Static magnetic field modulates rhythmic activities of a cluster of large local interneurons in *Drosophila* antennal lobe

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**Static magnetic field modulates rhythmic activities of a cluster of large local interneurons in *Drosophila* antennal lobe.** *J Neurophysiol* 106: 2127–2135, 2011. First published July 20, 2011; doi:10.1152/jn.00067.2011.—With the development of superconducting magnets, the chances of exposure to intense static magnetic fields (SMFs) have increased. Therefore, safety concerns related to magnetic field exposure need to be studied, especially the effects of magnetic field exposure on the central nervous system. Only a limited number of studies have provided direct evidence of the connection between magnetic fields and electrophysiological signal processing. Here we described a cluster of large local interneurons (LNs) located laterally to each antennal lobe of *Drosophila melanogaster*, which exhibit extensive arborizations throughout the whole antennal lobe. Dual recordings showed that these LNs demonstrated rhythmic spontaneous activities that correlated with other LNs and projection neurons (PNs) in the olfactory circuit. The results suggest that 3.0-T SMF can interfere with the properties of the action potential, rhythmic spontaneous activities of large LNs, and correlated activity in pairs of ipsilateral large LN/LN in the olfactory circuit. This indicates that *Drosophila* can be an ideal intact neural circuit model and that the activities of the olfactory circuit can be used to evaluate the effects of magnetic field stimulations.

**neural circuit; *Drosophila melanogaster***

1,230-G SMF in principal cells in the cat lateral geniculate body suggested that the spontaneous discharge frequency was decreased and the discharge pattern was changed on account of the distortion of ion channels (Rosen and Lubowsky 1990). Evidence has shown that the action of SMFs is mainly based on their effects on the molecular structure of excitable membranes, especially imbedded ion channels, which can interfere with the mechanism of calcium and sodium channel activation (Miyakoshi 2005; Rosen 2003b). A previous study has shown that exposure to a 120-mT SMF results in a slight reduction in the peak calcium current amplitude, a shift in the current-voltage relationship, and a slowing of the channel activation rate without any change in the inactivation rate (Rosen 1996). Since ion channels play important roles in cellular excitability and neural function, the effect of SMFs on the nervous system has attracted more scholarly attention. Exposure to 2- to 3-mT SMF has been proven to modulate synaptic excitability in a mouse hippocampal slice preparation (Wieraszko 2000). However, previous studies have mainly focused on cultured neurons and brain slice preparations; the effects of SMFs on intact neural circuit function are still far from being completely understood.

The antennal lobe of *Drosophila*, serving like the vertebrate olfactory bulb, provides an ideal intact neural network model to investigate neural circuit function (Ng et al. 2002). Three classes of neurons form synapses in the antennal lobe and participate in the processing of olfactory information, including olfactory receptor neurons (ORNs), projection neurons (PNs), and local interneurons (LNs). The ORNs expressing the same olfactory receptors project to the same glomerulus and connect to the PNs in the antennal lobe, modulated by LNs. Then PNs transmit olfactory information to the mushroom body and the lateral horn (Shang et al. 2007; Wilson et al. 2004). The morphology, physiology, and development of PNs and ORNs have been widely documented, while LNs have not received much attention (Chou et al. 2010; Das et al. 2008). A previous study has shown that correlated spontaneous activity exists in the *Drosophila* antennal lobe, which is essential for information processing by neural circuits (Kazama and Wilson 2009). Thus the *Drosophila* antennal lobe could be an ideal model for investigating the effects of SMFs on the central nervous system, especially on the network of neural circuits, and may provide more information about the mechanisms of SMF action and the risk factors associated with the use of intense SMFs.
To address these questions, we describe a cluster of large LNs in the *Drosophila* antennal lobe and report the effects of 3.0-T SMF on their extracellular and intracellular spontaneous activities by using a *Drosophila* whole brain recording system (Gu and O’Dowd 2007) in wild-type Canton-S female fly pupae 2 days before eclosion. Our results demonstrated that these large LNs generated rhythmic spontaneous activities that correlated with the activities of other LNs and PNs in the ipsilateral antennal lobe, and we found that 3.0-T SMF can modulate the rhythmic activities and correlated activity of large LNs.

