

Exposure to Static Electric Fields Leads to Changes in Biogenic Amine Levels in the Brains of *Drosophila*

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With 4 Figures

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Abstract

Natural and anthropogenic static electric fields are commonly found in the environment and can have both beneficial and harmful effects on many animals. Here we asked how the fruit fly responds to these fields and what the consequences of exposure are on the levels of biogenic amines in the brain. When given a choice in a Y-tube bioassay *Drosophila* avoided electric fields, and the greater the field strength the more likely *Drosophila* were to avoid it. By comparing wild type flies, flies with wings surgically removed and vestigial winged flies we found that the presence of intact wings was necessary to produce avoidance behaviour. We also show that Coulomb forces produced by electric fields physically lift excised wings, with the smaller wings of males being raised by lower field strengths than larger female wings. An analysis of neurochemical changes in the brains showed that a suite of changes in biogenic amine levels occurs following chronic exposure. Taken together we conclude that physical movements of the wings are used by *Drosophila* in generating avoidance behaviour, and are accompanied by changes in the levels of amines in the brain that in turn impact on behaviour.

Introduction

Electric fields in the environment can have profound effects on the behaviour of plants and animals. Considerable attention has focused on insects, and we now know that static electric fields can have both beneficial and detrimental effects, as well as affecting the behavioural responses of a number of insects to these fields. Bees, for example, produce static electric fields during flying and walking caused by friction charging. The fields are sufficiently large enough to induce movements of the antennae of conspecifics nearby, which are thought to act as a biologically relevant stimulus that may play a role in social communication [1]. These fields may also alter the behaviour of the bee parasite *Varroa jacobsoni* that can assist attack by attraction of the parasite to the charged bee [2]. Other studies have suggested that accumulation of charge during flight could assist foraging and pollination through the transfer of pollen grains onto plant stigmata [3-5]. Static electric fields can also lead to changes in walking [6,7] and avoidance behaviour [8,6] and influence locomotion and agitation [9-11].

Recent studies have suggested that while insects have not evolved a specialized sense for detecting static electric fields, they can do so by virtue of the displacement of long slender structures such as the antennae or mechanosensory hairs caused by Coulomb forces [7]. Such displacements are sufficient to evoke neural activity and thus leads to avoidance behaviour, as has also been demonstrated in bees [1]. The attractive and repulsive forces generated by static electric fields have proportionately more influence on smaller insects than larger insects [9] and this has led to the development of electric screens to prevent entry of small insects into glasshouses [12].

Electromagnetic fields (EMFs) have been shown to alter the neurochemistry of the brains of rats, raising the levels of amine metabolites and dopamine in specific regions of the brain [13,14]. EMFs have also been shown to increase the levels of heat shock proteins, hsp's, that often increase in response to stress [15]. Taken together these results raise the question as to whether static electric fields could also alter the brain's neurochemistry in insects. Amines play a major role in the behaviour of insects; from underpinning extreme changes in phenotypic plasticity in locusts where dopamine and serotonin have a major role in driving the swarming form of migratory and desert locusts [16-18], to social interactions between nest mates influencing brain biogenic amine homeostasis in stressed ants [19,20]. They also play a crucial role in aggressive encounters between dominant and submissive animals [21].

For the model organism *Drosophila*, an understanding of the effect of electric fields

on physiology and behaviour is crucially important given the wide scale use of plastics in the rearing of these insects. Plastics are well known for their ability to both charge and retain a charge for long periods through triboelectrification [8,22] and chronic exposure to these fields during rearing is inevitable. Moreover, understanding whether static electric fields have meaning to *Drosophila* as an environmental stimulus in the context of spontaneous choice and learning is important to establish. This study therefore asks if static electric fields affect *Drosophila* behaviour, how they might be detected, and how they change the levels of biogenic amines in the brain.

Materials and Methods

Wild-type *Drosophila melanogaster* (Oregon-R) were obtained from Blades Biological Supplies Ltd. (UK) and reared on a yeast/sugar medium with added live baker's yeast [23] in 50 ml glass bottles at $20 \pm 1^\circ \text{C}$ and under a light : dark cycle of 16:8hr.

