signaling genes such as *amnesiac* (*amn*), a PACAP-like adenylate cyclase-activating peptide), *pka-RI* (5), *rutabaga* (*rut*, Ca<sup>2+</sup>/CaM-responsive adenylate cyclase), and *dunce* (*dnc*, cAMP phosphodiesterase), there is functional evidence for the involvement of cAMP signaling in learning and memory (3), ovarian development (23), maintenance of circadian locomotor rhythmicity (9), and modulation of the sensitivity to ethanol intoxication (11) in *Drosophila*. In addition, PKA signaling functions in the widely used * hedgehog* signaling pathway (reviewed in Ref. 24).

Despite the aforementioned analyses, separable roles for PKAI *versus* PKAII signaling have been difficult to discern. In mouse, knockout of the RIIβ gene leads to experience-dependent locomotor defects and hypersensitization to repeated amphetamine exposures (14). These phenotypes could be due to the loss of the RIIβ subunit, but there are compensatory increases in other RI and RII subunits that could also explain the effects. Similar compensation is seen in RIIα-deficient mice (25). In flies, the *pka-RI* mutant shows a learning deficit, but this
may not be the full extent of pka-I functions, since this mut-
tant retains significant PKA-I expression (5).

Here we identify the Drosophila pka-RII gene and mutations that
virtually abolish its expression yet are homozygous-viable. We
used these PKAII-deficient mutants to analyze the involve-
ment of PKAII in various biological processes in Drosophila.

EXPERIMENTAL PROCEDURES

Fly Strains—The EP(2)2162 (26) line was obtained from the Berke-
ley Drosophila Genome Project (BDGP). For genetic back-
cross studies, randomly selected EP lines (EP(2)20844, EP(1)X1529, and
EP(1)X1192 of 10-12 days, as well as w
tartan retains significant PKA-RI expression (5).

Autophosphorylation Analysis—Autophosphorylation analysis was
conducted as described in Foster et al. (21) with modifications. Briefly,
protein extracts were prepared from whole flies by homogenizing the
frozen flies in Buffer A (5 mM EDTA, 50 mM Tris, pH 7.5) and
subsequently centrifuging at 10,000 × g for 30 min. The protein concentra-
tion of supernatants was determined by the Bradford method. A volume of
extract containing 30 μg of protein was incubated with 0.5 μCi of
[γ-32P]ATP (NEN Life Science Products) without additional cold ATP in
the presence or absence of 10 μM cAMP in a total volume of 20 μl of
Buffer B (10 mM β-mercaptoethanol, 10 mM MgCl2, 1 mM EDTA, 50 mM
Tris, pH 7.5) for 10 min on ice. The reaction was stopped by addition of
20 μl of 2X SDS gel loading buffer. 20 μl of assay mixture was separated
on a 10% SDS-polyacrylamide gel. The gel was fixed, dried, and
autoradiographed.

Western Blotting—A volume of whole fly extract containing 15 μg of
protein from each genotype was separated in an SDS-10% polyacry-
lamide gel and transferred to the nitrocellulose membrane. The blot was
blocked with 5% dried nonfat milk in TBS (25 mM Tris, 137 mM NaCl,
2 mM KCl, pH 8.0) for 30 min and incubated with anti-RII antiserum
(generously provided by Dr. D. Kalderon) for 2 h. After washing three
times with TBS, the membrane was incubated with horseradish perox-
idase-conjugated anti-rabbit goat IgG (Roche Molecular Biochemicals)
for 2 h at room temperature and then washed with TBS three times.
Immunoreactivity was visualized using the ECL system (Amersham
Pharmacia Biotech) following the manufacturer’s instructions. Kodak
Image Station 440 (Eastman Kodak Co.) was used to develop the
image and to analyze the relative density of signals.

cAMP Binding Assay—cAMP binding activity was measured by
the method described in Kumon et al. (27). A volume of extract containing
30 μg of protein was incubated with 1 μCi of 3’-5’-2’-8’-[3H]cAMP (ICN,
Costa Mesa, CA) in a total volume of 100 μl of Buffer C [50 mM sodium acetate, pH 4.5, 9 mM MgCl2, 2 mM ATP (0.25 mg/ml bovine serum
albumin, 50 mM Tris, pH 7.5) in the presence and/or absence of 10 μM cAMP and 1 μM of PKA inhibitor-(6–22)-amide (Life
Technologies, Inc.) for 5 min at 30 °C. Incorporation of phosphates to
Kemptide was measured by spotting the 20 μl of assay mixture onto
Whatman P-81 paper and subsequent washing with 1% phosphoric
acid. Calculations of kinase activity were based on the fraction inhibited
by the phosphate element had been inserted. Subsequent nucleotide sequencing of polym-

