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研究论文

机械分离的果蝇幼虫中枢神经元全细胞钾电流的特性

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摘　要：　培养的果蝇胚胎及幼虫中枢神经元已被广泛用于细胞膜离子通道、突触传递和胞内信使调节等电生理学研究。在本实验中，利用机械震荡分离方法获得了大量的果蝇幼虫中枢神经元，其中大部分为Ⅱ型神经元。运用膜片钳技术，鉴定了Ⅱ型神经元上五种具有不同动力学特性的全细胞钾电流。其中E型电流表型表现出与其它四种电流完全不同的‘钟形’激活特性。进一步的研究还表明该类型电流具有明显的钙依赖性，而且它具有与其它四种电流不同的衰减特性。

关键词：果蝇；机械分离；Ⅱ型神经元；钾电流
学科分类号：Q42，Q71

Properties of whole-cell potassium currents in mechanically dissociated *Drosophila* larval central neurons

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Abstract:　By electrophysiological methods, cultured *Drosophila* embryonic and larval central neurons have been widely used to study ion channels, neurotransmitter release and intracellular message regulation. Voltage-activated K^+ channels play a crucial role in repolarizing the membrane following action potentials, stabilizing membrane potentials and shaping firing patterns of cells. In this study, a mechanical vibration-isolation system was used to produce a sufficient number of acutely dissociated larval central neurons, of which the majority were type Ⅱ neurons (2～5 μm in diameter). Using patch clamp technique, the whole-cell K^+ currents in type Ⅱ neurons were characterized by containing a transient 4-AP-sensitive current (I_A) and a more slowly inactivating, TEA-sensitive component (I_K). According to their kinetic properties, five types of whole-cell K^+ currents were identified. Type A current exhibited primarily fast transient K^+ currents that activated and inactivated rapidly. The majority of the neurons, however, slowly inactivated K^+ currents with variable inactivation time course (type B current). Type C current, being present in a small number of the cells, was mainly composed of noninactivating components. Some of the neurons expressed both transient and slow inactivating components, but the slowly inactivating components could reach more than 50% of the peak current (type D current). Type E current showed distinct voltage-dependent activation properties, characterized by its "bell-shaped" activation curve. Type E

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current was inhibited by application of Ca^{2+}-free solution or 0.1 mmol/L Cd^{2+}. Moreover, this novel current ran down much more rapidly than other types. These results indicate that different K^+ channels, which have different kinetic and pharmacological properties, underlie the whole-cell K^+ currents in type Ⅱ neurons of *Drosophila* larval central nervous system.

Key words: *Drosophila*; mechanical dissociation; type Ⅱ neuron; K^+ current

由于果蝇生活周期短、培养条件简单、成本低，成为遗传学研究的理想模型。同时，果蝇也是电生理研究的良好材料，如培养的果蝇神经元被广泛地应用于细胞膜离子通道、突触传递和胞内信使调节等研究中。大部分果蝇神经元电压门控钾电流包括快速失活的 A 型钾电流(I_A)和慢失活的延迟整流钾电流(I_K)，两者具有不同的激活、失活及药理学特性。I_A 具有快速激活和失活的特性，对 4-氨基吡啶(4-AP)敏感；I_K 则激活缓慢，不失活或缓慢失活，对四乙胺(TEA)敏感。

果蝇三龄幼虫中枢神经元根据大小可以分为三种类型[1]：Ⅰ型神经元直径大于 8 μm，Ⅱ型神经元直径在 25 μm 之间，大小介于两者之间的为Ⅲ型神经元[1]。目前有关果蝇幼虫Ⅲ型神经元的特性研究较多[1,2]，但对Ⅱ型神经元的功能知之甚少。在本实验中，我们利用膜片钳技术记录了机械分离的果蝇三龄幼虫中枢Ⅱ型神经元的全细胞钾电流，并着重对其中一类钙依赖性的 E 型电流加以分析。

