Neuroscience Letters 472 (2010) 143–147

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

The differential effects of depression on evoked and spontaneous pain behaviors in olfactory bulbectomized rats

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ARTICLE INFO

Article history:
Received 26 December 2009
Received in revised form 21 January 2010
Accepted 28 January 2010

Keywords:
Depression
Evoked pain
Formalin
Olfactory bulbectomy

ABSTRACT

Although it has been accepted that depression and pain are common comorbidities, their interaction is not fully understood. The current study was aimed to investigate the effects of depression on both evoked pain behavior (thermal-induced nociception) and spontaneous pain behavior (formalin pain) using an olfactory bulbectomy (OB) rat model of depression. Emotional behaviors were assessed by open field and Morris water maze tests. The results showed that the depressed rats exhibited stronger tolerance to noxious thermal stimulation compared to non-depressed animals. In contrast, the spontaneous nociceptive behaviors induced by formalin injection were significantly enhanced in the OB rats in comparison to control rats. These results demonstrated that depression can have differential effects on stimulus-evoked pain and spontaneous pain, with alleviation in the former while aggravation in the latter. The present study has confirmed our previous findings that depression can inhibit evoked pain but facilitate spontaneous pain, and provides evidence that the OB depression model is a feasible model for studying the relationship between depression and pain.

In recent years, there has been an increasing interest in the relationship between pain and depression. Previous studies have shown that painful somatic symptoms can predict depression and vice versa [16,24]. On average, 65% of patients with depression experience one or more pain complaints, and depression is present in 5–85% (depending on the study setting) of patients with pain [2,13]. The combination of pain and depression can be costlier and more disabling than either condition alone.

To explore the relationship between pain and depression, experiments have been performed on patients with depressive disorders. Most studies regarding depressed patients found increased pain thresholds [3,15], while a few reports described a decrease in the experimentally evoked pain [17]. Thus, the correlation between depression and pain is still a matter of debate and has been far from clear. A few studies have also been carried out in animals. Similar results were obtained that the nociceptive behaviors were either reduced [20] or enhanced [1] in subjects exposed to chronic environmental stress, a condition that has been demonstrated to cause depression. Using unpredictable chronic mild stress animal model, our previous work has found that depression can enhance spontaneous pain but alleviate stimulus-evoked pain [21].

In the present study, a different animal model—olfactory bulbectomy (OB)—was employed. The OB rat is a well-characterized animal depression model that results in a number of behavioral, physiological, neurochemical, endocrinological, and immunological changes that are similar to human depression [14]. The present study was designed to explore the effects of depression on the evoked pain behavior (thermal-induced nociception) and spontaneous pain behavior (formalin pain, which is closer to clinical persistent pain) using an OB rat model. The aim of this study was to confirm our previous finding as well as to provide an alternative approach for investigating the neural mechanisms underlying the relationship between depression and pain.

Forty-eight male Sprague Dawley rats (weight on arrival: 200–220 g, Laboratory Animal Center of the Academy of Military Medical Sciences, Beijing, China) were used in this study and housed individually. Food and water were available *ad libitum*. The colony was maintained at 22 ± 2 °C with a standard 12 h light-dark cycle (lights on at 07:00 am). Animals were allowed to habituate to the environment for 1 week before experiments, and were handled daily by the experimenter. Adequate measures were taken to minimize pain or discomfort. Experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. The research protocol was approved by the Institutional Animal Care and Use Committee of Chinese Academy of Sciences

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doi:10.1016/j.neulet.2010.01.075