RESULTS

Molecular Characterization of a PKA-RII in Drosophila—We
identified a cDNA encoding a Drosophila homolog of a type II
regulatory subunit of cAMP-dependent protein kinase (PKA-
RII) in the BDGP EST data base through BLAST searches
based on the primary protein sequences of known PKA-RII proteins.
These searches identified a single cDNA clone, LD44591, in BDGP cDNA collections, which was subsequently
sequenced in its entirety. The cDNA encodes a deduced 42.7-
kd protein that retains significant similarities to vertebrate
PKA-RII proteins and the previously identified Drosophila
PKA-RI (Fig. 1). The most striking similarities are observed in
cyclic nucleotide-binding domain sequences (residues 151–167, 187–204, 289–285, and 309–326) that form two
cAMP/GMP-binding motifs (residues 124–239 and 242–362,
indicated by bars in Fig. 1). Also observed is a PKA autophos-
phorylation site (residues 81–84, indicated by an asterisk
in Fig. 1), a unique feature of type II PKA regulatory subunits (29).
It also contains amino acid sequences that have been
identified by peptide sequencing from biochemically purified
Drosophila PKA-RII (30), indicated by dashes in Fig. 1).

Characterization of pka-RII Gene and Identification of a pka-
RII Mutant—Genomic DNA sequences corresponding to Dros-
ophila PKA-RII cDNA were retrieved from the BDGP genomic
sequence data base, and polymerase chain reaction amplifica-
tion and subsequent DNA sequencing were carried out to
determine the full genomic DNA sequence. Ten exons are dis-
persed throughout the ~15-kilobase pair genomic region that is
localized in the proximal part of chromosome 2R in polytene
region 46D1–46D2 (Fig. 2
150-kDa proteins (Fig. 2

Ovary Staining—To visualize nuclei, 4’,6-diamidino-2-phenylindole
staining was performed according to the procedure of Ruohola et al. (48). To
highlight cell outlines and ring canals, rhodamine-conjugated phalloidin
was added at the same time as 4’,6-diamidino-2-phenylindole.

Locomotor Activity Assay—Male flies kept at 18 °C were exposed to
cyclic light-dark (LD) cycles with a photoperiod of 12:12 light–12–12 dark (LD, where zeitgeber time 0 (ZT0) is
lights on and 12 (ZT12) is light off), followed by 10–12 days in constant
dark (DD) conditions in a locomotor activity monitor (Trikinetics,
Waltham, MA). Wild type and mutant flies were tested simultaneously in a
given experiment. The free running periods of individual flies during
the light phase and subsequent DNA sequencing were carried out to
determine the full genomic DNA sequence. Ten exons are dis-
persed throughout the ~15-kilobase pair genomic region that is
localized in the proximal part of chromosome 2R in polytene
region 46D1–46D2 (Fig. 2A). The data base searches also led to the
identification of a P insertion mutant, EP(2)2162, inserted
98 base pairs upstream of the predicted transcription start site
corresponding to the sequence of LD44591 clone in a position
that could block transcription of pka-RII. This insertion line is
homozygous-viable and fertile with no obvious behavioral or
developmental phenotypes.