Responses to electric fields

A Y-tube consisting of three cylindrical glass chambers (2mm thick, 150 mm in length x 30mm inner diameter) fused together at 120° (Fig. 1A) was used to quantify the responses of *Drosophila* to static electric fields. Two copper rings, 5 x 28 mm (width x diameter), were positioned inside each arm 14 mm from the intersection with the vertical arm and attached to an insulated socket through a 7 mm hole in the surface (Fig. 1A). One copper ring was connected to a Brandenburg Alpha III power supply (Brandenburg, UK) and the other to ground. The ends of each arm were covered by two further tubes to capture insects as they passed through the Y-tube. One arm of a dual gooseneck cold light source (Schott KL 1500 LCD) was focused through each arm of the Y-tube to encourage upward, phototactic, movement of the flies. The vertical arm (C) was used as a release chamber and covered with an aluminium mesh (1mm square) connected to ground to prevent electric fields in this area. The Y-tube was secured by a metallic holder and placed inside a dark aluminium cage [7].

Groups of 20 flies (males and females) were placed into eight new tubes (50 ml) each containing a small piece of wet tissue to prevent desiccation. Flies were starved for 24 hr prior to experimentation to encourage locomotory behaviour. The stopper of the tube containing the flies was removed and the tube presented to the base of the Y-tube and flies allowed to move freely. Different voltages (0, 0.12, 0.25, 0.5, 1, 1.5, 2 and 3 kV) were applied to one arm of the Y-tube to test their effect on each group of flies. The responses of flies to each voltage were tested with 8 different tubes of flies ($n = 8$). After 5 min the distribution of the flies within each arm of the Y-tube was assessed and the number of flies remaining in the release arm was analysed using one-way ANOVA (SPSS software, v17). Flies were exposed to one voltage only. Preliminary studies showed that there was a constant number of flies remaining in the release arm at all voltages suggesting that the release arm was not affected by electric fields. The charged arm of the Y-tube was randomly switched between experiments to reduce the effects of experimental bias and after each experiment the apparatus was washed and dried.

Electric field modelling within the Y-tube apparatus

Maxwell SV two-dimensional software (Version 7, Ansoft Corporation, Pittsburgh, USA) was used to model electric fields within the Y-tube, based on the physical properties of the materials, their sizes and the applied voltages. This modelling provides accurate estimates of the magnitude of the electric fields within the Y-tube that can be correlated with the response of the flies at different field strengths [7]. In Maxwell SV the Y-tube was drawn as a simple 2D x-y model with a cross-section taken from each part of the Y-tube (Fig. 1B). The electric fields generated by different applied voltages (0, 0.25, 0.5, 1, 1.5, 2 and 3 kV) were modelled.

The role of the wings in detection and avoidance of static fields

To assess the role of the wings in the detection and avoidance of electric fields three groups of flies were exposed to static electric fields at different voltages (0, 0.5, 1, 2 and 3 kV) using the Y-tube apparatus. The flies included wild-type flies, cut wing flies in which the wings of wild-type flies were physically removed and vestigial winged mutants (Blades Biological Ltd., UK). Flies were grouped into 5 tubes (50 ml), each group consisting of 20 flies (males and females randomly selected). Flies were maintained in these tubes for 24hr to recover, with the addition of a small piece of wet tissue in the tube to prevent desiccation. Flies were allowed to move freely and the number in each arm of the Y-tube counted after 5 min and the experiment repeated 6 times for each fly group.

Wing displacement

To measure the direct effects of electric fields on the wings, flies were placed under a glass chamber (2 mm thick, 100 mm x 30 mm (L x D)) fixed in a metallic holder. A copper ring electrode (4 x 28 mm, W x D) was fixed at the entrance of the chamber and attached to a DC power supply (Brandenburg Alpha III, Brandenburg, UK) to generate a static field. Eight male and 8 female flies were tested individually. First, each living fly was fixed on a glass slide underneath the charged electrode using sticky paper (EasiStick' Traps, Fargro Ltd.) and then exposed to electric fields at different voltages. The fly was then killed using CO₂ and tested again. To measure wing displacement flies were photographed at each voltage using a Nikon digital camera (D80) and photographs analysed using Canvas X (ACD Systems Inc., USA) to calculate wing angle (the angle between a line that extended from the mid-point of the head to the wing hinge and another between the tip of the wing and the wing hinge). No difference was found

between live and dead flies (data not shown).