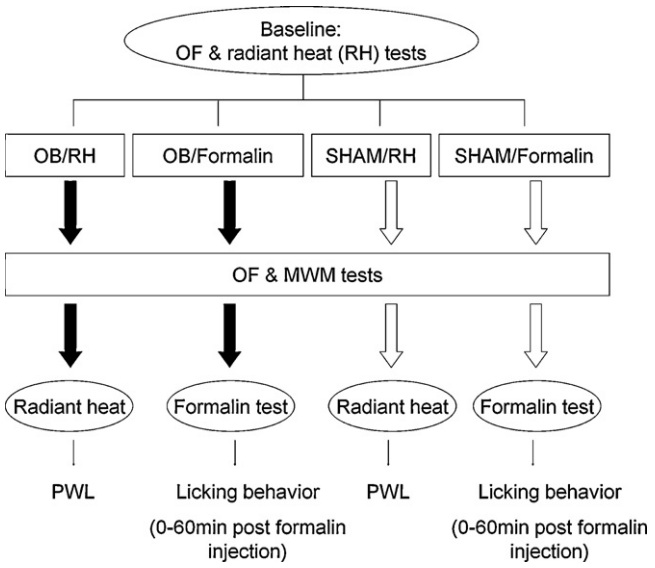

Fig. 1. Schematic diagram of the experimental protocol. Rats were divided into two groups (OB and SHAM groups, receiving olfactory bulbectomy or sham surgery, respectively) based on the results of the open-field test. Both groups were further divided into two sub-groups for either radiant heat (RH) or formalin test, i.e., OB/RH, OB/formalin, SHAM/RH, and SHAM/formalin groups. Baseline thermal thresholds were measured in the OB/RH and SHAM/RH rats. Then all animals were tested in the open field and Morris water maze. Evoked pain was assessed by the paw withdrawal latency (PWL) induced by heat stimulation. Spontaneous pain was evaluated by the nocifensive paw-licking behavior following formalin injection. OF: open field; MWM: Morris water maze.

Rats were tested pre-surgically in the open field and balanced over two groups (OB group and SHAM group, which received olfactory bulbectomy and sham surgery, respectively) according to their locomotor behaviors. These groups were further divided into two sub-groups: radiant heat (RH) evoked pain and formalin induced spontaneous pain, i.e., OB/RH group ($n = 14$), OB/formalin group ($n = 14$), SHAM/RH group ($n = 10$), and SHAM/formalin group ($n = 10$). The baseline pain thresholds of the OB/RH and SHAM/RH groups were examined before surgery. Then rats underwent bilateral olfactory bulbectomy or sham operation. After 2-week recovery period, open field and Morris water maze tests were employed to assess the depressive state of rats. The evoked pain by heat stimulation and spontaneous pain by formalin injection were measured on the following day. The experimental protocol was illustrated in Fig. 1.

The animals (270–300 g) were anesthetized with sodium pentobarbital (0.5 mg/kg, i.p.) and fixed on a stereotaxic apparatus (Stoelting, USA). A midline sagittal incision was made to expose the skull. Two 2-mm diameter holes were bored 8 mm rostral to the bregma and 2 mm lateral to the midline separately. The bilateral olfactory bulbs were sucked from the holes using a vacuum pump and the cavity was filled with gel foam (Coltene whaledent, Switzerland) to control bleeding. Special care was taken to avoid damaging the frontal cortex. Penicillin powder was sprinkled on the wound prior to closure. Sham-operated rats were treated similarly, except that no brain tissues were removed. At the end of the experiment, animals were dissected to check if all the olfactory bulbs were removed. If not, the data will be rejected in the final analysis.

The open field test was performed in an iron circular black base (180 cm in diameter) and applied to analyze the locomotor and rearing behaviors of rats. The wall surrounding the base consisted of a 50 cm high iron sheet. Illumination was provided by a 40 W bulb. On the 15th day after surgery, all animals were tested in the open field for 5 min. The distance traveled during the test was recorded by a computer-based system *Etho Vision* (Noldus Information Technology, Wageningen, the Netherlands). The number of rearing behaviors was recorded by the experimenter. In the interval between each two tests, the apparatus was cleaned with ethanol and water to remove olfactory cues.

Water maze training was conducted in a circular pool of 180 cm in diameter, containing water of 30 cm in depth. It was divided into four quadrants (I, II, III, and IV) of equal size, each having an entry point. A circular platform (12 cm diameter) was placed 2 cm beneath the water level. Water temperature was held at $22 \pm 1\,^{\circ}C$. The procedure consists of training for 4 days and testing for 1 day. On every training day, the platform was positioned in the II quadrants. Rats were put into the maze facing the wall at entry point in one of the three other quadrants over three trials. Animals were allowed to stay in the water for 120 s. Any rat that could not find the platform within 120 s was placed on the platform by the experimenter and allowed to stay there for 15 s. The swimming paths of the rats were tracked using a video camera suspended centrally above the pool. The distance swum and the time spent in finding the platform were recorded. On the testing day, the platform was removed from the pool and rats were allowed to swim freely for 60 s. The percentage of time that the animals spent in each quadrant was calculated.