To see the effect of this insertion on the expression of PKA-
RII protein, we tested the cAMP-sensitive autophosphorylation of
PKA-RII in whole fly extracts from wild type and EP(2)2162
lines (see “Experimental Procedures”). ATP concentration in
the reactions was maintained low enough to allow predominant
intramolecular RII autophosphorylation that is blocked by
cAMP addition and subsequent C subunit dissociation (31). The
autophosphorylation reaction leads to the phosphorylation of
~57- and ~70-kDa proteins (Fig. 2B). The 57-kDa protein,
which is phosphorylated in a cAMP-sensitive manner, is reduced by 95% in the EP(2)2162 extracts (Fig. 2B), and the phosphorylation of the 70-kDa protein is increased about 2-fold. Given that cAMP-sensitive phosphorylation occurs only on the PKA-RII that is electrophoretically determined to be about 52 kDa in its dephosphoform and 57 kDa in its phosphoform in Drosophila (21), this result indicates that EP(2)2162 does not express a functional PKA-RII. Consistent with this, by Western blotting analysis (Fig. 2C), RII immunoreactivity is reduced ~10-fold in EP(2)2162 extract. In addition, the EP(2)2162 extract contains significantly reduced cAMP binding activity (Fig. 2D) attributable to the absence of PKA-RII, a major cellular cAMP-binding protein (32). Taken together, the EP element in EP(2)2162 disrupts the normal expression of PKA-RII causing a severe, if not null, mutation of pka-RII, which we subsequently refer to as pka-RIIEP(2)2162. Since BLAST searches against the Drosophila euchromatic genomic sequence data base fail to show any additional pka-RII candidate genes (data not shown), pka-RIIEP(2)2162 likely represents the first animal in which PKA-II activity is effectively eliminated.

We note that the PKA-RII cDNA encodes a deduced 42.7-kDa protein, significantly smaller than the apparent size by electrophoretic mobility. A similar size discrepancy is observed in bovine PKA-RII, with deduced molecular mass of 45 kDa and apparent molecular mass of 58 kDa (33). This suggests that the structural properties of PKA-RII proteins that induce the size discrepancy are conserved among these diverse organisms.
pka-RIIEP(2)2162 Shows Altered PKA Activity—In mouse, loss of PKA regulatory subunits by genetic manipulations leads to reduction of cAMP-inducible PKA activity mainly due to proteolytic degradation of free PKA C subunits and compensatory up-regulation of the other PKA regulatory subunits (14). To assess the effect of selective loss of PKA-RII on PKA activity in pka-RIIEP(2)2162, we assayed PKA activity in the total protein extracts from wild type and pka-RIIEP(2)2162 whole flies (Fig. 3). In the absence of exogenous cAMP, the low basal level of PKA activity is significantly increased in the pka-RIIEP(2)2162 extract (Fig. 3a), consistent with an increase in free C subunits due to loss of RII subunits. However, in the presence 10 μM cAMP, the pka-RIIEP(2)2162 extract shows only ~40% of the activity seen in wild type extract (Fig. 3b). The inducibility of PKA activity by excess cAMP is ~70-fold in extracts from wild type flies and ~12-fold in extracts from pka-RIIEP(2)2162 with the reduction of cAMP action attributable to the loss of this major cAMP signaling component.

pka-RIIEP(2)2162 Affects Ovarian Development—Since defects in oogenesis have previously been reported for hypomorphic pka-C1, a PKA catalytic subunit mutant (23, 34), we investigated whether pka-RIIEP(2)2162 ovaries show similar phenotypes (Fig. 4). Approximately 43% (~n = 150) of ovarioles from pka-RIIEP(2)2162 flies show defects in follicle formation and maturation. These defects include multinucleate nurse cells, too many or too few nurse cells within a follicle, absence of...
interfollicular stalks, and an abnormally large number of somatic epithelial cells at the posterior end of the follicle. These phenotypes are very similar, but not identical, to those seen in pka-C1 mutants. In pka-C1 ovaries, multinucleate nurse cells often still contain the ring canal that would have connected the two nurse cells (34), but in pka-RIIEP(2)2162 ovaries, multinucleate cells (brackets, Fig. 4, b and d) have no internal ring canals. Follicles with multinucleate cells also seem to have fewer nurse cells than normal. pka-RIIEP(2)2162 homozygotes also have large follicles where double and triple the normal number of nurse cells is observed, and often, these follicles are not separated from the next follicle by an interfollicular stalk. The similarities between pka-C1 and pka-RIIEP(2)2162 phenotypes indicate that PKAII is a major contributor to PKA signaling during ovarian development.

