Biophysics and Physicobiology

Regular Article

Food deprivation changes chemotaxis behavior in *Caenorhabditis elegans*

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Exploring for food is important in food-deprived condition. Chemotaxis is one of the important behaviors to search food. Although chemotactic strategies in *C. elegans* have been well investigated: the pirouette and the weathervane strategies, the change of the chemotactic strategy by food deprivation is largely unclear. Here, we show the change of chemotactic strategy by food deprivation, especially for isoamyl alcohol. To compare chemotaxis under different food-deprivation period, we showed that worms change their chemotactic behaviors by food deprivation. The worms with 1-h food-deprivation change the weathervane strategy. On the other hand, 6-h food deprived animals change the pirouette strategy. These results demonstrate that worms change chemotactic strategy different way depend on period of food deprivation.

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Food deprivation is severe problem for animals. Food deprivation affects various behaviors including food taking in various organisms [1–8]. Under food deprivation, animals should explore for food efficiently. Although chemotaxis is one of the important behaviors to explore for food [9–12], effect of food deprivation on chemotaxis is largely unknown. In *C. elegans*, two chemotactic strategies are well known: the pirouette and weathervane strategies [12–15]. In the pirouette strategy, worms change their locomotive direction with sharp turns (pirouettes) when they detect a negative time derivative of the odor concentration ($dC/dt < 0$) [13]. In the weathervane strategy, animals gradually migrate to higher concentration regions [14,15]. These strategies have been investigated well [12–15]; however, the change of the chemotactic strategy by food deprivation is still unknown.

Here, we showed that the change of chemotactic strategy by food deprivation especially for isoamyl alcohol (IAA). To compare chemotaxis under different food-deprivation period, we found the change of strategy; 1-h food-deprivation animals modulate the weathervane strategy, but 6-h food-deprivation animals modulate the pirouette strategy. These results clearly show that worms change their chemotactic behaviors by food deprivation.

◀ *Significance* ▶

Exploring for food is important in food-deprived condition. Chemotaxis is one of the important behaviors to search food. Here, we show the change of chemotactic strategy by food deprivation. The worms with 1-h food-deprivation change the weathervane strategy. On the other hand, 6-h food deprived animals change the pirouette strategy. These results demonstrate that worms change chemotactic strategy different way depending on period of food deprivation.

Materials and methods

C. elegans strains

Worms were cultured at 20°C on nematode growth medium (NGM) agar plates with *Escherichia coli* OP50 bacteria under standard conditions [16]. Hermaphrodites of the Bristol, N2 strain were used for all experiments.

Chemotaxis assays

Behavioral assays were performed as previously described [12] (Fig. 1). Briefly, assay plates consisted of 8 ml of 1.8% agar, 1 mM $CaCl_2$, 1 mM $MgSO_4$ and 5 mM KH_2PO_4 in 10-cm petri dishes. Some young worms were transferred to the assay plates without food 1–6 h before the assay and were incubated at 20°C. In the control experiments, worms were not transferred to the assay plates. Then, worms were moved into S-basal buffer in a microtube with a sterilized platinum wire, and washed with S-basal buffer. Next, all worms were transferred to the assay plate with the buffer by a pipette to enable picking up of each worm. Then, 4 μl of distilled water was spotted in the center of another assay plate (rather than S-basal, to avoid the effect of salt taxis [17]). Worms were transferred to the spotted water with a sterilized platinum wire. Water was then removed using Kimwipes until the worms were not swimming. 1 μl of 10^{-2} dilution of isoamyl alcohol (IAA) in ethanol (EtOH) was spotted onto the plate; 1 μl of EtOH was also spotted onto the other side of the plate. To each spot, 1 μl of 500 mmol L^{-1} sodium azide was added as an anesthetic in advance so that animals were restrained once they reached the odor spot. Images were captured with a web camera (HD Pro Webcam C920, Logitech) every second for 30 minutes with a custom-made Matlab program (MATLAB 2016a, MathWorks).