The apparatus and test for thermal evoked pain were the same as described by Wang et al. [25]. The animals were put into a Plexiglas chamber on a glass floor beneath which the radiant heat apparatus (100-W projector lamp) was situated. A beam of light through a hole (4 mm in diameter) of the apparatus was focused on the plantar surface of the left hindpaw. Paw withdrawal latency (PWL) was defined as the length of time between the light onset and the paw lift. The intensity of light was adjusted so that the baseline PWL was around 10 s, with a cutoff time of 22 s to prevent tissue damage. Four trials were performed with at least 5 min interval. The last three trials were averaged to get a mean latency as the threshold of the thermal evoked pain.

The formalin test was carried out in a quiet room. The room was kept at an even temperature between 21 and 23 °C. Animals were put into a plastic test chamber (25 cm × 25 cm × 30 cm) for at least 30 min to accommodate to the environment prior to test. Then rats received a subcutaneous injection of formalin (5%, 50 μl) into the plantar surface of the left hindpaw. The nociceptive behaviors were videotape recorded throughout the following 60 min. Pain intensity was determined by measuring the time spent in licking the injected paw every 5 min after injection.

Statistica 5.1 and GraphPad prism 5.0 were used to analyze data and draw graphs. Data involving 2 factors was analyzed with multifactor analysis of variance (ANOVA). Duncan's test was employed for post hoc test. Student's *t*-test was used for comparing 2 groups. The data was presented as means ± SEM. The statistical significance was set at $P < 0.05$.

Body weights were measured the day before, and daily for 14 days after the surgery. The baseline body weights of animals did not differ between OB and SHAM groups (287.1 ± 1.7 g vs. 281.2 ± 5.7 g, $t(42) = 1.076$, $P = 0.2882$). Throughout the 2 weeks of recovery, significant reduction of weight gain was observed in the OB rats as compared to the control rats (two-way ANOVA, $F(14, 588) = 423.7$, $P < 0.001$) (Fig. 2A).

Additionally, the OB rats showed significantly higher level of locomotor and rearing behaviors in open field than that of control rats (4928 ± 422 cm vs. 1541 ± 173 cm, $P < 0.001$, see Fig. 2B; 12 ± 1 vs. 3 ± 1 times, $P < 0.001$, see Fig. 2C, for locomotor and rearing behaviors, respectively). Spatial learning and memory capability were measured with water maze task. As shown in Fig. 2D and E, both the distance swum and the time spent in finding the platform were longer in OB rats than in control group ($F(1, 42) = 44.7$, $P < 0.001$; $F(1, 42) = 33.6$, $P < 0.001$, for swimming distance and