In addition, the wings of individual flies were excised and placed on a glass slide to measure the field strength required to raise the wing toward the charged electrode. After each trial images of the wing were collected using a compound microscope (Zeiss Axiophot) with a digital camera (Roper Scientific RTE/CCD-1300-y). The length and width of the wings were measured using MetaMorph v6 software (Universal Imaging Corporation, PA, USA).

Statistical Analysis

During all experiments the number of flies in the uncharged and charged arms of the Y-tube were counted and a response index (RI) calculated based on the number of flies in the uncharged arm minus the number of flies in the charged arm and divided by the total number of flies [24,25]. An RI value above zero indicated that the flies avoided the electric fields. Data was tested for normality and homogeneity and the statistical significance assessed using one-way ANOVA.

Measurement of biogenic amines

The levels of biogenic amines in entire heads of *Drosophila* exposed to static electric fields of 70kV/m were measured by collecting the heads of 10 flies following freezing in liquid nitrogen. The heads were homogenised in 50µl of ice-cold 0.1M perchloric acid containing 5ng of 3, 4-dihydroxybenzylamine. After centrifugation of the homogenate (0°C, 15000rpm, 30 min), 40µl of the supernatant was collected. Amines in the brain and heads were measured using high-performance liquid chromatography with electrochemical detection as described elsewhere [18, 19].

Results

Avoidance behaviour of flies

Static electric fields were applied to the electrode of one arm of the Y- tube to determine the behavioural responses of flies. Separate gender groups of wild-type flies were exposed to static electric fields generated at 0 to 3kV for 5 min ($n = 8$ trials at each voltage with 20 flies per trial), and the flies in the uncharged and charged arms recoded. The results showed that both male and female wild-type flies significantly avoided electric fields (ANOVA $F_{4, 25} = 6.64$, $p = 0.0009$ and $F_{4, 25} = 20.41$, $p < 0.0001$) after 5 min of exposure (Fig. 2A,B). *Post hoc* analysis showed that the threshold for avoidance occurred at 1 kV (field strength of 34-43 kV/m, Maxwell modelling). The mean RI at this voltage was 0.59 ± 0.06 in males and 0.52 ± 0.05 in females, compared to 0.07 ± 0.14 in males and -0.10 ± 0.10 in females at 0 kV. The avoidance behaviour was also exhibited at higher voltages (1.5 kV and 2 kV) (Fig. 2A,B). No statistically significant difference in avoidance behaviour was found between male and female flies (ANOVA $F_{1, 60} = 0.808$, $p = 0.37$). It should be noted that not all flies avoided the static electric fields even at higher applied voltages, and not all flies moved out of the release tube (see Materials and Methods).

Given that the wings have been shown to vibrate during exposure to fields [26] we asked whether the wings play a role in detection of electric fields. To determine their role in generating avoidance the wings of wild-type flies were cut close to the wing hinge. Analysis showed that the flies with cut wings ($n = 6$ trials with 20 flies for each trial) did not avoid electric fields even after application of 3 kV (ANOVA $F_{4, 25} = 1.21$, $p = 0.33$) (Fig. 2C). By contrast, vestigial winged flies ($n = 6$ trials with 20 flies for each trial) showed significant avoidance (Fig. 2D) when electric fields of 2 kV (field strength 52-104 kV/m) and 3kV (95-164 kV/m) were applied (ANOVA $F_{4, 25} = 4.42$, $p = 0.004$). The mean responses after 5 min at 0 kV and 2 kV were -0.14 ± 0.13 and 0.38 ± 0.12 , respectively. Raising the applied voltage to 3 kV also led to greater avoidance compared to control (RI of -0.14 ± 0.13 and 0.46 ± 0.08).