pka-RIIEP(2)2162 Shows Arrhythmic Locomotor Activity—PKA is also involved in the maintenance of locomotor circadian rhythm in Drosophila, functioning downstream of the brain-based circadian pacemaker in an output pathway (9). We analyzed locomotor rhythm of pka-RIIEP(2)2162. About 42% of pka-RIIEP(2)2162 flies retain normal circadian rhythm in constant environmental conditions (Fig. 5C), consistent with the locomotor analyses of pka-C1 (9), a PKA catalytic subunit mutant. pka-RIIEP(2)2162 entrains normally to LD conditions but the rhythmity rapidly dampens through following DD conditions (Fig. 5B). In 42% of flies showing rhythmity, a normal period of ~23.7 h is observed. About 85% of wild type and randomly selected EP lines consistently show normal rhythmity (Fig. 5, A and C, data not shown).

pka-RIIEP(2)2162 Shows Altered Ethanol Intoxication—Functional evidence for the involvement of cAMP signal transduction pathway in the ethanol intoxification in Drosophila has been reported (11). Mutations in the gene amn, which is thought to encode a positive regulator of cAMP signaling, increase sensitivity to ethanol intoxification, as assessed by increased rate of passing through an inebriometer. We devised a simple apparatus that allows comparison of ethanol intoxification sensitivity of the two groups of flies in parallel, by measuring time for flies to fall to the bottom of a tube through which humidified ethanol vapor is pumped (see “Experimental Procedures”). Multiple alleles of amn were tested using this apparatus. Consistent with the previous report using the inebriometer (11), these amn alleles show shorter KO90 time than wild type or random EP lines (data not shown). In contrast, pka-RIIEP(2)2162 shows robust resistance to ethanol (Fig. 6A) with ~6 min longer KO90 time than wild type flies (Fig. 6B). To verify that the observed phenotype is not due to the altered ethanol permeability or metabolism rate, the amount of ethanol in the whole body extracts of pka-RIIEP(2)2162 and wild type lines was measured (see “Experimental Procedures”). There are no significant differences in permeability and metabolism rates between pka-RIIEP(2)2162 and wild type lines (data not shown).

pka-RIIEP(2)2162 Shows Altered Responsiveness to Cocaine—In vertebrates, PKA signaling is a key pathway modulating responses to chronic administration of cocaine. We tested the responsiveness of pka-RIIEP(2)2162 to cocaine, a potent motor stimulant in flies (28). The response of pka-RIIEP(2)2162 to the first dose of cocaine is much lower than that of wild type flies and other randomly selected EP lines (Fig. 7). Significantly higher doses of cocaine (180–200 μg) are required to elicit substantial initial responses, which are induced at ~5–90 μg of cocaine in wild type lines. Furthermore, subsequent doses of cocaine failed to elicit robust sensitization in pka-RIIEP(2)2162 flies, whereas wild type flies show prominent sensitization (Fig. 7), measured as an enhanced response to the second cocaine exposure.

Reversion of pka-RIIEP(2)2162—To ascertain that the phenotypes reported are due to the EP(2)2162 element, multiple complete and partial revertants were recovered by mobilizing the P element in pka-RIIEP(2)2162 (see “Experimental Procedures”). Phenotypes were analyzed against their background strain (y,w) and parental strain (pka-RIIEP(2)2162). Complete revertants, pka-RIIEP(2)2162 and pka-RIIEP(2)2162, show none of the behavioral and developmental defects that are observed in pka-RIIEP(2)2162 (data not shown). Similarly, imprecise excision of the P element resulting in deletion extending to the pka-RII gene yields biochemical and behavioral phenotypes indistinguishable from pka-RIIEP(2)2162, indicating that pka-RIIEP(2)2162 is a severely hypomorphic, if not a null, mutant.

**DISCUSSION**

**pka-RIIEP(2)2162 Is Deficient in PKAII—** Type II PKA is the predominant isoform in adult Drosophila (21, 22), yet the pka-RII gene has not been isolated previously. We detect a unique pka-RII gene and a PKA-RII protein species that is autophosphorylated in vitro in a cAMP-sensitive manner. We have not detected other PKA-RII candidate genes in BDGP nucleotide sequence data base that represents most of Drosophila euchromatic DNA sequence. A P element-induced mutation, pka-RIIEP(2)2162, lacks at least 95% of the cAMP-sensitive autophosphorylation activity, characteristic of RII. Furthermore, deletions that extend into pka-RII as a result of imprecise excision of this P element show phenotypes that are no more severe than the initial mutant. Surprisingly, all of these mutants show full viability. These mutants are the first animals in which PKAII activity is essentially abolished, giving substrates for examining the in vivo roles of PKAII signaling.

