Neuronal Polymorphism among Natural Alleles of a cGMP-Dependent Kinase Gene, foraging, in Drosophila

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Natural variation in neuronal excitability and connectivity has not been extensively studied. In Drosophila melanogaster, a naturally maintained genetic polymorphism at a cGMP-dependent protein kinase (PKG) gene, foraging (for), is associated with alternative food search strategies among the allelic variants Rover (for¹; higher PKG activity) and sitter (for²; lower PKG activity). We examined physiological and morphological variations in nervous systems of these allelic variants isolated from natural populations. Whole-cell current clamping revealed distinct excitability patterns, with spontaneous activities and excessive evoked firing in cultured sitter, but not Rover, neurons. Voltage-clamp examination demonstrated reduced voltage-dependent K⁺ currents in sitter neurons. Focal recordings from synapses at the larval neuromuscular junction demonstrated spontaneous activity and supernumerary discharges with increased transmitter release after nerve stimulation. Immunolabeling showed more diffuse motor axon terminal projections with increased ectopic nerve entry points in sitter larval muscles. The differences between the two natural alleles was enhanced in laboratory-induced mutant alleles of the for gene. The pervasive effects of the for-PKG on neuronal excitability, synaptic transmission, and nerve connectivity illustrate the magnitude of neuronal variability in Drosophila that can be attributed to a single gene. These findings establish the consequences in cellular function for natural variation in an isoform of PKG and suggest a role for natural selection in maintaining variation in neuronal properties.

Key words: Drosophila; foraging; neuronal polymorphism; natural variation; cultured neurons; neuromuscular junction; membrane excitability; K⁺ currents; cGMP-dependent protein kinase; focal recording; patch clamping

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ents, as well as in synaptic activities and nerve terminal morphology. These differences in neuronal phenotypes found in naturally occurring alleles were further enhanced in laboratory-induced mutant sitter alleles.

Parts of this paper have been published previously in abstract form (Renger et al., 1997).

**MATERIALS AND METHODS**

**Fly stocks.** The for strains used in this work have been described previously (de Belle et al., 1989; Osborne et al., 1997). We found that the naturally occurring all mutant sitter alleles display mild, but noticeable, electro-induced leg-shaking behaviors that were clearly distinguishable from the ShD locus (Zhao et al., 1995). The ShD transgenic lines used in this study were generated by P-element insertion of a vector containing ShD cDNA fused to a hsp-70 promoter into the first, second, or third chromosome in the Sh genetic background (Zhao et al., 1995).

All stocks were grown on standard medium and were raised at 20–22°C. The cell culture and whole-cell recording. The procedure for culturing Drosophila “giant” neurons and the whole-cell patch-clamp technique have been reported previously (Wu et al., 1990; Saito and Wu, 1991; Zhao and Wu, 1997; Yao and Wu, 1999a). Briefly, gastrulae embryos were homogenized and suspended in Schneider medium (Life Technologies, Grand Island, NY) supplemented with 200 ng/ml insulin (Sigma, St. Louis, MO), 20% fetal bovine serum (100 μg/ml streptomycin and 50 U/ml penicillin. After washing, cells were resuspended in the above medium containing 2 μg/ml cytochalasin B (Sigma) and plated on glass coverslips. Cultures were maintained in humidified chambers at room temperature for 2–3 d before recording. Recording bath solution (Jan and Jan, 1976a) contained (in mM): 128 NaCl, 2 KCl, 4 MgCl₂, 1.8 CaCl₂, and 35.5 sucrose, buffered with 5 HEPES at pH 7.1. Patch pipettes were filled with solution containing (in mM): 144 KCl, 1 MgCl₂, 0.5 CaCl₂, and 5 EGTA, buffered with 10 HEPES, pH 7.1. K⁺ currents were isolated by adding tetrodotoxin (TTX) (0.2 μm) and Cd²⁺ (0.2 mm) to the bath solution. In pharmacological experiments that test the PKG modulation on K⁺ currents, the PKG inhibitor guanosine 3',5'-cyclic monophosphorothioate, 8-(4-chloro-phenylthio)-Rp-isomer (Rp-8-pCPT-cGMPs) (Calbiochem, La Jolla, CA), was applied to the bath after control recordings. All recordings were obtained at room temperature from isolated neurons with a patch-clamp amplifier (Axopatch 1B; Axon Instruments, Foster City, CA). Data acquisition and analysis were performed using pClamp software (Axon Instruments), and continuous data were stored on a frequency modulation tape recorder (Store 4D; Lockheed Electronics, Plainfield, NJ).