Behavioral analysis

The same analysis methods in previous research were employed [12] using Matlab programs modified from *parallel worm tracker* [18]. If worms arrived at the high odor concentration area, their subsequent trajectories were removed from the dataset because the animals were restrained by sodium azide. For worms that reached the edge of the plates, tracking data before arrival at the edge were used for analysis. In the analysis, pirouettes were defined as turns with an absolute turning rate over 90°. The chemotaxis index was calculated as follows: [(# of animals within a 1 cm radius of the odor spot) − (#of animals outside this area)]/total # of animals on the plate. For calculation of the circular variance of bearing (Fig. 2D), bearing as the angle between odor direction and locomotive direction was calculated [14]. Circular variance of bearing angle V [19] was evaluated as

$$V = 1 - \sqrt{\left(\frac{1}{N}\sum_{i=1}^{N}\cos\theta_i\right)^2 + \left(\frac{1}{N}\sum_{i=1}^{N}\sin\theta_i\right)^2}$$

where θ_i is bearing angle at time point i. Circular variance V varies from 0 to 1. The odor concentration for Figure 3 was estimated by the numerical simulation as previous research [12,20]. For calculation of the direction change ($\Delta\cos\theta$, Fig. 4B) is evaluated as $\cos\theta_{post} - \cos\theta_{pre}$ (Fig. 4A). θ is an angle between worm's direction and odor source. $\cos\theta_{pre}$ indicates the angle at initiation of pirouette and $\cos\theta_{post}$ indicates the angle at termination of pirouette. $\Delta\cos\theta$ takes a value within the range of −2 to 2 ($-2<\Delta\cos\theta<2$). The positive values indicate that the direction is improved by the pirouette, and the negative value indicates that the direction is worsened by the pirouette.

Statistical tests

Statistical analysis was performed using Wilcoxon rank-sum test (Fig. 2; R version 3.5.2. with the wilcox_test
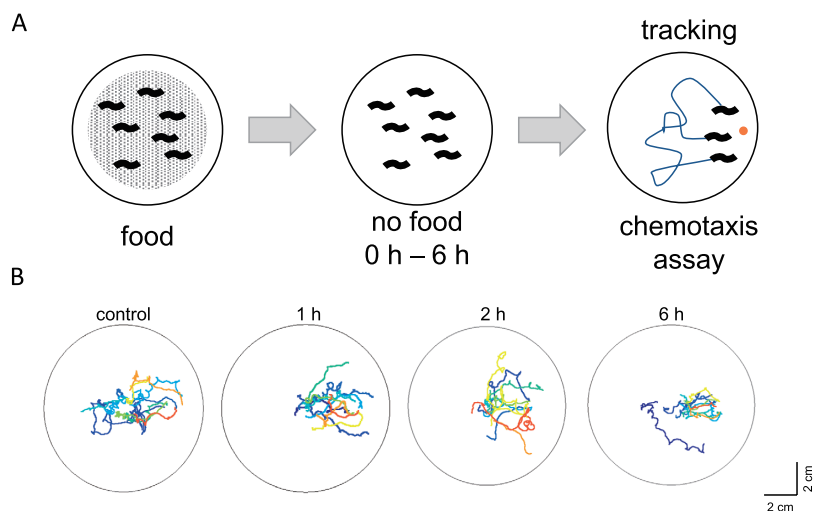

Figure 1 Chemotaxis under food deprivation. (A) Experimental scheme. Chemotaxis under 0-h (control), 1-h, 2-h, and 6-h food deprivation was investigated. (B) The representative tracks of animals on one assay. Each animal is represented by a different color.

function in the coin library) and Kolmogorov-Smirnov test (Fig. 3, 4; R version 3.5.2. with the ks.test function). Holm method (R version 3.5.2. the p.adjust function) was used for controlling the family-wise error rate [21]. The number of assays (N) and animals (n) for all analyses were: control: N=11, n=81; 1 h: N=10, n=82; 2 h: N=9, n=72; 6 h: N=8, n=67.

Results

Food deprivation affects chemotactic behavior

To identify the modulation of chemotactic strategy by food deprivation, we performed the chemotaxis assay for an attractive odor, isoamyl alcohol (IAA), with worms after 0–6 hours of food deprivation (Fig. 1A). Almost all worms reached the odor spots; however, approaching trajectories seems modulated by food deprivation (Fig. 1B). For further analysis, several parameters were quantified [12–14,17,22]. As seen in the trajectories, the chemotaxis index, which shows how much worms are attracted to odors, was not modulated by food deprivation (Fig. 2A). Velocity was

decreased as previously reported by 6-h food deprivation (Fig. 2B) [11], and turn (pirouette) frequency was also decreased (Fig. 2C). However, circular variance of bearing angle, which shows how worms do not approach to an odor straight, decreased only in 1-h food-deprived worms (Fig. 2D). These results demonstrate that food deprivation affects chemotactic behaviors, especially weathervane strategy.