**METHODS**

Fly strains. *Drosophila melanogaster* stocks were reared on standard cornmeal agar medium supplemented with dry yeast at 24°C and 60% relative humidity. All experiments were performed on wild-type Canton-S female flies 2 days before eclosion. In our lab, flies produce new adults in 14 days. To accurately record the time point, flies that are 2 days before eclosion are double-identified by red eyes and transparent wings in the puparium.

3.0-T SMF exposure. The experimental group was deposited in a box filled with a 2-cm sound-absorbing sponge to eliminate potential noise. The box was tightly stuck to the center of the magnetic resonance spectrometer (Siemens MAGNETOM Trio A Tim System 3.0T MRI machine) with a 3.0-T SMF, and there was no movement of pupae in the field. Although an electromagnetic wave shield barrier was constructed between the examination room and the operation room of the MRI machine, a mT-level magnetic field still existed in the operation room. To ensure that the control group was free from magnetic field, we deposited the control group in our own lab. The temperatures of the examination room of the MRI machine and our lab were both controlled at 24°C, and the humidity was 60%. Other influence factors of these two groups were consistent.

Electrophysiological recordings from large LNs in isolated whole brain. All brains were obtained from female flies 2 days before eclosion. The entire brain, including optic lobes, was removed and prepared for recording in standard external solution containing 20 U/ml papain with 1 mM L-cysteine as previously described (Gu and O’Dowd 2006, 2007). The standard external solution contained (in mM) 101 NaCl, 1 CaCl₂, 4 MgCl₂, 3 KCl, 5 glucose, 1.25 NaH₂PO₄, and 20.7 NaHCO₃, pH 7.2, 250 μmol/kgH₂O. Then the dissected brains were mounted in an RC-26 perfusion chamber (Warner Instruments, Hamden, CT) containing the recording solution bubbled with 95% O₂ and 5% CO₂ (2 ml/min) throughout the experiments with the anterior face of the brain up. Pipettes were targeted to LNs in the dorsal neuron cluster in the antennal lobe.

Cell-attached and whole cell recordings were performed with pipettes (9–12 MΩ) filled with an internal solution containing (in mM) 102 K-glucanote, 0.085 CaCl₂, 1.7 MgCl₂, 17 NaCl, 0.94 EGTA, and 8.5 HEPES, pH 7.2, 235 osmol/kgH₂O. Gigaohm seals were achieved before recording in cell-attached configuration. Recordings were made at room temperature, and only a single large local neuron or a pair of neurons was examined in each brain.

All electrophysiological recordings were carried out with a BX51WI upright microscope (Olympus, Lehigh Valley, PA). Signals were acquired with an EPC10 amplifier (HEKA Elektronik, Lambrecht/Pfalz, Germany), filtered at 5 kHz with a built-in filter, and digitized at 5 kHz.

**Biocytin staining and fluorescence imaging.** The morphology and identity of a single large LN were confirmed by post hoc staining with biocytin. In some cells, 0.4% biocytin was added to the internal pipette solution. To make sure that biocytin could be injected into the soma and the terminals, the recording pipette was maintained in the whole cell configurations for at least 30 min. After electrophysiological recording, the brain was fixed in phosphate buffer containing 4% formaldehyde at 4°C for 10 h and subjected to biocytin staining. Then the brain was washed in 1% PBS three times, blocked, and incubated in a blocking buffer (0.1 M PBS, 0.1% Triton X-100, 1% BSA) containing streptavidin-Cy3 (Molecular Devices) for 3 h at room temperature. After incubation, the brain was washed three times with 5-min intervals in PBS. A BX51WI microscope with a ×40 objective and an Imag-Pro plus 7.0 (Olympus) camera was used to acquire photos of dendritic arborization of the large local neurons in the antennal lobe.