**Heat-shock protocol for ShD current induction.** Expression of transgenic ShD channels in cultured ShM host neurons was induced by exposure to 38.5°C for 30 min (Zhao et al., 1995). For isolation of the heat shock-dependent ShD currents, a twin-pulse protocol was used to extract the fast inactivating (τ < 20 msec), slow recovering (τfast = 5 sec) A-type ShD current by subtracting the currents elicited by the second pulse from that elicited by the first pulse delivered 1 sec apart (Zhao et al., 1995).

**Extracellular focal recording.** Postfeeding third instar larvae were dissected in Ca²⁺-free bath saline (see above). The neuromuscular junction was visualized with differential interference contrast optics through a 40× water immersion objective on an upright compound microscope (Zeiss, Jena, Germany). Focal recording electrodes were pulled from glass capillary tubes (75 μl, 1.5 mm outer diameter; VWR Scientific, West Chester, PA) on a pipette puller (model pp-85; Narishige, Tokyo, Japan) and then polished and bent on a microforge (model de fonbrune; Lockheed Electronics, Plainfield, NJ). Focal electrodes had an inner diameter from 4 to 8 μm and were filled with extracellular solution. Boutons recorded were from type 1 terminal arborizations of larval leg segment 3 (Zhong et al., 1992; Wang et al., 1994). The segmental nerves were stimulated at the cut end with a suction electrode (5–10 μm inner diameter). Focal recordings (Dudel, 1977) were made with a loose patch-clamp amplifier (model 8510; Zeitz Instruments, Munich, Germany) and stored on video cassette recording tapes with a pulse code modulator (Neuro-Corder DR-384; Neuro Data, New York, NY). Seal resistances were determined to correct for attenuation of synaptic current amplitude caused by leakage at the pipette tip (Stühmer et al., 1983; Renger, 1997). Data analysis was performed with the software Axograph (versions 2.0 and 3.0; Axon Instruments), and for presentation, some traces were digitally filtered at 1 kHz.

**Confocal microscopy.** The procedures for immunostaining and confocal microscopy have been described previously (Wang et al., 1994). Postfeeding third instar larvae were dissected in Ca²⁺-free saline and immediately immersed in Bouin’s fixative. Preparations were washed in 0.1 m phosphate buffer, pH 7.2, with 0.2% Triton X-100 (PBT) for 20 min, three to five repetitions. Rabbit anti-HRP conjugated to FITC (Cappel, Durham, NC) was applied at 1:100 in PBT to preparations overnight in a humidified chamber and were washed again in PBT for three repetitions of 20 min each. Preparations were then mounted on glass slides in Vectashield H-1000 (Vector Laboratories, Burlingame, CA). Confocal scanning laser microscopy was performed on a Bio-Rad (Hercules, CA) MRC model 600 or 1200 in conjunction with an upright microscope equipped with a 60× oil immersion lens. Serial images were overlaid using the accompanying Bio-Rad software.

**RESULTS**

**Neuronal firing properties and K⁺ current amplitude in natural and mutant for alleles.**

We examined neuronal phenotypes of the two naturally occurring alleles, for⁸ and for⁹, as well as a number of sitter mutant alleles of the for locus (Osborne et al., 1997). The giant neuron culture system was used to examine the extent of phenotypic variation in neuronal membrane properties between Rover and sitter neurons. A hallmark of neurons cultured from sitter variants, with reduced PKG activity, was membrane hyperexcitability. Spontaneous nerve firing occurring in the absence of stimulation was observed in 36% of for⁸ neurons examined (Fig. la). In addition, supernumerary, aftershock nerve spikes were evident after cessation of current injections in 18% of for⁸ neurons (Fig. 1b). Significantly, such events were absent in all for⁹ neurons examined (Fig. 1). The mutant allele for⁸, induced on a for⁹ genetic background, has a significantly lower PKG enzyme activity level than the natural for allele (Osborne et al., 1997). This mutant allele displayed even greater hyperexcitability than the naturally occurring sitter for allele. Spontaneous and supernumerary action potentials were found in 42% of for⁹ neurons (Fig. 1).