The role of the wings in the detection and avoidance of static electric fields

The movements of the wings were determined from flies fixed below a copper electrode. The wings of female and male flies ($n = 8$ for both groups) were displaced by static electric fields (ANOVA $F_{6, 49} = 73.56$, $p < 0.0001$ and $F_{6, 49} = 55.01$, $p < 0.0001$) with greater field strengths causing greater angular displacements of the wing (Fig. 3). *Post hoc* tests showed significant wing elevation at 2 kV (57-96 kV/m) and above in males compared

to controls (Students t-tests $p < 0.0001$ at 2, 3, 4 and 5kV), and at 3 kV (96-115 kV/m) and above in females (Students t-tests $p < 0.0001$ at 3, 4 and 5kV). There was also significantly greater elevation in males ($14.48 \pm 1.90^\circ$) than females ($20.44 \pm 4.20^\circ$) when 5 kV was applied (field strengths of 163-183 kV/m ($p = 0.0095$)).

To determine whether active movements contributed to wing elevation during exposure to electric fields the wings of dead male and female flies were also analysed. There were no statistical differences in the elevation angles between the dead and live flies. For example, the mean (\pm SEM) deflections of the wings of live male flies at 0, 2 and 5kV were $-26.4 \pm 0.99^\circ$, $-16.55 \pm 3.3^\circ$ and $32.9 \pm 4.55^\circ$, compared to $-27.8 \pm 1.55^\circ$, -18.54 ± 2.48 and $25.55 \pm 3.05^\circ$ for dead flies (Students t-tests, $p = 0.438$, $p = 0.559$ and $p = 0.21$).

The effect of static electric fields on excised wings

Excised wings were exposed individually to electric fields at different voltages to determine the threshold required to raise the wings. The results demonstrated that male excised wings ($n = 8$) were raised by application of 0.68 ± 0.05 kV (mean \pm SEM) corresponding to a modeled electric field strength of 28-38 kV/m compared to 1.06 ± 0.09 kV (mean \pm SEM) (38-48 kV/m) for excised wings of females. The differences between males and females were statistically significant (Students t-test, $p = 0.004$).

To determine whether the difference between the sexes may be related, in part, to the morphology of the wings, the length and width of excised wings of both males and females flies were measured. The length and width of the wings in males were significantly smaller than those of females, with a mean length in males of 1.29 ± 0.036 mm compared to 1.49 ± 0.036 mm in females, and a mean width of 0.89 ± 0.024 mm in males compared to 0.98 ± 0.022 mm in females (Students t-tests, $p = 0.0019$ and 0.012 , respectively).

Neurochemical changes in brain amines during exposure to static electric fields

The levels of four key amines in the brains of *Drosophila* exposed to static electric fields at 70kV/m for 4hr, 24 and 72hr were measured and compared to time-matched controls (Fig 4). Serotonin levels decreased only slightly following 4 hr exposure from 1.14 ± 0.21 (mean \pm SEM) to 0.93 ± 0.15 pmol/brain, but to significantly lower levels following 24hr exposure from 0.8 ± 0.05 to 0.173 ± 0.021 pmol/brain (Students t-test, $p < 0.0001$, d.f. = 17). After 72 hr exposure the levels of serotonin returned to control levels (0.89 ± 0.15 and 0.93 ± 0.015 pmol/brain, respectively, $p = 0.834$, d.f. = 17).

Dopamine levels decreased significantly in treated flies at all exposure times. After 4 hr exposure dopamine levels decreased from 198.5 ± 26.24 to 48.85 ± 5.54 pmol/brain.

After 24 hr exposure dopamine levels declined from 142.7 ± 6.58 pmol/brain to 66.58 ± 3.97 pmol/brain, while after 72 hr exposure dopamine levels fell from 293.5 ± 19.88 to 48.85 ± 5.54 pmol/brain (Students t-test, $p < 0.0001$, d.f. = 17, for all exposure times).

By contrast octopamine levels increased following exposure to static electric fields. After 4 hr exposure octopamine levels increased from 38.86 ± 9.97 to 467.9 ± 40.25 pmol/brain. After 24 hr exposure octopamine levels increased from 53.5 ± 6.61 pmol/brain to 207.3 ± 14.16 pmol/brain, while after 72 hr exposure octopamine levels increased from 29.65 ± 4.24 to 467.9 ± 40.25 pmol/brain (Students t-test, $p < 0.0001$, d.f. = 17, for all exposure times). The levels of tyramine at all exposure times were low and below detection levels.