Data analysis. All data were analyzed by Clampfit 10.2 (Molecular Devices). Two or more spikes with interval time <100 ms were defined as a burst, and a single spike or a burst was regarded as an event. Low-frequency oscillations <10 Hz were isolated by low-pass filtering from voltage traces acquired in whole cell current-clamp configuration. Cross-correlation function was performed by Clampfit 10.2 with 2,000-ms lag time.

Statistics. The differences between control and SMF treatment were evaluated by independent *t*-test and Mann-Whitney rank sum test where appropriate. Values of *P* < 0.05 were considered significant.

**RESULTS**

A cluster of large LNs in the *Drosophila* antennal lobe demonstrated rhythmic activities. Previous studies have shown that LNs in the *Drosophila* antennal lobe are diverse in their neurotransmitter profiles, connectivity, and physiological properties, and they play important roles in information processing by olfactory neural circuits (Chou et al. 2010; Das et al. 2008). The whole brain of female fly pupae 2 days before eclosion was dissected and observed. A cluster of three or four large LN somata was found located laterally to each antennal lobe, with an impressively larger size than other cell bodies nearby, around 9.68 ± 0.31 μm in diameter (Fig. 1, A and B). These neurons were approached with a standard glass electrode. To show detailed morphology of these neurons, biocytin was used when whole cell patch-clamp recording was applied. The cluster of large LNs was intrinsic to the ipsilateral antennal lobe, with thick processes extending from the laterally situated cell body into the center of the lobe and exhibiting extensive arborizations that almost covered the entire antennal lobe (Fig. 1, A and C). These features were consistent with some types of LNs previously reported (Das et al. 2008).

Electrophysiological properties of these large LNs were monitored by patch-clamp recording. The cell-attached recording showed that they demonstrated spontaneous rhythmic spikes (3.02 ± 0.47 Hz) 2 days before eclosion (Fig. 2A). Under whole cell current-clamp recording, these neurons revealed a mean resting membrane potential (RMP) of −48.22 ± 1.80 mV and fired spontaneous action potentials (APs) actively with a mean amplitude of 32.09 ± 3.40 mV and a mean firing rate of 3.39 ± 0.92 Hz, including bursting pattern with consecutive spikes (Fig. 2B). The spikes could be blocked by tetrodotoxin (TTX) (data not shown). Whole cell voltage-clamp recording demonstrated the presence of spontaneous oscillatory activities (Fig. 2C).

Large LNs showed correlated activity with other LNs and projection neurons in the antennal lobe. Correlated spontaneous activities have been widely documented in neurons in the brain, which are important in information coding and processing by neural circuits (Kashiwadani et al. 1999; Kreiter and Singer 1996; McCarthy et al. 2011). A previous study has shown that homotypic PNs produce highly correlated spikes in

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Drosophila antennal lobe (Kazama and Wilson 2009). To determine whether these large LNs showed correlated activity with other neurons in the olfactory circuit, dual recordings from pairs of ipsilateral large LN/PN and large LN/LN in the antennal lobe were performed. In cell-attached recording, large LNs, PNs, and LNs generated spontaneous spikes (Fig. 3A and B; Fig. 4A and B). Two or more spikes with an interval time <100 ms were defined as a burst, and a single spike or a burst was regarded as an event. Then the cumulative frequency distribution of the interval time between events was detected. No significant differences in pairs of large LN/PN (P = 0.095, Kolmogorov-Smirnov test; Fig. 3E) and pairs of large LN/LN (P = 0.12, Kolmogorov-Smirnov test; Fig. 4E) were found.