For an initial exploration of the ionic basis underlying the contrast in membrane excitability, we undertook voltage-clamp studies of voltage-activated outward K⁺ currents, which could be readily measured in cultured giant neurons. We found striking differences in K⁺ currents between Rover and sitter neurons. Inward Na⁺ currents were eliminated by TTX and inward Ca²⁺ currents and outward Ca²⁺-activated K⁺ currents were abolished by Cd²⁺ added to the saline (Saito and Wu, 1991; Zhao et al., 1995; Yao and Wu, 1999a). Depolarizing voltage pulses from a holding potential of −80 mV were used to elicit voltage-activated K⁺ currents, which demonstrate a transient peak of fast-inactivating current, followed by a sustained current plateau (Fig. 2a). Clear differences in both components of the K⁺ current were apparent between the Rover and sitter neurons (Fig. 2a,b). The more excitable for⁸ neurons demonstrated significantly lower levels of both peak and sustained outward currents compared with for⁹. A more striking contrast in neuronal phenotypes was found when the natural for⁸ allele was compared with the mutant allele for⁸ [maximum peak and sustained conductances in for⁸: 523.9 ± 51.9 and 254.4 ± 28.8 pS/pF (mean ± SEM; n = 29); for⁹: 410.0 ± 41.5 and 205.6 ± 20.2 pS/pF (n = 27); p < 0.05; for⁸: 347.0 ± 37.2 and 159.0 ± 13.2 pS/pF (n = 24); p < 0.05] (Fig. 2b).

The half-activation voltages of the peak (but not sustained) K⁺ currents in for⁸ and for⁹ also shifted significantly toward positive potentials compared with for⁹ (see half-activation voltages V1/2 in Fig. 2 legend). The slopes of voltage-dependent activation, however, were similar among the three alleles for both the peak and sustained currents (see Vslope in Fig. 2 legend).
that identified Sh channels are sensitive to Rp-8-pCPT-cGMPS modulation, we used ShD transgenic lines in which a fast-inactivating, slow-recovering Sh current can be readily induced by heat shock (Zhao et al., 1995). The extremely slow recovery kinetics allowed extraction of the ShD currents by a twin-pulse protocol (see Materials and Methods). We found that ShD currents were significantly inhibited by Rp-8-pCPT-cGMPS (Fig. 2e,f). The above results suggest that the Sh product may be a major target for PKG modulation.

**Figure 1.** Variations in neuronal firing properties observed among alleles of the for gene, which encodes a PKG in *Drosophila*. a. Spontaneous bursts of action potentials in the absence of stimulation occurred in sitter (for\(^{R}\) and for\(^{S}\)) neurons but not in Rover (for\(^{R}\)) neurons. b. Aftershock supernumerary spike activity, after the cessation of current injection pulses, appeared in neurons of the naturally occurring for\(^{R}\) allele and was more extreme in the for\(^{S}\) mutant neurons. c. Comparison of spontaneous and aftershock supernumerary nerve activities among different alleles. Spontaneous action potentials occurred in for\(^{R}\) (n = 4 of 11) and for\(^{S}\) (n = 5 of 12) but not in for\(^{R}\) (n = 0 of 15) neurons. Current injections evoked supernumerary action potentials that outlasted the current pulse in for\(^{S}\) (n = 2 of 11) and for\(^{S}\) (n = 5 of 12) but not in for\(^{R}\) (n = 0 of 15) neurons. All recordings were made under whole-cell current-clamp conditions at rest (−50 to −60 mV). Current injection was 60 pA. Only cells capable of firing all-or-none spikes were included in the analysis.

Voltage-activated K\(^{+}\) currents in *Drosophila* are encoded by several related genes, including Sh, Shal, Shab, and Shaw, generating currents with distinct kinetics and voltage dependence when expressed in heterologous systems (Butler et al., 1989). Available mutants and transgenic lines of the Sh gene enabled an examination of PKG modulation on Sh channels. A membrane-permeant PKG antagonist, Rp-8-pCPT-cGMPS (10 μM), was found to suppress both the peak and sustained K\(^{+}\) currents in Rover (Fig. 2c) and other laboratory wild-type strains (data not shown). Significantly, neurons of a null allele, Sh\(^{−}\), showed a markedly reduced sensitivity to this inhibitor (Fig. 2d,f). To further confirm
Reduced voltage-activated K\(^+\) currents in neurons of for\(^R\) and for\(^s\) and altered PKG modulation of Sh currents. 