Discussion

Here we show that *Drosophila* avoid static electric fields and that exposure to static fields of 26kV/m and above led to displacement of the wings. Removal of the wings reduced avoidance suggesting that the wings were involved in the detection of static electric fields. We also found that exposure to levels of static electric fields that can be encountered in the environment lead to changes in the neurochemistry of the brain.

Are the levels of static fields used in the laboratory representative of those in the natural environment?

Natural electric fields in the environment range between 0.1- 0.3 kV/m [27] so that the fields experienced by insects in their environment are normally less than those used in the experiments described here to elicit avoidance behaviour and, as such, one might argue about the relevance of such stimuli in the normal behavioral repertoire of insects. Static fields of far greater magnitude occur around man-made structures such as electricity transmission lines [28] or through friction charging [29]. Dezelak et al. [30] calculated the field at ground level underneath 400kV transmission lines and found that it was 9-11 kV/m, which is of the same order of magnitude of the fields that evoke avoidance of *Drosophila* used here. Maxwell models of the electric fields around transmission lines show that forces are much higher near the operating wires (up to 95kV within 1.5 m of the wires) suggesting that such fields are likely to have a far greater impact on flying insects.

Avoidance movements

Drosophila, like cockroaches [6], and other Diptera [31] show clear avoidance of static electric fields [32]. What is notable about *Drosophila* is that the wings had a major role to play in avoidance, while the antennae are involved in cockroaches [7]. While intact wild type flies avoided static fields at relatively low levels, wild-type flies with excised wings could not, even at much higher applied voltages. By contrast vestigial winged mutant *Drosophila* showed avoidance but at field levels higher than intact wild-type flies. Thus, the surface area of the wings appears to be a major determinant of avoidance.

Previously we suggested that electric fields could be detected via electrical forces causing deflection of sensory appendages resulting in mechanical stimulation [7]. Any electrically neutral object has a random distribution of negative and positive charges over the surface, and when that object enters an electric field it will experience forces on the

electrons that cause an uneven distribution (polarisation) of charges. The electric forces can generate physical movement of the object towards or away from the electric field region as a result of interaction between charges [33,34]. We found that the wings could be moved by electric fields of the same magnitude as those found to evoke avoidance behaviour. When a fly was placed underneath a negatively charged electrode, the static electric field forces caused polarisation leading to passive elevation of the wing toward the electrode, as unlike charges were attracted.

The wings of intact male flies and excised male wings required significantly lower electric fields to lift them compared to female wings. This is arguably counter-intuitive given that avoidance in general appears to be related to the surface area of the wing. However, it should be noted that the wings of females, while larger, are also likely to be heavier meaning that there may be a trade-off between force to raise the wing and the mass of the wing opposing lift.

Detection of static electric fields

Movements of the wings are detected by three key mechanoreceptors, campaniform sensilla, the tegula, and stretch receptors [35-37] located in and on the wings. Sensory neurones from these sensors transmit information about the wings position and deformation to the thoracic ganglia. Locally, responses are processed by interneurones and motor neurones that control the wing muscles [38], while intersegmental interneurones may also receive sensory inputs from the wing, which ultimately lead to limb movements [39]. Thus deflection of the wings by Coulomb forces generated by static electric fields has the potential to generate or modify limbs movements that could form the basis of avoidance.

Aminergic control of behaviour

The levels of serotonin and dopamine in the heads of flies found in this study were of the same order of magnitude found in previous studies [40]. The levels of dopamine, while being an order of magnitude higher than that described by Watson et al. [40], were lower than the levels found by others [41]. Monoamines are known to underpin behavioural states, often caused by social interaction between conspecifics [20] or by environmental conditions [16]. For example, the levels of octopamine in insects have been correlated with active and stressful states [42-45] and aggression [21]. Octopamine is also associated with flight and can both increase the likelihood of flight [46] and induce adipokinetic hormone

which mobilises lipids as fuel for flight [47]. Moreover, amines play a key role as reinforcement signals during learning and memory in insects [48]. That octopamine levels increase in flies exposed to static fields could be related to both active and stressful states caused by Coulomb forces acting on the flies and increased activity required to overcome those forces, either by enhanced locomotion or flight.