1 材料和方法

1.1 果蝇品系 野生型果蝇北大 1 号(F.PH1)由北京阜外医院麻醉科刘进教授赠送，在玉米-酵母-琼脂培养基、恒温(2123℃)条件下培养。

1.2 溶液配制 去 Ca^{2+}-Mg^{2+} 溶液(mg/100 ml)：NaCl 800，KCl 20，NaH_2PO_4 5，$NaHCO_3$ 100，glucose 100；用 HCl 将 pH 调至 7.27.4。细胞外液(mmol/L)：NaCl 130，KCl 6，$CaCl_2$ 2.5，$MgCl_2$ 5，HEPES 5，glucose 10，用 Tris-base 将 pH 调至 7.27.4。电极内液(mmol/L)：KCl 140，$CaCl_2$ 0.5，$MgCl_2$ 2，EGTA 5，HEPES 5，用 Tris-base 将 pH 调至 7.27.4。鉴定 K^+ 电流时所用 CsCl 电极内液(mmol/L)：CsCl 130，$MgCl_2$ 4，HEPES 10，EGTA 10，pH 值用 Tris-base 调至 7.27.4。TEA 细胞外液(mmol/L)：NaCl 130，TEA 6，$CaCl_2$ 2.5，$MgCl_2$ 5，HEPES 5，pH 值用 Tris-base 调至 7.27.4。4-AP 细胞外液(mmol/L)：4-AP 5，NaCl 125，KCl 6，$CaCl_2$ 2.5，$MgCl_2$ 5，HEPES 5，用 Tris-base 将 pH 调至 7.27.4。无钙细胞外液(mmol/L)：NaCl 130，KCl 6，$MgCl_2$ 5，HEPES 5，glucose 10，用 Tris-base 将 pH 调至 7.27.4。0.1 mmol/L $CdCl_2$ 溶液，在正常细胞外液中加入 0.1 mmol/L $CdCl_2$。所有化学试剂均购自 Sigma 公司。

1.3 神经元分离和电生理记录 神经元的分离采用 Wu 等[3]的方法，简言之，将果蝇三龄幼虫的脑和腹神经节在去 Ca^{2+}-Mg^{2+} 溶液中分离后置于盛有 3 ml 果蝇细胞外液的培养皿中，在低频振荡器下分离细胞。分离后的细胞在培养皿中静置 10 min 使之贴壁，然后在倒置相差显微镜下观察，挑选Ⅱ型神经元进行膜片钳记录。果蝇Ⅱ型神经元胞体较小，当电极尖端接触胞膜，无须施加负压或略加负压，便可形成巨阻封接(> 1 GΩ)。电极由电极拉制仪(Narishige，Japan)两步拉制而成，外径约 1.21.5 mm，尖端电阻 79 MΩ。数据由 Axopatch 200B 膜片钳放大器(Axon Instruments，Foster city，CA)采集，采样频率为 2 kHz，2 kHz 低通滤波。利用 Clampfit 软件(Version 8.1，Axon Instruments，Foster City，CA)进行数据处理和分析。在实验过程中对电极电容、膜电容、串联电阻均作了补偿。钳制电位为 − 70 mV。施药沿用 Y 管加药系统[4]，施药管管口距细胞约 50-100 μm，该系统换药时间可达 20 ms。细胞分离以及膜片钳记录均在 25℃室温下进行。

2 结果

同酶解所得幼虫神经元[1]类似，机械分离的果蝇三龄幼虫中枢神经元按大小也可分为Ⅰ型、Ⅱ型、Ⅲ型三类神经元(图 1)。但是，用机械分离方法得到的神经元中，Ⅱ型神经元占多数(约 75%)，其直径在 25 μm 之间[3]。我们挑选在相差显微镜下周围光滑、明亮、有立体感的Ⅱ型细胞作膜片钳记录。

2.1 Ⅱ型神经元全细胞钾电流表型

用膜片钳记录的果蝇中枢神经元的全细胞电流几乎均为外向钾电流[5-7]，即使在电极内液和细胞外液中分别加入 Cs^+ 和 TEA 以阻断钾电流，也无明显的内向钠和钙电流。我们所记录的 94 例细胞中，