**a**, Both transient and sustained outward K\(^+\) currents were attenuated in for\(^R\) and for\(^s\) compared with for\(^R\). The voltage-activated K\(^+\) currents were elicited by depolarization steps (950 msec) from a holding potential of −80 mV to voltages between −60 and +60 mV in 20 mV increments. 

**b**, G–V curves of the peak and sustained K\(^+\) currents for the three for allelic variants. 

The membrane conductance (in picosiemens per picofarads; mean ± SEM) was obtained using the formula $G = I/(V - V_0)$, where $I$ is the current density and $V$ the reversal potential of the K\(^+\) current (−75 mV). The current density (in picoamperes per picofarads) was determined by normalizing the K\(^+\) current to membrane capacitance. The conductance was fit to the Boltzmann relationship $G = G_{\text{m}}/(1 + \exp((V - V_0)/V_{\text{m}1/2}))$, where $G_{\text{m}}$, $V_{\text{m}1/2}$, and $V_{\text{m}1/2}$ are the maximum conductance, half-activation voltage, and limiting slope, respectively. 

**c**, The peak and sustained currents were significantly larger in Rover than the sitter alleles ($p < 0.05$; see Results). $V_{\text{m}1/2}$ of the peak K\(^+\) currents in for\(^R\) and for\(^s\) shifted significantly toward positive potentials compared with for\(^R\) (for\(^R\), $-6.22 ± 1.71$ mV; for\(^s\), $-0.34 ± 1.88$ mV; $p < 0.01$; for\(^s\), $-0.17 ± 2.07$ mV; $p < 0.01$). 

**d**, $V_{\text{m}1/2}$ of the sustained K\(^+\) currents was not significantly different ($p > 0.05$) among the three alleles (for\(^R\), $1.75 ± 1.41$ mV; for\(^s\), $3.55 ± 2.41$ mV; for\(^s\), $2.63 ± 2.11$ mV). However, $V_{\text{m}1/2}$ of the sustained K\(^+\) currents was not significantly different ($p > 0.05$) among the three alleles (for\(^R\), $14.23 ± 0.85$ and $13.26 ± 0.8$ mV/e-fold; for\(^s\), $12.66 ± 0.45$ and $13.11 ± 0.64$ mV/e-fold; for\(^s\), $13.3 ± 0.64$ and $14.6 ± 1.2$ mV/e-fold for peak and sustained K\(^+\) currents, respectively). 

**e**, Modulation of Sh K\(^+\) currents by Rp-8-pCPT-cGMPS, a PKG inhibitor. Addition of 10 μM Rp-8-pCPT-cGMPS to the bath resulted in suppression of voltage-activated K\(^+\) currents (compare traces 1 and 2 in each panel). 

**f**, Fraction of remaining transient and sustained currents after 10 μM Rp-8-pCPT-cGMPS treatment. All data are mean ± SEM for the number of cells indicated in parentheses. 

DISCUSSION 

This report demonstrates that a natural polymorphism of a single gene in wild populations can generate considerable variations in basic neuronal properties and that electrophysiology provides a sensitive probe to detect allelic variation. Natural variation of the foraging gene results in discrete patterns of food-searching behaviors, indicating that natural selection has acted to fine-tune the activity of for-PKG in response to the ecological and evolutionary history of Drosophila populations. Laboratory-induced mutant alleles of the for locus revealed extreme phenotypes in neuronal excitability and connectivity. This raises several questions about the developmental and functional roles of for-PKG and the mechanism by which it gives rise to the behavioral polymorphism.
PKG isoforms and expression patterns

The upstream signaling systems that regulate enzymatic activity levels of PKG may include both nitric oxide (NO)-dependent and -independent mechanisms, which are mediated by the soluble and membrane-bound guanylyl cyclase (GC), respectively (Koesling et al., 1991; Garbers, 1992; Simpson et al., 1999).