PKA activity in pka-RIIEP(2)2162 extracts shows reduced cAMP-mediated inducibility, as would be expected for loss of this major CAMP regulatory subunit. This reduction in cAMP inducibility is due to two effects. First, these extracts show increased basal PKA activity measured in the absence of exogenous cAMP relative to wild type, presumably due to an increase in free C subunits that are not coupled with R subunits. Second, pka-RIIEP(2)2162 extracts show reduced PKA activity relative to wild type in the presence of saturating CAMP. In vertebrates, loss of R subunits leads to destabilization of C subunits, making them more susceptible to proteolytic degradation (14, 25). PKA activity at saturating cAMP measures the

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**Fig. 3. Altered PKA activity of pka-RIIEP(2)2162.** PKA activities of the whole fly extracts from pka-RIIEP(2)2162 and wild type (WT) flies were measured in the absence (a) and presence (b) of 10 μM cAMP. Three homogenates of each genotype were assayed in duplicate. The data points represent the mean ± S.E. The differences in PKA activities are statistically significant between genotypes as determined by one-tailed t test. *, p < 0.05, and **, p < 0.001.
total C subunit content, which is therefore reduced in 

**FIG. 5.** Arrhythmic locomotor activity of pka-R1I<sup>Ep(2)2162</sup>. Locomotor activity of individual flies kept at 18 °C was recorded for 4 days in light/dark (LD) cycles followed by 10 days in constant darkness (DD). Average locomotor activities of wild type (WT, A) and pka-R1I<sup>Ep(2)2162</sup> (B) flies (n = 32 each) in the last day of LD and the 5 subsequent days of DD are shown. Open and solid bars indicate light and dark conditions, respectively. The fractions of flies showing normal rhythmicity from the pool of three independent experiments for each genotype are shown in C. Statistical significance was determined by a χ<sup>2</sup> test; **, p < 0.001, n = 88 for wild type flies, and n = 105 for pka-R1I<sup>Ep(2)2162</sup>.

**FIG. 4.** Abnormal ovarian development of pka-R1I<sup>Ep(2)2162</sup>. Ovarioles from wild type (WT, a and c) and pka-R1I<sup>Ep(2)2162</sup> (b and d) females were stained with 4',6-diamidino-2-phenylindole (DAPI, a and b) to visualize nuclei and rhodamine-conjugated phalloidin (c and d) to show cell outlines. The brackets in b and d indicate an example of a multinucleate nurse cell.

Altered Cocaine Responsiveness of pka-R1I<sup>Ep(2)2162</sup>—PKAII-deficient pka-R1I<sup>Ep(2)2162</sup> flies show two defects in cocaine responsiveness. First, they are very resistant to cocaine, requiring much higher doses than normal to stimulate locomotor responses. Second, they are defective in sensitization, even when exposed to high enough cocaine doses to yield behavioral responses sufficient to evoke sensitization in wild type flies.

In vertebrates, stimulation of D1-like dopamine receptors subsequent to cocaine exposure results in activation of G-protein-dependent adenylate cyclase and PKA (reviewed in Refs. 35 and 36), and pharmacological activation of adenylate cyclase by cholera toxin produces an augmented behavioral response to an acute injection of cocaine (37). Consistent with these effects, there is an increase in the basal activity of adenylate cyclase and PKA in the nucleus accumbens of the forebrain after repeated cocaine administration (38). The PKA-mediated second messenger system regulates the activity of immediate early genes, such as c-fos, via cAMP-responsive element binding protein (reviewed in Ref. 13). Additional PKA targets proposed to play a role in behavioral sensitization include (S)-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (39) and voltage-dependent sodium channels (36). All of these changes subsequent to psychostimulant exposure are interpreted as occurring in neurons postsynaptic to the dopamine neurons that project to this region, although G-protein signaling and transmitter synthesis and release in the dopamine cells...
themselves can also be altered by activation of dopamine autoreceptors (8, 40, 41). The first phenotype, resistance to cocaine, is what would be expected of a major disruption in cAMP signaling in postsynaptic neurons. Mice lacking PKA-RIIβ show normal responsiveness to amphetamine (14), which targets the same amine transporters as cocaine. It is premature to conclude that PKAII signaling is not involved in amphetamine responsiveness in mice, since mice contain two each of the RI and RII genes, and there is significant up-regulation of other R subunits in compensation for the lack of RIIβ during development (14).