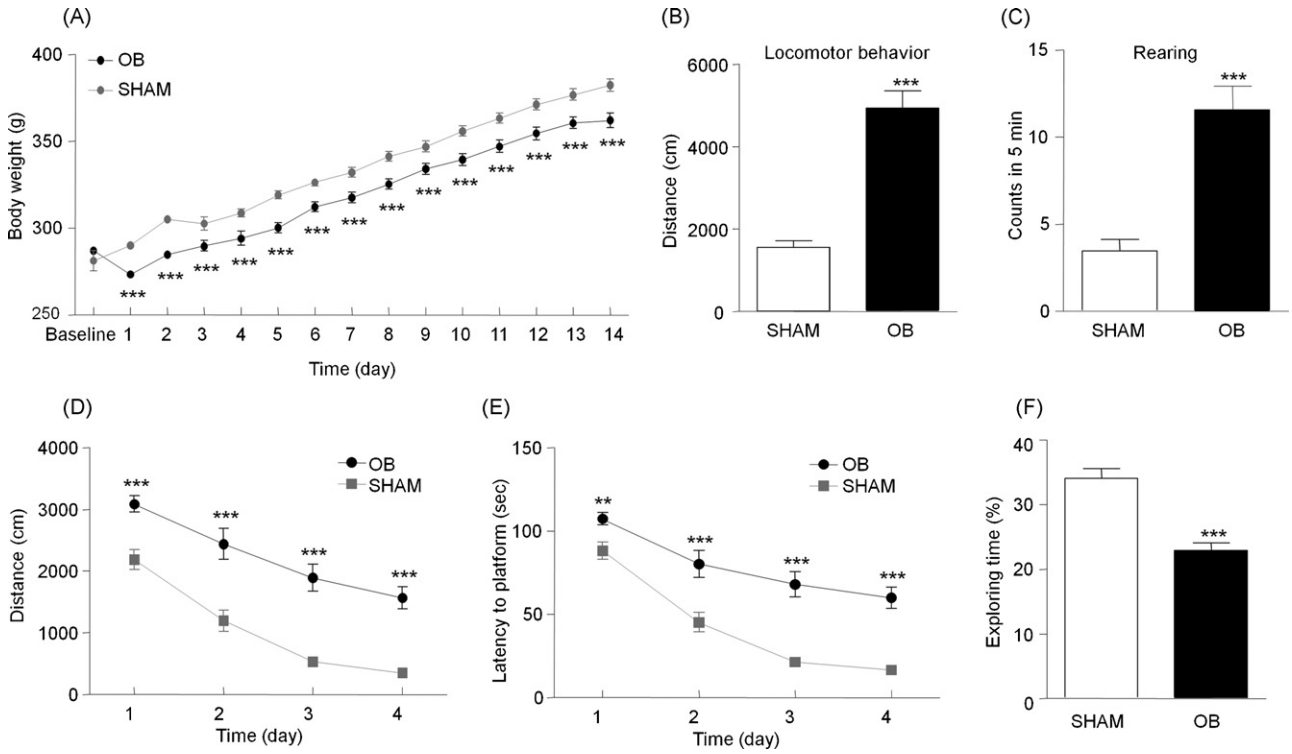

Fig. 2. Behavioral outcome of the OB model for depression. (A) Body weights. The body weights of rats showed significant decrease in the OB group in comparison to the control group over the 14-day post-operation period. (B and C) Open field test. Significant higher level of locomotor activity and rearing behaviors were found in the OB rats than in the control rats. (D and E) Morris water maze training. In four training days, rats exhibited significant more activity and longer time to explore the platform in the OB rats than in the control group. (F) Morris water maze test. The percentage of time spent in the quadrant II where the platform was located was significantly lower in the OB group than in the SHAM group. Data are presented as mean ± SEM (n = 20–24). $^{**}P < 0.01$; $^{***}P < 0.001$.

exploring time, respectively) throughout the four training days. On the testing day, the platform was removed. The percentage of time spent in exploring the platform in quadrant II where the platform was located is significantly lower in OB group than in control group (22.9 ± 1.1 s vs. 34.0 ± 1.5 s, $P < 0.001$, see Fig. 2F), demonstrating an impaired spatial memory capability in OB rats. These results indicate that the OB rats have exhibited depressive-like behaviors and the animal model for depression has been successfully established.

Evoked pain behaviors were measured before (baseline) and 3 weeks after olfactory bulbectomy surgery. As shown in Fig. 3A, animals in OB group displayed longer PWLs compared to control group (16.19 ± 0.97 s vs. 12.83 ± 0.81 s, $P < 0.01$), suggesting that depressed rats had higher thermal pain thresholds than non-depressed rats. On the other hand, subcutaneous injection of formalin into the hind paw induced a typical biphasic pattern of licking behavior (Fig. 3B). In contrast to the control group, the licking behaviors of OB rats were significantly increased over the entire observation hour ($F(1, 21) = 12.63$, $P < 0.01$). Cumulative licking time clearly represented the augmentation in phase I (0–5 min, 105.6 ± 5.99 s vs. 60.69 ± 11.15 s, $t(19) = 3.605$, $P < 0.01$, Fig. 3C), interphase (5–15 min, 66.19 ± 14.9 s vs. 17.02 ± 6.83 s, $t(21) = 2.719$, $P < 0.05$, Fig. 3D), and phase II (15–60 min, 562.8 ± 73.51 s vs. 298.5 ± 45.25 s, $t(21) = 2.841$, $P < 0.01$, Fig. 3E). These results suggest that the spontaneous pain behavior was enhanced following olfactory bulbectomy.