Figure 3. Focal loose patch-clamp recordings from individual synaptic boutons in larval neuromuscular junctions of different for alleles. a. Spontaneous ejcs in the absence of nerve stimulation. In the naturally occurring Rover (for\textsuperscript{R}) allele, only spontaneous miniature ejcs were seen. In contrast, the natural (for\textsuperscript{S}) and mutant sitter strains displayed spontaneous ejcs (0.5 mM Ca\textsuperscript{2+}). In the transgenic rescue line dg2-cDNA in which four copies of the for\textsuperscript{R} version of dg2 are placed in the for\textsuperscript{S} background (Osborne et al., 1997), the larger spontaneous ejcs were suppressed. b. Supernumerary ejcs after nerve stimulation (filled dots) in saline containing 0.5 mM Ca\textsuperscript{2+}. Supernumerary discharges after the enhanced initial release were most extreme in the sitter mutant for\textsuperscript{s92} but were absent in the for\textsuperscript{R} and transgenic dg2-cDNA lines. c. Supernumerary ejcs in the sitter allele for\textsuperscript{s92} could be suppressed by reducing membrane excitability with subthreshold doses (10 nM) of TTX. [Ca\textsuperscript{2+}], 0.2 mM. d. Occurrence of ejcs was determined in a 125 msec poststimulus period (left; n = 50 or 100 trails, 1 Hz, 0.5 mM Ca\textsuperscript{2+}). Number of ejcs per trial period (mean ± SEM) are shown. Shaded bars indicate excess ejcs with respect to the ejcs time-locked to the stimulus (unshaded bars). No supernumerary ejcs in excess of the time-locked ejc was observed except for R, whereas for\textsuperscript{s92} had the most supernumerary ejcs among all sitter alleles. The amplitude and number of supernumerary ejcs were restored in transgenic rescue line dg2-cDNA to a level near that of for\textsuperscript{R}. The mean ± SEM amplitude of the time-locked ejcs for each genotype are shown in the right (filled bars). One-way ANOVA shows that the for\textsuperscript{R} strain had significantly lower mean amplitude than did for\textsuperscript{S}, for\textsuperscript{s189Y}, for\textsuperscript{s}, and for\textsuperscript{s92} (Student–Neuman–Keuls test groupings: *p < 0.05).

Figure 4. Neuronal polymorphism in synaptic terminal morphology among Rover and sitter alleles. a. Immunohistochemically stained third instar larval neuromuscular junctions. Anti-horse radish peroxidase staining demonstrated that Rover larvae displayed stereotypical branching patterns in muscles 12 and 13 of abdominal segment 3, similar to those previously described. However, larval muscles of the natural for\textsuperscript{R} and mutant for\textsuperscript{s2} alleles contained ectopic nerve entry points (open arrows) associated with atypical branches that deviated from the usually restricted single nerve entry points (open triangles). b. Bar graph comparing the occurrence of ectopic nerve entry points found within the neuromuscular junction in sitter and Rover larvae. Occurrences of ectopic nerve entry points (mean ± SD) found in muscles 12 and 13 of the third abdominal hemisegment were determined in the number of larvae indicated. One-way ANOVA showed significant differences (F(3,24) = 8.72; p < 0.0004) in the mean number of nerve entry points (Student–Neuman–Keuls test groupings: *p < 0.05) with for\textsuperscript{R} having fewer nerve entry points than all of the sitter strains, for\textsuperscript{S}, for\textsuperscript{s189Y}, and for\textsuperscript{s92}.

PKG isoforms and expression patterns

The upstream signaling systems that regulate enzymatic activity levels of PKG may include both nitric oxide (NO)-dependent and -independent mechanisms, which are mediated by the soluble and the membrane-bound guanylyl cyclase (GC), respectively (Koesling et al., 1991; Garbers, 1992; Simpson et al., 1999).
K⁺ channels as potential targets for PKG modulation

The influence of PKG on neuronal excitability (Fig. 1) is likely mediated by modulation of downstream targets, e.g., ion channels. Several reports have described modulation of different K⁺ channels by PKG, including an inward rectifier (Kubokawa et al., 1998) and a Ca²⁺-activated maxi-K (Alioua et al., 1998) channel. However, mutations of the slo gene, which encodes a Ca²⁺-activated K⁺ channel subunit (Atkinson et al., 1991) mediating maxi-K⁺-like outward currents in Drosophila (Komatsu et al., 1990), do not replicate the sitter phenotypes but predominantly affect action potential duration (Saito and Wu, 1991), which does not vary among different for alleles.