Food deprivation affects pirouette strategy

We investigated pirouette behaviors further. Previous research shows worms use the pirouettes which include sharp turns and subsequent short migrations to approach to an odor, and it allows worms to significantly change direction towards an odor source [12,13]. The time derivative of the odor concentration (dC/dt) at pirouette initiation is crucial factor for chemotactic strategy. Therefore, we calculated the probability distributions of pirouette initiation against dC/dt as previous research [12]. The probability distributions of pirouette initiation against dC/dt is modulated by food deprivation over 2 h (Fig. 3A), and the cumulative probabilities became steeper in food-deprived animals over
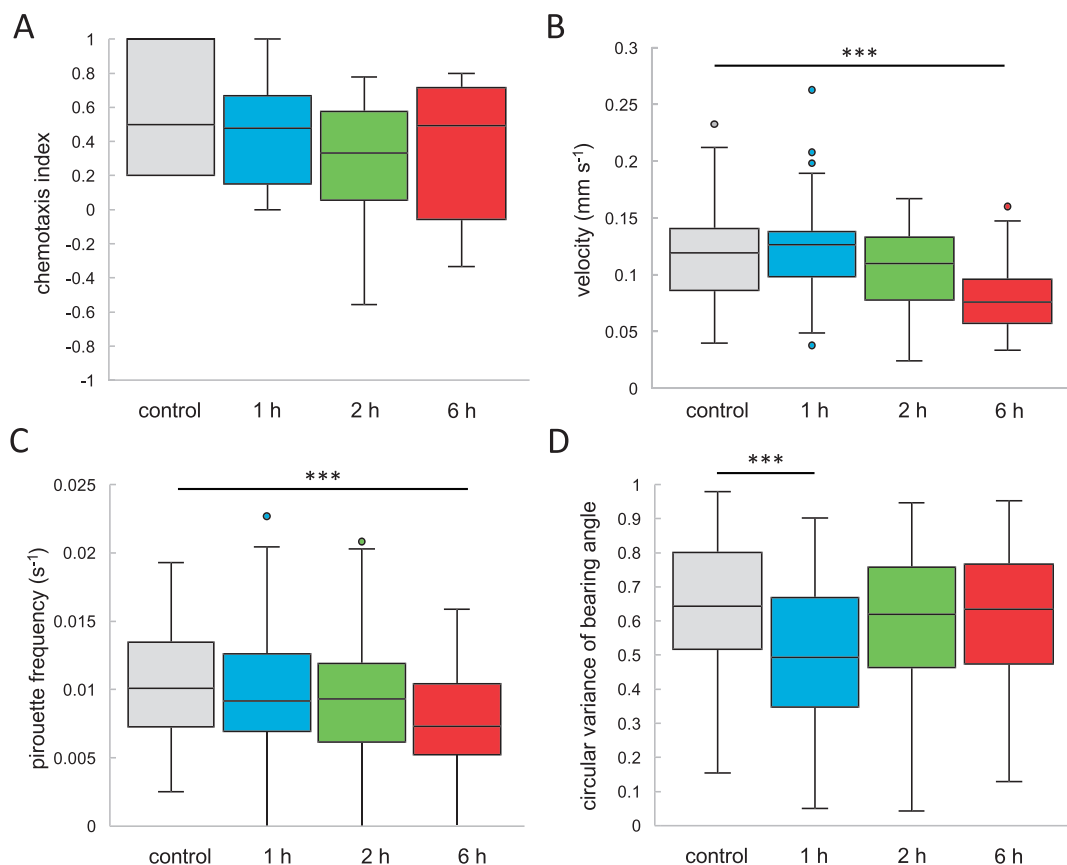