In whole cell current-clamp recording, these three types of neurons revealed spontaneous APs and oscillations (Fig. 3C, Fig. 4C). Considering that oscillations are a common feature of spontaneous activity in the olfactory system and are thought to play an important role in information processing and memory in a variety of brain areas (Galan et al. 2006; Gelperin 2006; Ravel et al. 2003), low-frequency oscillations <10 Hz were isolated by low-pass filtering from voltage traces acquired in whole cell current-clamp configuration (Fig. 3D, Fig. 4D). The timing of low-frequency oscillation was found to be correlated in both pairs of ipsilateral large LN/PN and large LN/LN by computing the cross-correlation function. The cross-correlation analysis presented a clear peak with mean value at 0.62 ± 0.10 (lag time = 0 ms) (n = 12) in pairs of large LN/PN (Fig. 3F) and 0.79 ± 0.17 (lag time = 0 ms) (n = 16) in pairs of large LN/LN (Fig. 4F). Low-frequency oscillation correlation was largely decreased from 0.78 ± 0.01 to 0.20 ± 0.09 by 20 µM curare (n = 3, P < 0.05, paired t-test) but unaffected by 10 µM picrotoxin (n = 3, P = 0.15, paired t-test).

These results indicated that large LNs showed correlated activity with other ipsilateral LNs and PNs in the antennal lobe, suggesting that the activities of large LNs can partially reveal the activities of the entire local olfactory circuit.

3.0-T static magnetic field-modulated rhythmic activities of large LNs. The effects of SMFs on the central nervous system have been widely investigated in cultured neurons and brain slice preparations (Saunders 2005). Previous studies have demonstrated that weak SMF can change the properties of APs of neurons (McLean et al. 1995; Nikolic et al. 2008). As large LNs showed correlated activity with other LNs and PNs in olfactory circuit of antennal lobe, the effects of 3.0-T SMF on the rhythmic extracellular activities and APs of large LNs as well as correlated activity in large LN/LN pairs were monitored in order to investigate the alterations of the neural circuit under intense SMF exposure.

Cell-attached and whole cell recording were performed on the large LNs in isolated whole brain of female fly pupae 2 days before eclosion. Two or more spikes with an interval time <100 ms were defined as a burst. The changes of spike and burst frequency induced by exposure to 3.0-T SMF for 8 h were detected. In cell-attached configuration, the mean fre-
frequency of extracellular spikes decreased significantly from 3.02 ± 0.47 Hz in the control to 1.49 ± 0.41 Hz in the 3.0-T SMF treatment (P < 0.05, Mann-Whitney test; Fig. 5G). Additionally, the extracellular activities of three cells (n = 13) were silent after SMF exposure even though gigaohm seals were achieved (Fig. 5A). The effects of 3.0-T SMF on the properties of APs were further investigated (Fig. 5B). In whole cell configuration, the mean RMP of large LNs was -48.22 ± 1.80 mV in the control. Even though RMP seemed to decrease to -50.05 ± 3.65 mV after exposure to 3.0-T SMF for 8 h, it was not significantly different (P = 0.633, independent t-test). The mean frequency of APs was significantly reduced from 3.39 ± 0.92 Hz to 1.87 ± 0.85 Hz (P < 0.05, Mann-Whitney test; Fig. 5C), and the mean amplitude of APs decreased significantly from 32.09 ± 3.40 mV to 20.78 ± 3.21 mV (P < 0.05, independent t-test; Fig. 5D) after 3.0-T SMF treatment. Moreover, the mean frequency of bursts decreased from 0.58 ± 0.08 Hz to 0.20 ± 0.09 Hz (P < 0.01, Mann-Whitney test; Fig. 5E), while the mean duration of bursts decreased from 119.58 ± 14.12 ms to 50.97 ± 18.86 ms (P < 0.05, Mann-Whitney test; Fig. 5F). During dual whole cell recording performed on the ipsilateral large LN/LN pairs, the mean cross-correlation function value of low-frequency oscillation decreased from 0.79 ± 0.17 (n = 16) in the control group to 0.69 ± 0.26 (n = 11) in the SMF group (P < 0.01, ANOVA) after exposure (Fig. 5, H and I).

These results indicate that 3.0-T SMF can interfere with rhythmic spontaneous activities of large LNs and correlated activity in large LN/LN pairs, which may influence the activities and functions of the olfactory circuit in Drosophila antennal lobe.