Among different ionic currents in Drosophila neurons, the role of voltage-activated K⁺ currents in excitability patterns has been more extensively characterized in K⁺ channel mutants in Drosophila, allowing a comparison with those seen in different for alleles. Interestingly, the hyperexcitability patterns in the sitter alleles do not completely coincide with any of the K⁺ channel subunit mutants known to affect neuronal firing patterns, including Hk, eag, and Sh (Saito and Wu, 1991, 1993; Yao et al., 1998; Yao and Wu, 1999a). Nevertheless, some aspects of the K⁺ channel mutant phenotypes appeared to correspond to those of the hyperexcitability in sitter alleles. For example, the spontaneous bursting activity observed in sitter neurons is distinct from the rhythmic firings in Hk and eag neurons (Yao et al., 1998; Yao and Wu, 1999a) but resembles the spontaneous bursting in some Sh neurons (Yao and Wu, 1999a). Upon current injection, the supernumerary spikes can be seen in a substantial portion of eag neurons (Yao et al., 1998), reminiscent to those seen in sitter neurons. Our results demonstrate variation in voltage-activated K⁺ currents among different for alleles and suggest Sh subunits to be a major target for PKG modulation (Fig. 2). However, a role of other K⁺ channel subunits in PKG signaling cannot be ruled out (cf. Zhong and Wu, 1993).

Rover and sitter larvae are distinct mainly in food-searching behaviors. Differences in their locomotion patterns are evident when traversing yeast-covered surfaces containing the food stimulus (Sokolowski and Riedl, 1999). In contrast, compared with wild type, all the above K⁺ channel mutant larvae show differences in locomotion pattern on agar-covered surfaces lacking the food stimulus (Wang et al., 1997; J. Wang and C.-F. Wu, unpublished observations). It is likely that regulation of behavior depends on fine-tuning of multiple K⁺ channel types through complex mechanisms. In addition, inward Na⁺ and Ca²⁺ currents play important roles in action potential generation. The potential modulatory effects of PKG on these currents need to be further investigated in Drosophila neurons.

Synaptic transmission, nerve connectivity, and the evolution of signaling pathways

Our data indicate that for-PKG regulates not only neuronal excitability but also synaptic transmission and nerve connectivity, which take part in regulating behavioral expressions. The spontaneous ejcs and evoked supernumery afterdischarges in sitter alleles are likely caused by increased motor axon excitability (Fig. 3). However, the increased evoked transmitter release in the time-locked ejcs of sitter alleles indicates variation in transmitter release machinery per se. An NO-mediated cGMP cascade can influence long-term potentiation (Zhuo et al., 1994) and depression (J. Wu et al., 1998), and capsaicin-dependent enhancement of neurotransmitter release has been linked to PKG-dependent processes (Sluka and Willis, 1998) in vertebrate preparations. Indeed, variations in activity-dependent synaptic efficacy was found in the pair-pulse paradigm among for alleles (Renger, 1997).

The profound effects of allelic variation on the for-PKG activity are reminiscent of changes in neuronal and behavioral plasticity by mutational perturbations of the cAMP (Zhong and Wu, 1991; Zhong et al., 1992; Dubnau and Tully, 1998) and Ca²⁺/calmodulin-dependent protein kinase II (CaMK) (Griffith et al., 1994; Wang et al., 1994; Yao and Wu, 1999b) in cascades in Drosophila. In general, disruptions of both cAMP and CaMK pathways also alter neuronal excitability, synaptic transmission and facilitation, motor axon terminal projection, and behavioral plasticity. However, unique neuronal phenotypes result from genetic manipulations of each of the three pathways. For example, spontaneous activity in dissociated neurons and at the larval neuromuscular junction is far more striking in sitter mutants than in mutants defective of the cAMP or CaMK II cascade (Zhong and Wu, 1991; Wang et al., 1994; Zhao and Wu, 1997). The ectopic entry points of the motor neuron projection in sitter alleles are distinct from the increased nerve terminal branching in the cAMP cascade mutant, dnc, which displays ramifications of higher order branches (Zhong et al., 1992). Thus, the three genetically separable signaling pathways might differentially regulate distinct functional aspects of activity-dependent neuronal properties that are relevant to specific behavioral tasks, e.g., foraging, learning, and memory (C.-F. Wu et al., 1998).
REFERENCES


Basel: Birkhauser.


Yao W-D, Wu C-F (1999b) Regulation of firing pattern through modu-