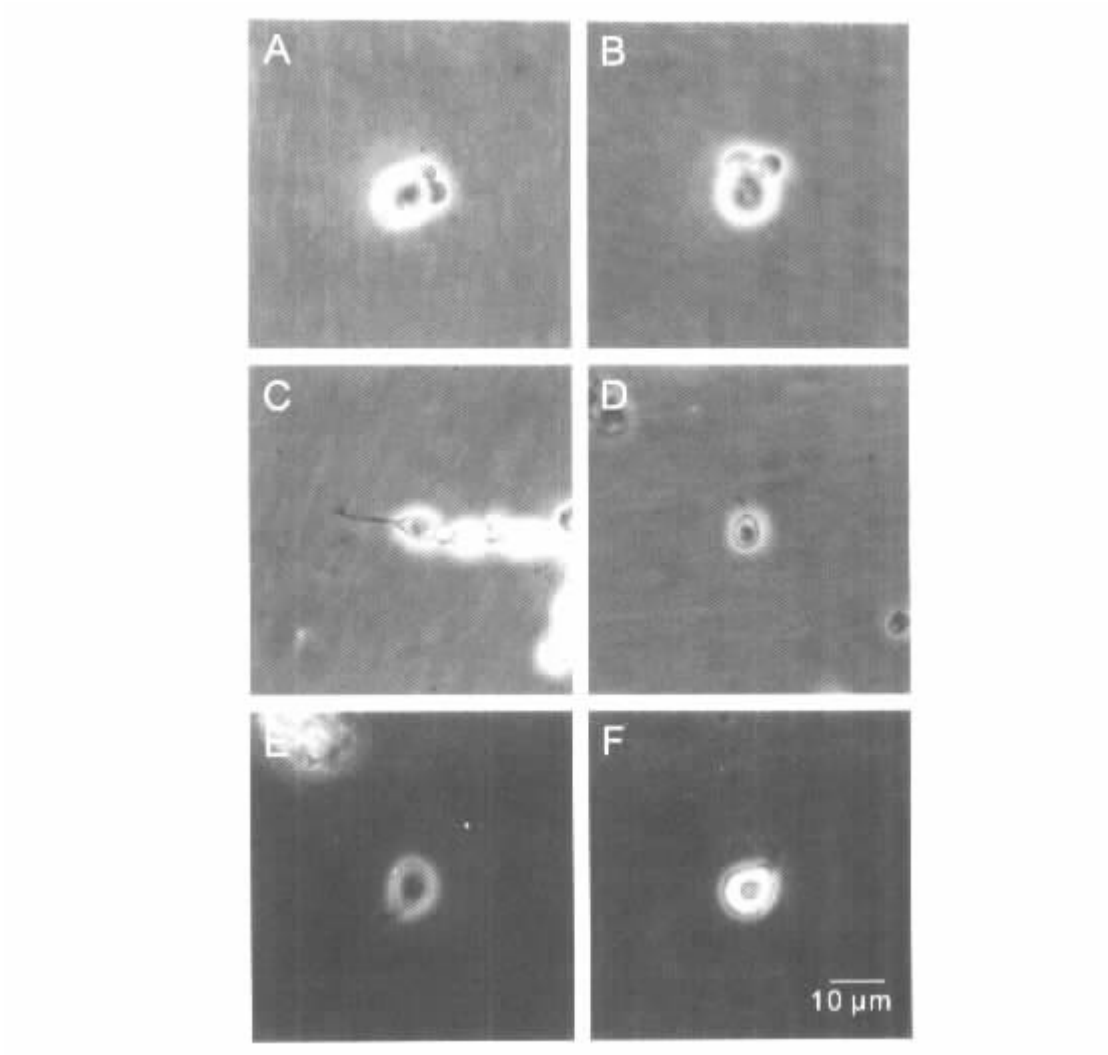

图　1.　机械分离的果蝇幼虫中枢神经元

Fig. 1.　Neurons mechanically dissociated from *Drosophila* larval CNS. The cells could be divided into three types according to the size. *AB*: Type Ⅰ consisted of neuroblast-like large cells and most of them were accompanied with smaller cells. *CD*: Type Ⅱ small cells comprised the largest population. *EF*: Type Ⅲ cells were intermediate-sized. Some of them were oval- or spindle-shaped with short processes. All images were taken at half to 1 h after dissociation.

