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RESEARCH ARTICLE

Gait-specific adaptation of locomotor activity in response to dietary restriction in *Caenorhabditis elegans*

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ABSTRACT

Locomotion is crucial for the survival of living organisms, as it allows foraging, flight and mating behaviour. In response to environmental cues, many organisms switch between alternative forms of locomotion, referred to as gaits. The nematode *Caenorhabditis elegans* exhibits two gaits: swimming in liquids and crawling on dense gels. The kinematics and patterns of muscle activity differ between the two gaits, with swimming being less efficient than crawling. We found that *C. elegans* when grown on dietary restriction (DR) plates and then tested immediately for swimming activity exhibit an accelerated frequency of body-bending swimming compared with *ad libitum*-fed worms, resulting in an increased swimming speed. This response is independent of the presence or absence of food bacteria in the assay liquid. In contrast, the crawling speed of DR worms on assay agar plates is decreased and influenced by food availability. Because DR also attenuates the disturbed swimming activity of worms that are deficient in the presynaptic dopamine transporter DAT-1, our data link DR-induced alterations of the swimming gait to synaptic processes. This strongly suggests a biochemical rather than a biomechanical response to DR provoked by changes in the worm's body structure. We conclude that the increase in locomotor activity in response to DR is specific to the swimming gait and might represent a survival strategy, allowing food-deprived nematodes to exit unfavourable environments.

KEY WORDS: Dietary restriction, Locomotory gait, *C. elegans*, Dopamine transporter

INTRODUCTION

A well-developed locomotion system is crucial in animals from a wide variety of taxa, allowing reactions to environmental changes and ensuring survival. Locomotion is generally coordinated by neuronal systems that generate rhythmic neuromuscular activity, which can be either symmetrical or asymmetrical (Zmyslowski and Kasicki, 1982). Adaptation to different environments is often accompanied by qualitatively distinct types of locomotion, referred to as locomotory gaits. For example, shrimp react to changes in temperature or salinity by altering their locomotion. Similarly, fish adjust their locomotion pattern and speed according to the mechanical load exerted by their surrounding medium (Beveridge et al., 2010; Yu et al., 2010).

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Caenorhabditis elegans (Maupas 1900) is an excellent model organism for studying adaptive locomotion, as the kinematic parameters of its locomotion behaviour have been well described. *Caenorhabditis elegans* shows two distinct functional locomotory gaits: swimming in liquids and crawling on dense gels (Gray and Lissmann, 1964; Niebur and Erdös, 1991; Pierce-Shimomura et al., 2008; Cohen and Boyle, 2010). The nematode occurs in habitats that are characterised by rapid environmental fluctuations such as decaying fruits and vegetables, where the worm is confronted with terrestrial and aquatic microniches (Kiontke and Sudhaus, 2006; Felix and Braendle, 2010). Hence, both locomotory gaits are used in the wild. Adaptive locomotion behaviour in response to environmental cues has previously been described in *C. elegans*. For example, swimming nematodes decrease their turning rates in liquids containing an attractive odorant (Luo et al., 2008), exhibit chemotaxis behaviour (Pierce-Shimomura et al., 2008) and respond to temperature variations by performing reorientation behaviours (Clark et al., 2007). Crawling nematodes exhibit food choice behaviours and food-quantity-dependent locomotion strategies such as roaming and dwelling (Shtonda and Avery, 2006; Ben Arous et al., 2009).

Depending on the animal species examined, both decreases and increases in locomotor activity have been described in response to limited food supplies (Pirke et al., 1993; Weed et al., 1997; Geng et al., 2007; Teske and Kotz, 2009; Boehm et al., 2010; Gingerich et al., 2010; Yu et al., 2010). However, gait-specific effects have not been considered. To address this issue, we used the model organism *C. elegans*. We found that the functional activity of the swimming gait increased in response to food deprivation. This activity was not influenced by food availability during a locomotion assay. In contrast, the functional activity of the crawling gait decreased under food restriction and depended on food availability. Furthermore, our dietary restriction (DR) regimen mitigated the dopamine-dependent swimming-induced paralysis (SWIP) phenotype observed in *C. elegans* dopamine transporter (*dat-1*) loss-of-function mutants linking DR-induced alterations of swimming activity to synaptic processes.