Dopamine is often associated with motor control and arousal in insects [49] and has a dual role in learning in *Drosophila* being involved in both appetitive and aversive learning [50]. We found that the exposure to static electric fields led to decreased dopamine levels. In *Drosophila* dopamine signalling can act via cryptochrome to increase arousal [51]. Interestingly cryptochrome also plays a role in the light-dependent magnetosensitive responses in *Drosophila* [52], raising the possibility that static electric fields might also directly impact on cryptochrome.

Changes in serotonin levels in flies exposed to static electric fields were not consistent over all time periods of exposure and were not significantly lower except following 24 hr exposure. Such a patterns of expression may suggest a reduced role of serotonin in electric-field induced behaviour, as it is in aggression [53,54]. For example, depletion of serotonin has no effect on aggression in crickets [54].

Taken together it is clear that a suite of changes in amine levels occurs during exposure to electric fields at levels present in the environment, many of which can act to modulate changes in behaviour. It is clear, therefore, that electric fields present in the environment have the potential to cause changes in insect behaviour and neurochemistry. Moreover, given that changes in amine levels produced in response to electric fields persist over time then the use of *Drosophila* from cultures maintained in plastic rearing tubes, as commonly occurs in many research laboratories, that can charge through triboelectrification could provide flies with an altered neurochemical background, and inappropriate for behavioural analysis.

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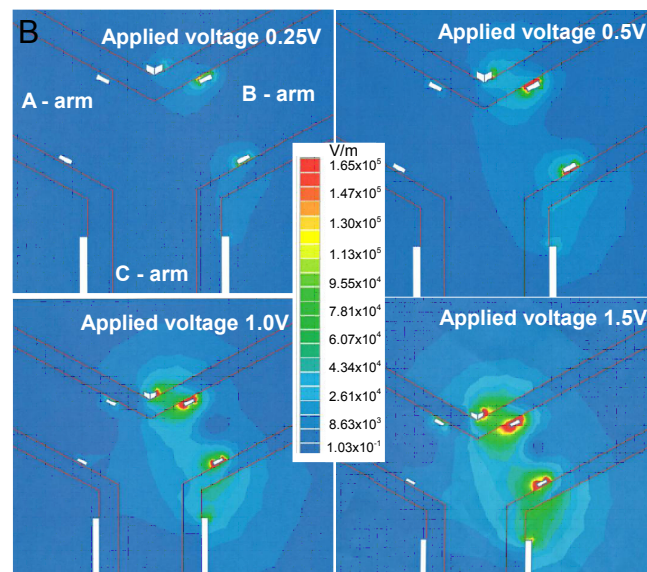
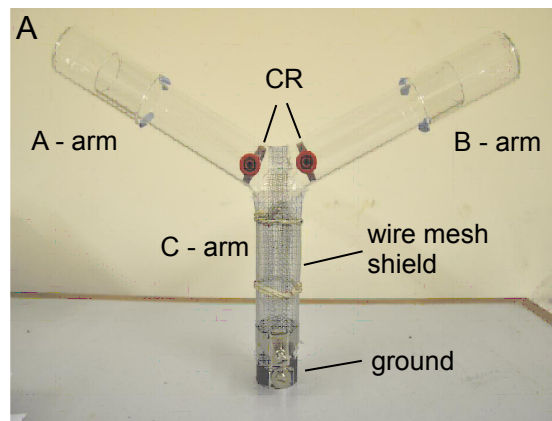
Figure Legends

Figure 1. The Y-bioassay and static electric field distribution. **A.** Photograph showing the Y-tube and the copper ring electrodes (CR). **B.** Maxwell FEA models of the electric field distribution around the Y-tube at 0.25, 0.5, 1 and 1.5kV.