Figure 2 Evaluation of chemotactic behaviors under food deprivation. (A–D) Chemotaxis index (C. I., A), velocity (B), number of pirouettes per unit time (pirouette frequency, C) and circular variance of bearing angle (D). Box plots show median (center line), quartiles (boxes), and range (whiskers). The statistical metrics are as follows: C. I. 1 h: p=0.52; C. I. 2 h: p=0.14; C. I. 6 h: p=0.48; velocity 1 h: p=0.39; velocity 2 h: p=0.37; velocity 6 h: p=1.7×10^{-8}; pirouette frequency 1 h: p=0.48; pirouette frequency 2 h: p=0.21; pirouette frequency 6 h: p=2.4×10^{-4}; circular variance 1 h: p=6.8×10^{-4}; circular variance 2 h: p=0.50; circular variance 6 h: p=0.52 (control: N=11, n=81; 1 h: N=10, n=82; 2 h: N=9, n=72; 6 h: N=8, n=67). Wilcoxon rank-sum test comparing with control with Holm correction. ***p<0.001.
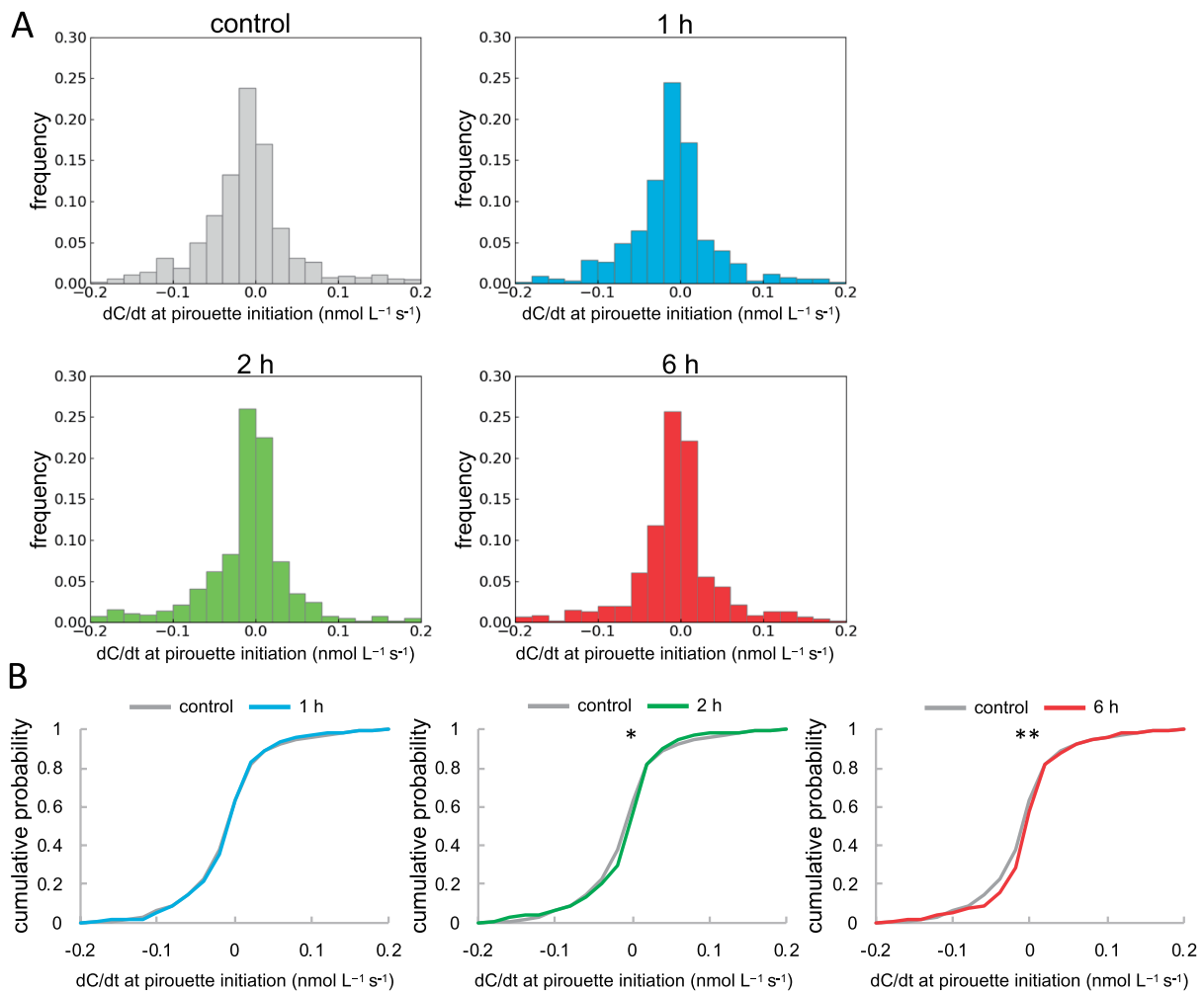
Figure 3 Time derivative of the odor concentration (dC/dt) eliciting pirouette behaviors. (A) Histograms for dC/dt at pirouette initiation. (B) Cumulative probability of the frequencies in A: left, control and 1-h food-deprived animals; middle, control and 2-h food-deprived animals; right, control and 6-h food-deprived animals. The statistical metrics are as follows: 1 h: p=0.77; 2 h: p=0.041; 6 h: p=0.0015 (control: N=11, n=81; 1 h: N=10, n=82; 2 h: N=9, n=72; 6 h: N=8, n=67). Kolmogorov-Smirnov test comparing with control with Holm correction. *p<0.05, **p<0.01.