均只观察到外向电流。这种外向电流主要包括快速失活 I_A 的和几乎不失活的 I_K，进一步药理学实验表明 I_A 可被 4-AP 特异性地阻断，而 I_K 则主要对 TEA 敏感。按不同神经元中 I_A 及 I_K 所占比例不同，即动力学特性不同，将电流表型分为 5 类(图 2)：A 型($n = 25$)，I_A 为主要成分，I_K 较小，电流的激活和失活均较快；B 型($n = 39$)，I_A、I_K 均较大，有较大的失活时间常数，但激活时间常数在不同细胞间相差较大；C 型($n = 4$)，I_K 为主要成分，几乎不含有 I_A；D 型($n = 12$)，I_K 相对较大，可达峰电流的 50%以上；E 型($n = 14$)，具有与上述不同的电压依赖性，到达峰值电压约为 30 mV，而其它 4 种电流表型峰值激活在 60 mV 以上(图 3)。

2.2 全细胞钾电流不同的衰减(run-down)特性

Ⅱ型神经元的 E 型电流具有不同其它几种类型电流的衰减特征。通过 50 min、并每隔 3 min 的间断性刺激的记录，发现 E 型电流衰减明显快于其它四型，其它四型之间则无明显差别。如图 4 所示，将各个时间点的电流峰值与首次记录峰值的比值作图，然后进行曲线拟合，可看出 E 型电流衰减明显加快，其电流衰减一半的时间为 3 min，而非 E 型电流为 18 min。

2.3 E 型钾电流的钙依赖特性

进一步实验使我们还观察到 E 型电流具有明显钙依赖特性。在细胞外液加入 0.1 mmol/L $CdCl_2$ 能可逆地阻断大部分 E 型电流。在无 Ca^{2+} 细胞外液中，E 型电流的幅度也明显减小(图 5)。