Figure 2. Avoidance behaviour of wild-type male and female flies to static electric fields at different voltages. Males (**A**) and females (**B**) showed significant avoidance behaviour to electric fields at 1 kV and above ($P < 0.05$ in both cases). There were no significant differences between male and female flies ($P > 0.05$ in all cases). **C.** With wings removed there was no significant difference in avoidance of static electric fields at any applied voltage (one-tailed t-test, $p > 0.05$ in all cases). **D.** Vestigial wing flies. Flies significantly avoided the electric field at 2 kV and above. Bars on graphs represent means \pm SEM.

Figure 3. The effect of static electric fields on intact wings. **A.** The magnitude and distribution of static electric fields around the charged electrode (CE) at 2kV were modelled using Maxwell SV software. The 2 kV voltage applied to the electrode generated a field strength between 57-96 kV/m around the fly's position (insect). (GT: glass tube; GS: glass slide; EE: earth electrode). **B.** Photographs of the effect of static electric fields at different voltages on wing deflection on a live *Drosophila*. **C.** The elevation of *Drosophila* wings in response to fields at different voltages. Symbols represent means \pm SEM. Different letters indicate significant differences between applied voltages.

Figure 4. Analysis of three biogenic amines isolated from *Drosophila* heads. Serotonin levels significantly decreased only following exposure to a static electric field for 24hr. Dopamine levels were significantly reduced compared to controls at all exposure times and octopamine levels increased markedly in the isolated heads at all exposure times. Results are based on $n = 9 - 10$ heads and tested at each time point against time-matched controls using Students t-tests. Significant differences are indicated by a line above each graph. Bars on graphs represent means \pm SEM.