2 h (Fig. 3B). These results indicate that food-deprived worms initiate pirouettes for smaller changes in odor concentration.

Next, we also analyzed the direction change by pirouette. Comparing the angle between worm's direction and odor source before and after the pirouette, the improvement by pirouette was evaluated (Fig. 4). In 6-h food-deprived animals, the directional change by pirouette became worse than one in control animals. This result indicates 6-h food-deprivation affects control of pirouette behavior.

Discussion

We investigated chemotactic behaviors in food-deprived animals. The chemotaxis index was not changed by food deprivation (Fig. 2A), but circular variance of bearing angle decreased in 1-h food deprived animals (Fig. 2D). This result indicates that worms change the weathervane strategy. In the weathervane strategy, animals gradually migrate to higher

odor concentration [14]. Change of variance of bearing angle suggests the change of the weathervane strategy. On the other hand, worms with food deprivation over 2 h change their sensitivity of pirouette initiation (Fig. 3). In pirouette strategy, worms use pirouettes to approach to an odor to change locomotive direction to an odor source [12,13]. Our result reveals that over 2-h food-deprived animals change the pirouette strategy. Moreover, 6-h food-deprivation worsen the directional change of pirouette (Fig. 4). These results indicate that worms change chemotactic strategy in different manner depend on their internal state related to the duration of starvation. There are various studies about food deprivation including starvation [1,2,8,11,23]; however, the chemotactic behavior, which is an important behavior for foraging is largely unknown. Our report should provide the novel insights to food deprivation.