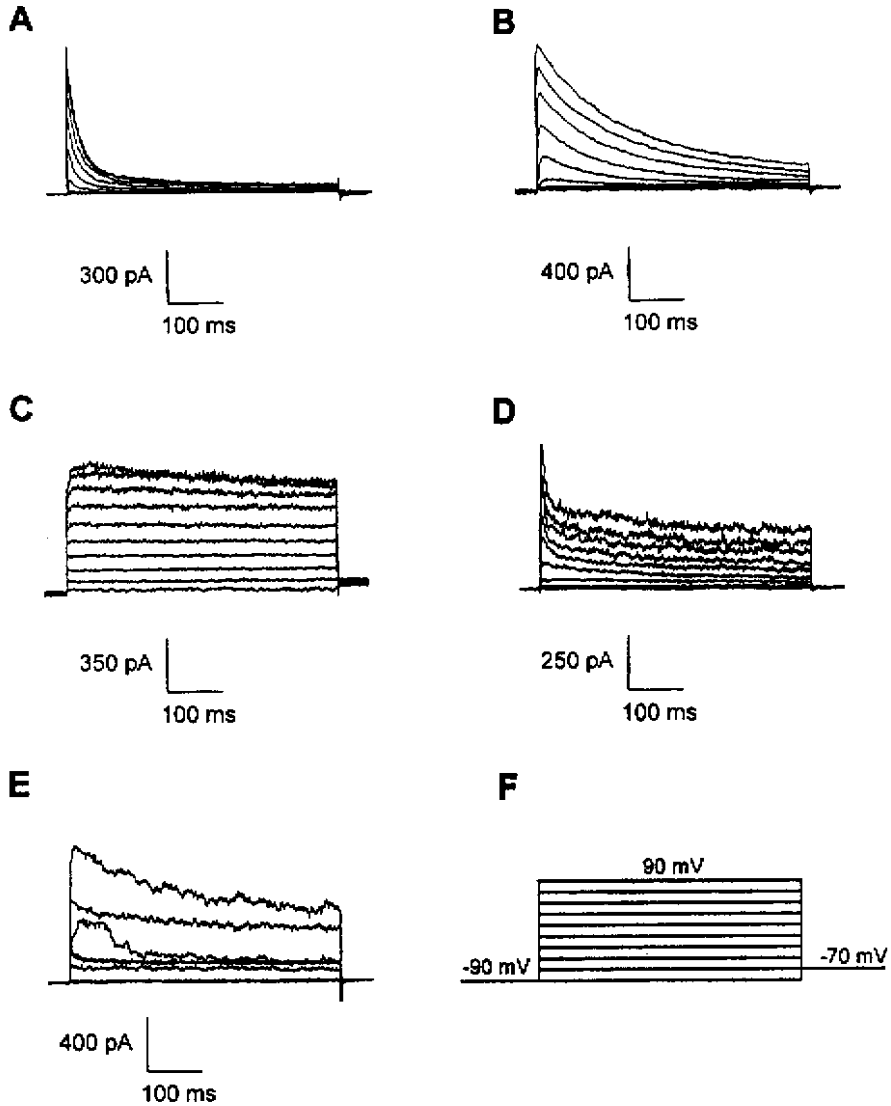

图 2. 果蝇幼虫Ⅱ型神经元的全细胞钾电流

Fig. 2. Whole-cell K^+ current phenotypes in acutely dissociated type Ⅱ neurons of *Drosophila* larval CNS. *AE*: Five types of K^+ current in type Ⅱ neurons. The two components, I_A and I_K, are different in the five types of K^+ currents. *F*: The membrane voltage was depolarized from a 200 ms prepulse voltage (V_P = −90 mV) to successively more depolarized command voltages between −90 and 90 mV at 20 mV steps. Holding potential was −70 mV. Command step duration was 500 ms.

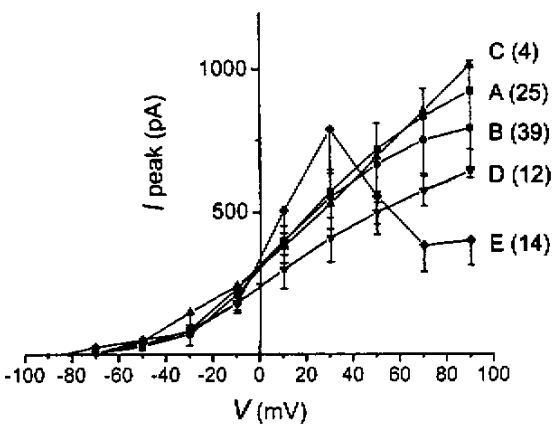

图 3. 五种类型全细胞钾电流的 $I-V$ 曲线

Fig. 3. The $I-V$ curves of the five types of K^+ currents. All of these phenotypes showed outward rectifier properties. However they had different voltage-dependence. The $I-V$ curves of the type E phenotype displayed bell shape. The activation voltage to peak current of type E current was about 30 mV while that of other type currents was over 60 mV. In this and following figures, each bar is the mean ± SD. Numbers in the parentheses indicate the sample size.

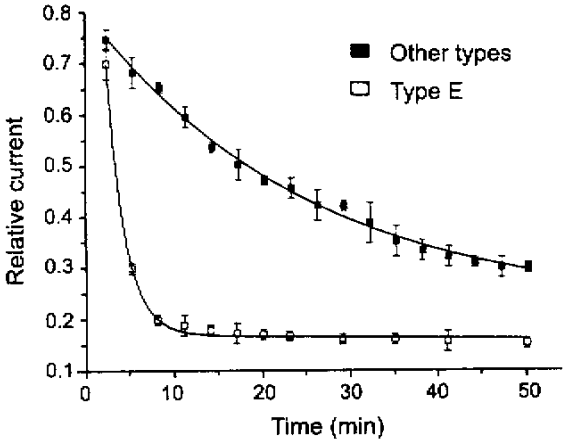

图 4. 全细胞钾电流的衰减特性

Fig. 4. The run-down properties of whole-cell K^+ currents. The type E currents (■, $n = 4$) exhibited a faster run-down than other types of currents (□, $n = 6$) with the recording time as long as 50 min. No obvious difference was shown between other types (data not shown). The time of whole-cell K^+ currents run-down by 50% of type E was 3 min, much shorter than that of other types (18 min).