The second phenotype seen in flies is a failure to sensitize to repeated cocaine exposures. In flies, sensitization to cocaine can be blocked by ectopic G-protein expression in dopamine and serotonin neurons in the central nervous system, indicating a crucial role for these neurons and this signaling pathway in sensitization (42). However, sensitization results in enhanced nerve cord responses to dopamine agonists (43), most simply interpreted as enhanced responsiveness in neurons postsynaptic to these neurons, analogous to observations in vertebrates (36). Determining whether the failure of pka-RIIEP(2)2162 to sensitize results from deficient cAMP signaling in the aminergic neurons themselves or in neurons postsynaptic to these neurons will be addressed by further studies.

Mice lacking RIIβ show a distinct phenotype of sensitizing more intensely than wild type mice to repeated amphetamine exposures (14). This observation clearly indicates an involvement for modulated PKA signaling in sensitization, but due to the complications of redundant genes and compensation mentioned above, more precise interpretations are not possible. Our results in flies indicate, however, involvement of PKA-RII in sensitization in a manner that any potential compensation by the remaining pka-RI gene cannot overcome.

Since pka-RIIEP(2)2162 lacks PKA-RII protein, the major gene product that mediates proper subcellular localization of PKA activity in cells, it is possible that abnormal redistribution of subcellular localization of PKA activity in the pka-RIEIEP(2)2162 could be partly responsible for the abnormal responsiveness to cocaine. Potentially relevant is the recent report that dopaminergic modulation of a voltage-gated Na⁺ current which is involved in cocaine sensitization in rat hippocampal neurons requires PKAII anchoring (44, 45).

Resistance of pka-RIIEP(2)2162 to Ethanol—We studied responses of pka-RIIEP(2)2162 to ethanol since a previous study indicates involvement of cAMP signaling in modulating sensitivity of Drosophila to ethanol (11). Mutations in amnesiac, rutabaga, and pka-C1, each thought to decrease cAMP levels or signaling, all show hypersensitivity to ethanol. Furthermore, the hypersensitivity of ann can be rescued by feeding of forskolin, an activator of adenylate cyclase (11). However, this study also indicated some complexity in the signaling pathways involved in these responses, since double mutant combinations of cAMP activating and repressing mutants gave paradoxical
responses. This complexity is most readily explained by proposing the existence of neuronal signaling pathways, in which given neurons can positively or negatively affect activity of other neurons in the circuitry.

In the present study, we found that pka-RIIEP(2)2162 flies show robust resistance to ethanol. This is most simply interpreted as indicating that responses to cocaine and ethanol are mediated through a common PKA-dependent signaling step. However, further study will be required to confirm this interpretation.

Determination of whether the resistance of pka-RIIEP(2)2162 to ethanol is consistent with the previous observations of ethanol hypersensitivity of cAMP signaling deficient strains of Drosophila (11) is complicated. Recall that pka-RIIEP(2)2162 shows both an increased basal level of PKA as assayed in the absence of cAMP and a reduced level in the presence of cAMP, yielding overall reduced induction of PKA activity by cAMP. Thus, the simplest explanation is that these flies should be hypersensitive to ethanol since they show reduced cAMP-inducible signaling. However, this simple explanation may not hold for several reasons. First, flies with selectively altered PKAII signaling may show very different responses to ethanol than flies with overall reductions in PKA signaling. Since the hedgehog and Notch mutants also suppress pka-RIIEP(2)2162, flies, since a weak rhythm lasts at least for the 2–3 days in DD conditions. This observation is similar to the phenotype of pka-C1 flies, which show obvious locomotor arrhythmicity but normal period protein oscillation (9). Our observation supports the view that PKA is involved in the locomotor output pathway and indicates that PKAII is a major player in this process.

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Type II PKA-deficient Drosophila

Type II cAMP-dependent Protein Kinase-deficient Drosophila Are Viable but Show Developmental, Circadian, and Drug Response Phenotypes
Sang Ki Park, Stacey A. Sedore, Claire Cronmiller and Jay Hirsh

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