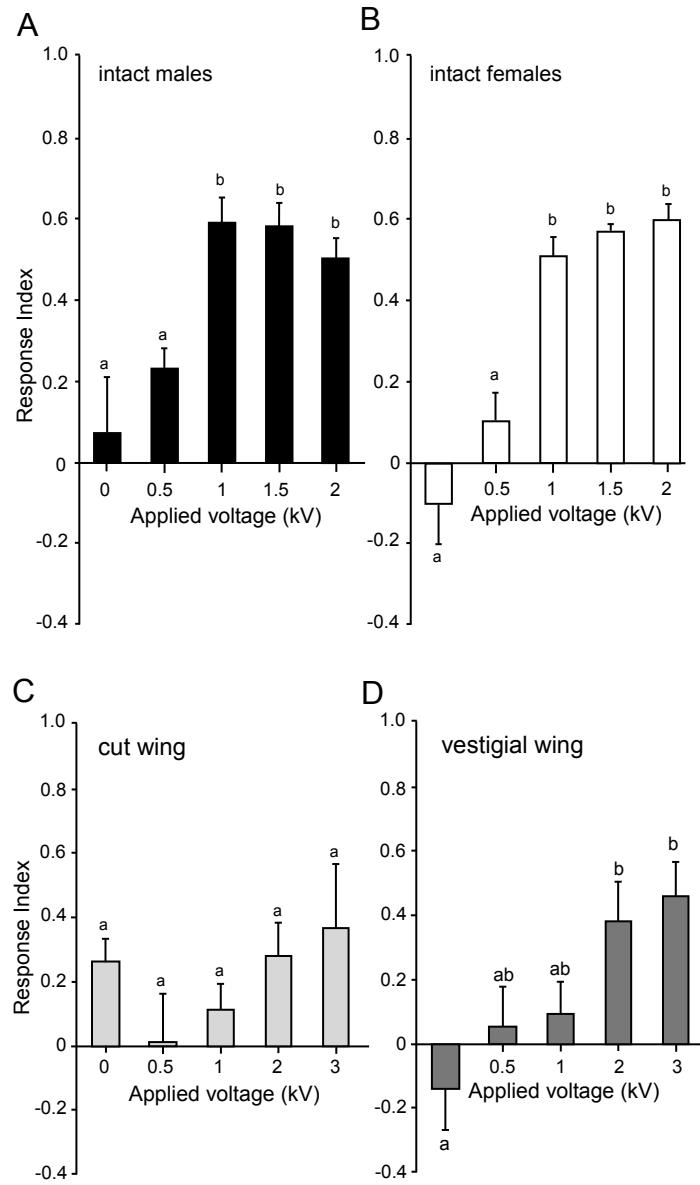


Figure 2

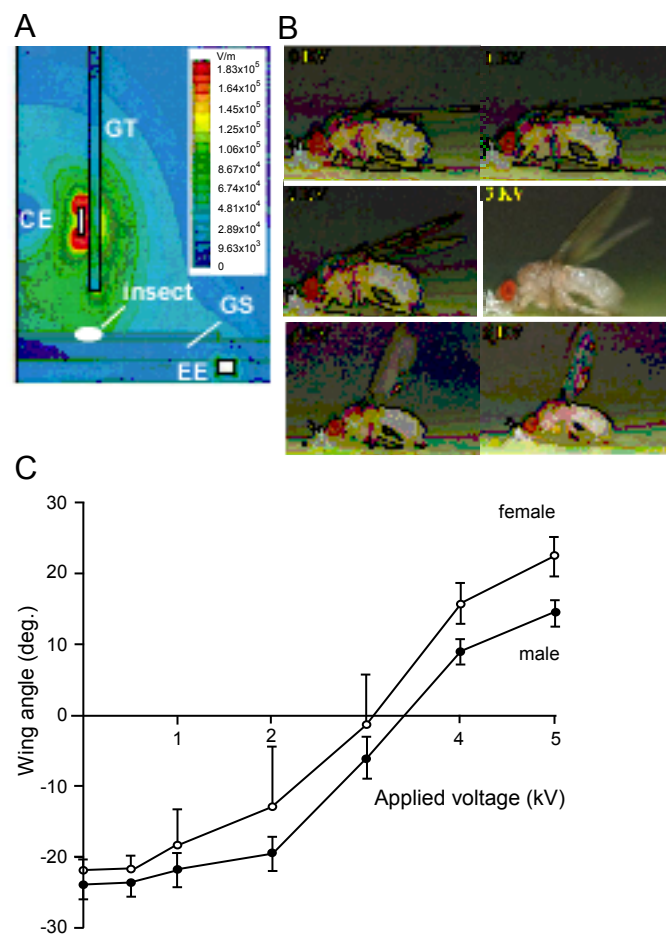


Figure 3

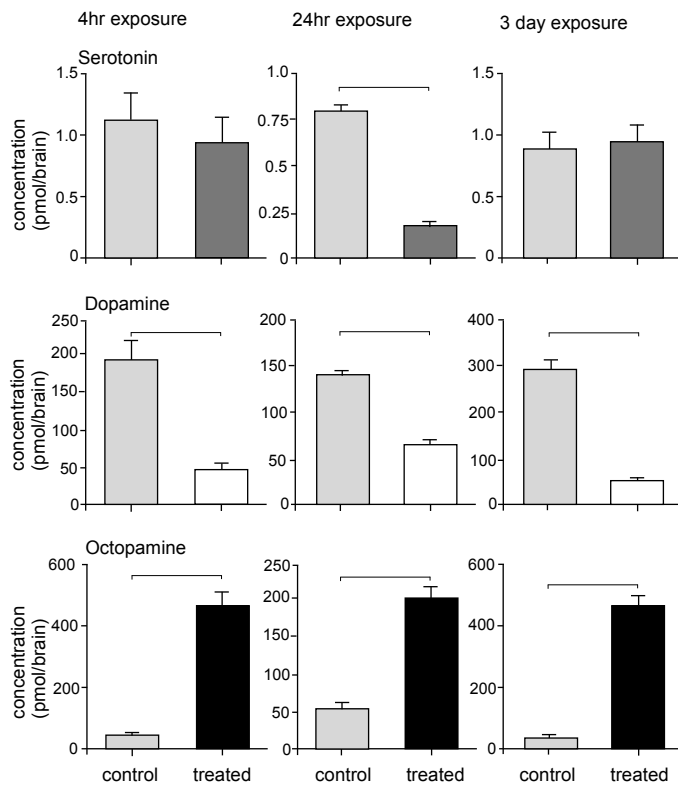


Figure 4