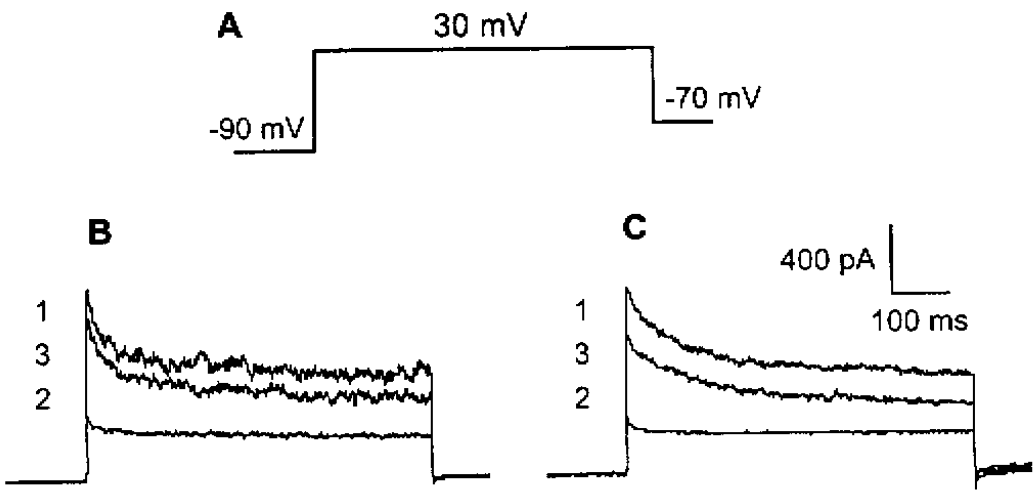

图 5. E 型钾电流的 Ca^{2+} 依赖特性

Fig. 5. Ca^{2+}-dependence of type E whole-cell K^+ currents. *A*: K^+ currents were activated by depolarizing to 30 mV for 500 ms, preceded by a 200 ms prepulse of −90 mV. Holding potential was −70 mV. K^+ currents were reversibly blocked by 0.1 mmol/L $CdCl_2$ (*B*) and Ca^{2+}-free solution (*C*). Traces 1, 2 and 3 stand for pre-treatment, treatment and post-treatment respectively.

3 讨论

机械性分离方法是一种经济、简便、有效的方法。与常规酶解和培养方法相比，该方法能避免酶对细胞表面蛋白，包括离子通道的损伤以及在培养环境下通道蛋白特性的改变。最近本实验室还利用此方法进一步获得了果蝇成虫中枢神经元，并初步研究了其全细胞钾电流特性[3]。

实验分离的果蝇三龄幼虫神经元中，Ⅱ型较多，且活性稳定。虽然Ⅱ型神经元在果蝇中枢神经系统占大多数，但有关其电生理学特性的研究尚未见系统报道。我们利用全细胞电压钳技术对Ⅱ型神经元的全细胞钾电流进行研究的结果表明，与酶解和培养方法得到的果蝇幼虫神经元电生理特性相似，Ⅱ型神经元的全细胞电流几乎均为外向钾电流，这种钾电流主要由稳态失活钾电流(I_A)和延迟整流钾电流(I_K)两种成分组成，两者具有不同的动力学特征。根据两种电流的相对组成，我们将Ⅱ型神经元的全细胞钾电流主要分为 5 种电流表型，其中 E 型电流含有钙依赖性钾电流，其激活及衰减特性均不同于其它 4 种表型。

编码果蝇中枢神经元电压门控钾通道 α 亚基的基因包括 *Shaker*、*Shal*、*Shaw* 以及 *Shab*，编码果蝇钾通道 β 亚基为 *Hyperkinetic* 基因[8]。编码基因的多态性决定了钾电流表型的多态性。我们记录的Ⅱ型神经元钾电流表型多样，提示Ⅱ型神经元钾通道可能

由多种基因编码。由于钾电流的不同表型决定了神经元不同的发放特性[9,10]，具有不同钾电流表型的Ⅱ型神经元可能在果蝇中枢神经系统兴奋性中起到不同的作用。E 型电流在无 Ca^{2+} 细胞外液中明显减小，并可被 $CdCl_2$ 阻断，表明其可能含有钙激活钾电流成分。果蝇中钙激活钾通道由基因 *Slowpoke* 编码[11]，其羧基端具潜在调节功能的序列，表现激酶活性，可能是 ATP 结合位点，可通过磷酸化进行调节[11,12]。E 型电流的快速衰减可能就是由于电极液对细胞内液的冲洗所致，但其是否为钙激活钾电流还需进一步的电生理学、药理学证据。E 型电流的衰减特性不同于其它四型钾电流，表明Ⅱ型神经元的不同钾电流受胞内调节机制不同。

果蝇的基因测序已经完成[13]，随着后基因组时代的到来，分析其基因产物(如细胞膜离子通道)的结构和功能必将成为以后的研究重点。钾电流对神经系统的兴奋性有重要的调节作用，而Ⅱ型神经元在果蝇中枢神经系统中占有很大比例。因此，深入研究果蝇以及各突变体中Ⅱ型神经元钾电流电生理学特性，将进一步增进我们对果蝇乃至哺乳动物神经系统整体作用机制的了解。

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