DISCUSSION

LNs are essential for information coding and processing in neural circuits (Chou et al. 2010; Lledo et al. 2008; Palhalmi et al. 2004). Here we described a cluster of large LNs located laterally to each antennal lobe of Drosophila, exhibiting extensive arborizations throughout the whole antennal lobe. The significantly large size of this cluster of LNs makes it possible to target the recording electrode to the same type of neurons every time. These large LNs exhibited rhythmic spontaneous activities that correlated with the activities of other LNs and PNs in the olfactory circuit. Since correlated activity plays important roles in neural functions of the intact brain, the results further suggest that Drosophila antennal lobe can be an ideal model to investigate the network of neural circuits. Additionally, the activities of these large LNs can partially reveal the activities of local olfactory circuits in the antennal lobe.

With the development of superconducting magnets, the chances of exposure to intense SMFs have increased. However, safety concerns related to magnetic field exposure remain unclear, especially its effects on the central nervous system (Schenck 2000; Silva et al. 2006). Evidence has shown that membrane calcium channels are the primary sites of moderate SMF effects, and sodium channels may also be involved. The channel activation kinetics can be altered, while channel inactivation is not expected to be influenced (Miyakoshi 2005; Rosen 2003a). Both of these two channels are key structures for excitability and...
activity in the central nervous system (Catterall and Few 2008), modulating a wide range of cellular events. Various studies carried out to detect the effects of SMF on neural function have mainly investigated cultured neurons and brain slice preparations. However, the effects of intense SMF on intact neural circuit functions still need to be further studied. Therefore, the present study used the antennal lobe of isolated Drosophila whole brain as a model neural circuit in order to reveal the effects of 3.0-T intense SMF on the activities of olfactory circuit. This was done by monitoring the neural activity alterations of the cluster of large LNs induced by SMF.

The properties of APs are important bioelectric parameters for characterization of spontaneously firing neurons. APs can be blocked by TTX in the large LNs, indicating that sodium channels mediate the process of AP generation. Our data demonstrate that 3.0-T SMF can interfere with the rhythmic

Fig. 3. Large LNs and ipsilateral projection neurons (PNs) produced correlated spontaneous activities. A and B: dual cell-attached recording of spontaneous spikes from a pair of ipsilateral large LN/PN. Rasters of spikes are shown in B. C: dual whole cell current-clamp recording of the action potentials from a pair of ipsilateral large LN/PN. D: low-frequency oscillations <10 Hz isolated by low-pass filtering from voltage traces acquired in dual whole cell current-clamp configuration from a pair of ipsilateral large LN/PN. E: cumulative frequency distribution of interval time between events acquired in cell-attached configuration from a pair of ipsilateral large LN/PN. There was no significant difference in cumulative probability distribution between large LN and PN (P = 0.095, Kolmogorov-Smirnov test). F: cross-correlation function (CCF) for low-frequency oscillations <10 Hz filtering from voltage traces acquired in whole cell current-clamp configuration from a pair of ipsilateral large LN/PN. The mean value was 0.62 ± 0.10 (lag time = 0 ms) (n = 12). The gray band represents ±SE across pairs.
spontaneous activities and the properties of APs, as the mean frequency of extracellular activities was significantly reduced after exposure to 3.0-T SMF for 8 h. RMP showed no significant difference, indicating that the potassium channel-mediated current may be intact. Previous studies have shown that the amplitude of the evoked APs of neurons increased after exposure to mT-level SMF (Nikolic et al. 2008; Ye et al. 2004). However, the results of this study showed that the frequency and amplitude of spontaneous APs, as well as the burst frequency and duration of these large LNs, were significantly reduced after exposure to 3.0-T SMF. This was consistent with a prior study that examined the effects of a 1,230-G SMF on spontaneous discharge frequency and discharge pattern of principal cells in the cat’s lateral geniculate body in a whole brain preparation (Rosen and Lubowsky 1990). Furthermore, the detected changes could persist for 4 h after SMF exposure.