In this study, the chemotactic strategy was differently changed by duration of food deprivation. In 1-h food depri-
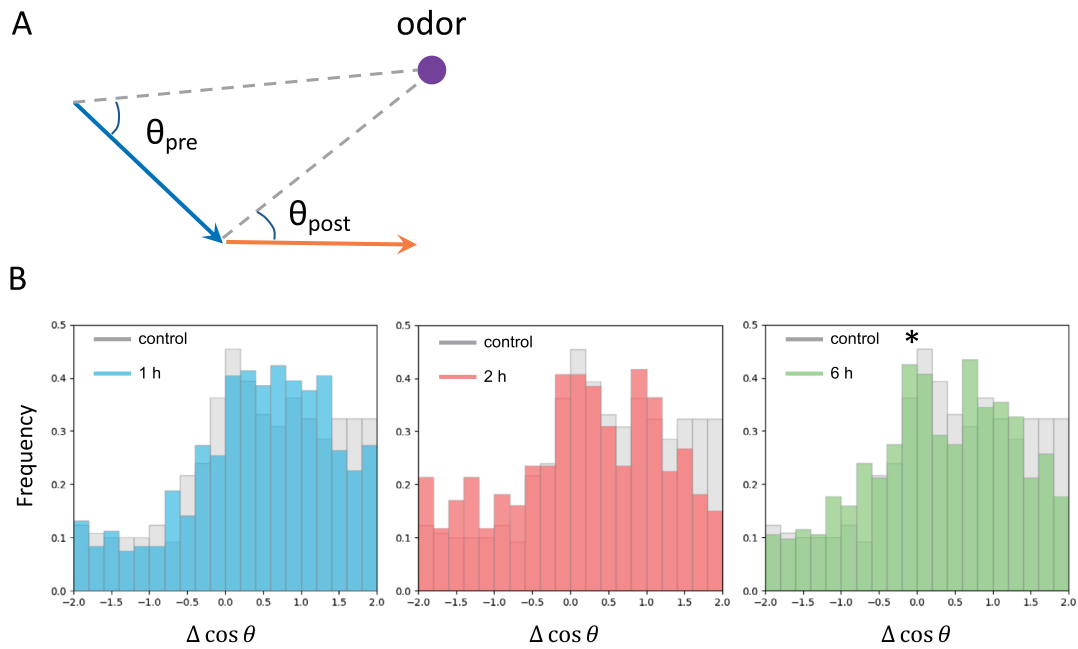
Figure 4 The direction changes by pirouette behaviors. (A) Schematic image of calculating the direction. The difference of $\cos\theta$ before and after pirouette ($\Delta\cos\theta$) was calculated (see also Materials and Methods) (B) Histogram of direction changes ($\Delta\cos\theta$): left, control and 1-h food-deprived animals; middle, control and 2-h food-deprived animals; right, control and 6-h food-deprived animals. The statistical metrics are as follows: 1 h: $p=0.51$; 2 h: $p=0.13$; 6 h: $p=0.016$ (control: N=11, n=81; 1 h: N=10, n=82; 2 h: N=9, n=72; 6 h: N=8, n=67). Kolmogorov-Smirnov test comparing with control with Holm correction. *$p<0.05$.

vation, worms reduced the variance of the locomotion angle. This indicates that worms could move straightly toward the odor. This indicates that worms would rather exploit the food than explore. In 6-h food deprivation, the velocity and pirouette frequency were decreased. This result suggests that worms would rather reduce their energy consumption than reach the food immediately. There was no difference for chemotaxis index itself in our experiments. This indicates that worms could change chemotactic strategy for more subtle concentration because worms can reach toward odor even on a condition that odor concentration is very low [22]. Under such condition, change of chemotactic strategy could induce starved worms to reduce their energy consumption.

We showed that food deprivation changes chemotactic strategy. There are several mechanisms could be hypothesized. Modulation by neuromodulators is one candidate. Dopamine has been known as an important factor for foraging and chemotactic behavior, and also food deprivation [1,10,17,24]. Therefore, the modulation of dopamine could change chemotactic behaviors. Starvation also induces sleep in *C. elegans* to reduce energy consumption [8,25–27]. Neuronal communication consumes large amount of energy, global neuronal activity is modulated by metabolic state via insulin signaling [8,26]. Therefore, insulin signaling could also affect chemotaxis. Combination of these mechanisms could explain the mechanisms of the change of chemotactic strategy by food deprivation. Moreover, these mechanisms could change the responsiveness of pirouette against dC/dt through modulating sensitivity of dC/dt itself (such as [28])

or changing their threshold of dC/dt to initiate the pirouette (such as [29]).

In this research, we only focused on the chemotaxis for IAA. However, worms show chemotaxis for various odor such as diacetyl [10]. For these odors, different sensory neurons senses the odor, and downstream pathway of neuroprocessing should be different [30,31]. Therefore, the effect of food-deprivation could also differ from the results that we showed.

Conclusion

In this study, we show the change of chemotactic strategy by food deprivation. To compare chemotaxis under different food-deprivation period, we showed that worms change their chemotactic behaviors by food deprivation. The worms with 1-h food-deprivation change the weathervane strategy. On the other hand, 6-h food deprived animals change the pirouette strategy. These results demonstrate that worms change chemotactic strategy different way depend on period of food deprivation.

Acknowledgements

Conflicts of Interests

No conflict declared.

Author Contribution

K. S., K. A., K. H. and K. O. designed the experiments. K. S. performed the experiments. K. S. and K. A. performed data analysis; K. A. wrote the original draft of paper and K. A., K. H. and K. O. reviewed and edited the paper. K. A., K. H. and K. O. supervised the work.

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