In the present study, we investigated the pain-related behaviors in olfactory bulbectomized rats. The results showed that the thermal stimulus-evoked pain and formalin induced spontaneous pain were suppressed and enhanced, respectively, following OB treatment. Our data confirmed our previous findings that depressed subjects tend to exhibit decreased sensitivity to experimental pain but increased intensity of ongoing pain (clinical pain complaints).

Most studies employing experimentally induced pain demonstrated that depressed patients are less sensitive to experimental noxious stimuli in contrast to the high frequency of clinical pain complaints [3,4,9]. In our study, depressed rats showed less sensitivity to noxious radiant heat applied on the hindpaw, which was consistent with the data from human subjects. Bar et al. investigated 30 patients suffering from a major depressive disorder and found hypoalgesia to heat and electrical pain in comparison to control subjects [3]. Lautenbacher and his colleagues reported that patients with depression had significantly higher pressure pain thresholds than the healthy controls [15]. These findings together with our results demonstrate that depression has an inhibitory effect on the stimulus-evoked pain. However, there are also contradictive findings. Some studies applying different methods and indicators of pain perception or employing minor depressed patients found that the pain thresholds of the depressed patients were unchanged or even decreased [19].

The occurrence of pain complaints in patients suffering from major depressive disorder has been shown to be significantly higher than that in the general population [8]. Primarily depressed patients exhibit a high degree of pain complaints [15]. Up to 92% of these patients reported clinical pain symptoms [8]. In the current study, the OB rats showed significantly more spontaneous pain response than the SHAM rats after formalin injection, consistent with the clinical observations. It has been reported that patients with depression had a more frequent, more intense, longer duration pain [7], and greater likelihood of non-recovery than healthy control subjects [15]. As is the fact that the memory of patients with emotional problems can be selectively biased toward the recall of negative events such as pain [10], it is true that an increased likelihood of remembering painful events in the past seems to be associated with an increased likelihood of suffering from pain in the