Fig. 4. Large LNs and ipsilateral LNs produced correlated spontaneous activities. A and B: dual cell-attached recording of spontaneous spikes from a pair of ipsilateral large LN/LN. Rasters of spikes are shown in B. C: dual whole cell current-clamp recording of the action potentials from a pair of ipsilateral large LN/LN. D: low-frequency oscillations <10 Hz isolated by low-pass filtering from voltage traces acquired in dual whole cell current-clamp configuration from a pair of ipsilateral large LN/LN. E: cumulative frequency distribution of interval time between events acquired in cell-attached configuration from a pair of ipsilateral large LN/LN. There was no significant difference in cumulative probability distribution between large LN and LN ($P = 0.12$, Kolmogorov-Smirnov test). F: cross-correlation function for low-frequency oscillations <10 Hz filtering from voltage traces acquired in whole cell current-clamp configuration from a pair of ipsilateral large LN/LN. The mean value was $0.79 \pm 0.17$ (lag time = 0 ms) ($n = 16$). The gray band represents $\pm$SE across pairs.
exposure, indicating that some metabolic processes such as enzyme activities may be involved in the observed changes induced by SMF. These results suggest that the inconsistent changes of neuronal activity induced by SMF may be related to the intensity of SMF and the different membrane properties of neurons. A previous study proposed that reorientation of membrane phospholipids during SMF exposure will result in the deformation of imbedded ion channels (Rosen 2003b). Therefore the decrease in AP amplitude suggests that sodium channels, which participate in the depolarizing phase of AP, may be affected more easily by SMF exposure. The reduction in the frequency of APs indicates that the 3.0-T SMF can inhibit large LN activities, which is consistent with previous findings (McLean et al. 1995; Nikolic et al. 2008). The bursting pattern
with consecutive spikes of neuron activity is related to calcium-dependent ion currents (Izhikevich 2000). Our results demonstrated that burst firing frequency and duration were significantly decreased after exposure to 3.0-T SMF. It is possible that alterations of bursting patterns of large LNs may result from the effects of the intense SMF on the calcium-dependent ion currents in some direct or indirect ways. Correlated spontaneous activities are important in information coding and processing by neural circuits. A previous study demonstrated that there were correlated activity and reciprocal chemical/ electrical connections between olfactory neurons in Drosophila antennal lobe (Huang et al. 2010). A recent study showed that electrical connections between olfactory neurons in processing by neural circuits. A previous study demonstrated ion currents in some direct or indirect ways. Correlated spon-
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strate that 3.0-T intense SMF can modulate the rhythmic 
activity, and cholinergic input was required for synchronous membrane activity whereas GABA can modulate firing pattern (McCarthy et al. 2011). In this study, we found that 3.0-T SMF decreased the correlated activity in pairs of ipsilateral large LN/LN. Whether this relates to the inhibited effects of SMF on single large LN activity, as we showed, the potential interaction between SMF and reciprocal chemical and/or electrical connections of neuron pairs, or possible effects of SMF on the synaptic input to large LNs needs to be studied further.

Local neurons are essential for transformation and integration of olfactory information through the glomerular relay between PNs and ORNs in antennal lobe, playing important roles in the functions of neural circuits (Ng et al. 2002; Wilson and Laurent 2005). The results of the present study demonstrate that 3.0-T intense SMF can modulate the rhythmic spontaneous activities of large LNs and correlated activity of ipsilateral pairs of large LN/LN in Drosophila antennal lobe, indicating that the activities of the local olfactory circuit may be affected by SMF. The data also suggested that calcium channels and sodium channels may be related to the activity alterations induced by SMF, which also needs to be investigated further. Our findings are, in a sense, the first step in detecting the effects of intense SMF on neural circuit functions. Drosophila antennal lobe, with genetic tractability and functional organization, provides an ideal model not only for investigating the network of neural circuits but also for linking the gaps between neural alterations and SMF stimulation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


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