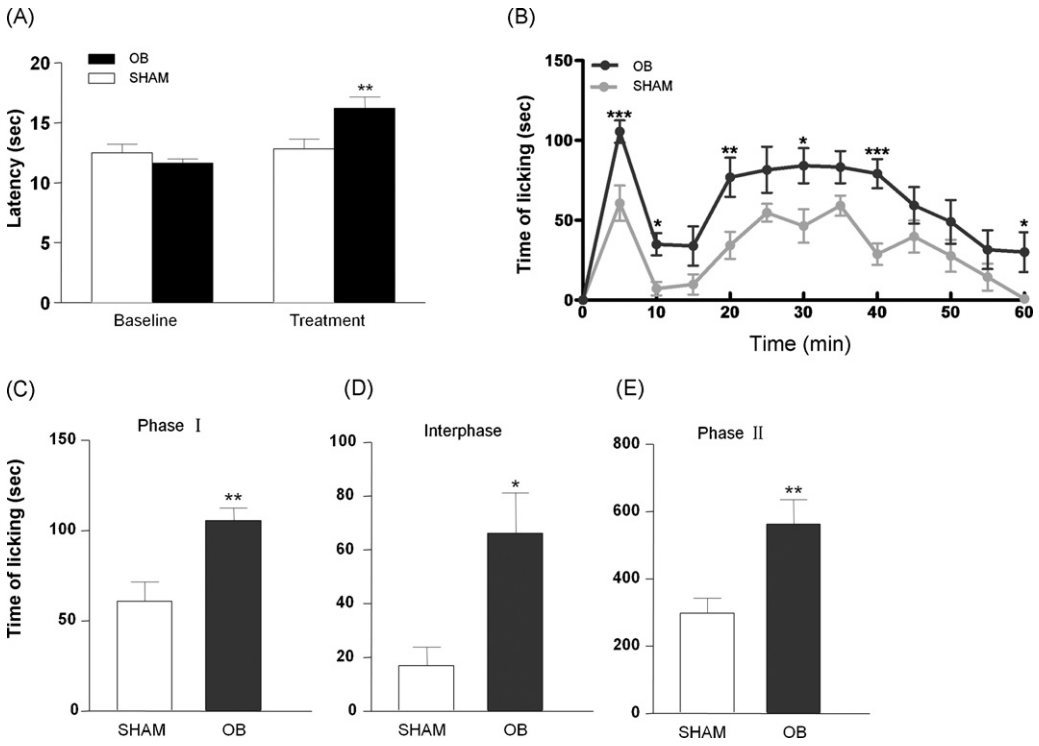

Fig. 3. Influence of depression on pain behaviors. (A) Evoked pain by thermal stimulation. Rats displayed significantly longer PWL to noxious heat stimuli in OB group than in SHAM group, suggesting a reduction of the thermal evoked pain in the depressive state ($n = 10–11$). (B–E) Spontaneous pain by formalin injection. Rats in the OB group exhibited significantly increased licking behaviors in the early and late phases as well as in the interphase following formalin injection, indicating that OB surgery resulted in an enhancement of the spontaneous ongoing pain ($n = 10–13$). Data are presented as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, compared with their respective control group.

present [11]. Indeed, depressed subjects holding negative anticipation causes some brain areas (such as the anterior cingulate gyrus) to activate, and the subjects then appear to focus, attend to, and rate the pain stimuli as more severe [2].

Although it is generally understood that depression and painful symptoms are common comorbidities, the underlying mechanisms of the association are far from clear [2]. Several previous studies have indicated that pain and depression share common neurochemical mechanisms, such as the dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis [6]. Serotonin and norepinephrine pathways, which ascend to affect traditional emotional symptoms of depression and descend through the brainstem and spinal cord to inhibit pain, may explain this coexistence of pain and depression to a certain extent [12]. Recent research has provided evidence of a central pain modulation system that can either dampen or amplify nociceptive signals from the periphery. Both serotonin and norepinephrine have been shown to dampen peripheral pain signals. This explains how depression, which is associated with a dysregulation of these key modulating neurotransmitters along a shared pathway, may contribute to the frequent presence of painful symptoms [2]. Meanwhile, functional imaging studies in depressed patients demonstrated a maladaptive activation of the neural network that is involved in pain and emotion modulation during the application of heat pain. In particular, it was suggested that the enhanced activation of prefrontal brain regions might be associated with reduced pain perception on the skin [5]. Nonetheless, these potential mechanisms need to be further investigated.

Olfactory bulbs have extensive connections with limbic system and other higher brain centers [14]. Olfactory bulbectomy results in an abrupt loss of inputs to these regions. Open field test was used to detect the changes in the sensitivity of rats to a stressful novel environment, and the Morris water maze has been widely used to test the ability of spatial learning and memory [22,23]. The hyperactivity performance in open field apparatus, as shown in our and others' research, emphasized that OB rats had reduced ability to adapt to sudden environmental changes. Besides, the impaired spatial learning and memory had been found, as revealed by the water maze test that the OB rats took more time to escape and spent longer time wandering in the target quadrant.

In our previous study, we used a UCMS model to reveal the depression-induced changes in the rodent pain behavior. Since it is believed that long-term exposure to multiple, inescapable stressors can induce and maintain clinical depression in humans, UCMS is considered a realistic means of producing an animal model of depression [26]. In addition, it has good face validity as it can elicit depression-like symptoms such as a lack of sucrose preference, interpreted as anhedonia, a core symptom of depression. However, the reliability of the UCMS model has been questioned, because a decrease in sucrose consumption is not consistently observed following the stress procedure between and/or within experiments, among various laboratories, with animal strains used, and according to specific procedures [18]. Therefore, each of the two models mentioned has its strong points and weak points in the research of depression. In light of our present and previous results, both models can be employed to study the effects of depressive disorder on pain behaviors. One may consider it according to his specific research interest.

In summary, our studies demonstrated that depression inhibited evoked but facilitated spontaneous pain behaviors in rats. The results have confirmed our previous finding and are consistent with most clinical manifestations. It is reasonable to suggest that OB is a feasible animal model in addition to UCMS to be used in exploring the mechanism underlying the relationship between pain and depression. Because of its less complex procedure, OB would have promising use in the domain of pain research.

Acknowledgement

This work was funded by an undergraduate student project from the IPCAS for YX, a NNSF grant (30700223) to JYW, NNSF grants (30770688, 30970959), a Beijing NNSF grant (5082008), the 100 Talented Plan of the Chinese Academy of Sciences, the National Hi-Tech Research and Development Program of China (Grant No. 2008AA022604), and a grant from NIH Fogarty International Center (R03-TW 008038) to FL.

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