RESULTS

DR leads to enhanced swimming activity in *C. elegans*

We investigated whether DR alters the locomotory rate of *C. elegans*. *Ad libitum* (AL)-fed adult worms grown on standard NGM agar plates swam with a body bending swimming frequency (BBSF) of 1.95±0.03 Hz and a speed of 0.29±0.03 mm s^{-1} when transferred to M9 assay buffer (Fig. 1A,B; supplementary material Movie 1). When worms that were grown on agar plates under increasing DR (i.e. decreasing bacteria concentrations; for details, see Materials and methods) were immediately assayed for their swimming activity, we observed a dose-dependent elevation in their BBSF (Fig. 1C). For our standard DR condition, the BBSF was accelerated by ~50% compared with the AL condition (2.81±0.09 Hz; Fig. 1A;

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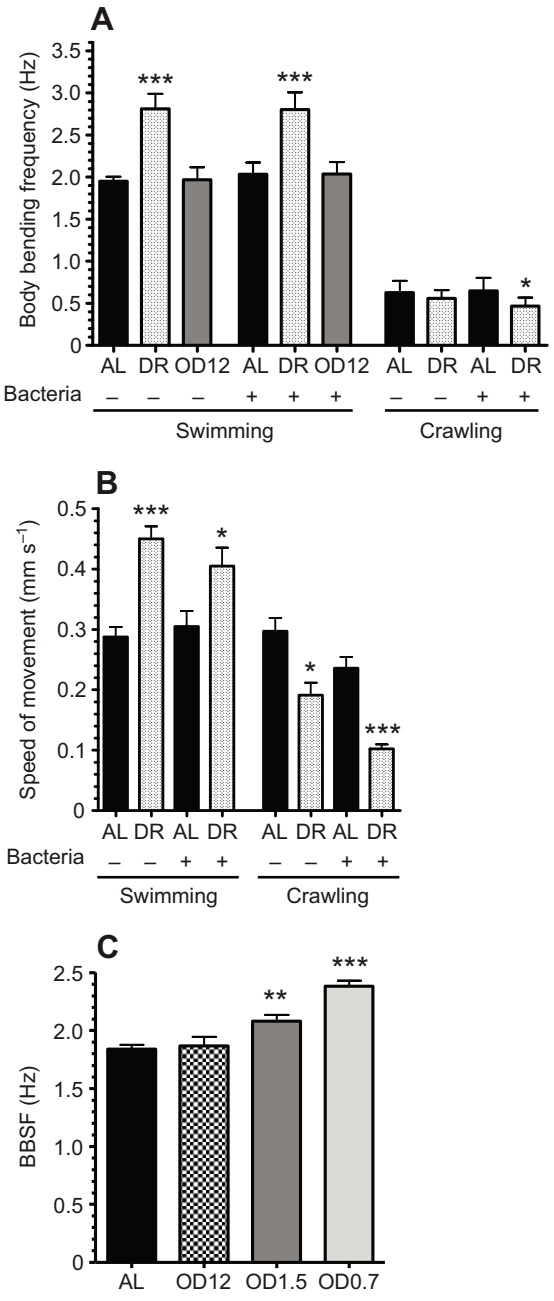

Fig. 1. Influence of dietary restriction on the locomotory activity of adult wild-type *Caenorhabditis elegans*. (A) Body bending swimming frequency (BBSF) and body bending crawling frequency (BBCF) (body bends per second; Hz) of *ad libitum* (AL)-fed and dietary restricted (DR) adult wild-type worms. AL worms were grown on bactopeptone containing standard NGM plates in the presence of OP50 concentrations equivalent to approximately OD_{600}=25. DR and OD12 worms were grown on bactopeptone-free agar plates seeded with concentrations of the food bacteria OP50 photometrically adjusted to OD_{600}=1.5 and 12, respectively. At young adult stage (72 h), worms were transferred to assay swimming and crawling activity. Data are presented for worms swimming in M9 buffer (–) or bacteria in suspension (+, OD_{600nm}=1.5). The crawling assay was performed on 5 g peptone NGM-agar plates with (+) or without (–) a bacterial lawn. Mean (±s.d.) values were obtained from *N*=4 independent experiments, with *n*=5–12 worms tested in each experiment and condition to determine BBSF and swimming velocity. *N*=8 experiments were performed for BBCF, using *n*=4–7 worms for each experiment and condition. *N*=7 independent experiments were performed for crawling velocity, with *n*=5–6 worms for each experiment and condition. The AL group was statistically compared with the DR group under the same conditions. (B) Corresponding swimming and crawling speeds (mm s^{-1}) of worms depicted in A. (C) The effect of increasing dietary restriction on BBSF of young adult wild-type *C. elegans*. A defined number of worms (*n*=100) were grown on bactopeptone-free agar plates seeded with different concentrations of the food bacteria OP50. Bacteria suspensions were photometrically adjusted to OD_{600}=0.7, 1.5 and 12. AL worms were grown on bactopeptone containing standard NGM plates in the presence of OP50 concentrations equivalent to approximately OD_{600}=25. At young adult stage (72 h), worms were transferred to M9 buffer to assay swimming activity. *N*=4; *n*>20 animals. **P*<0.05; ***P*<0.01; ****P*<0.001.

supplementary material Movie 2). The higher swimming frequency of the DR worms translated into an increased swimming speed of 0.45±0.04 mm s^{-1} (Fig. 1B). The efficiency of forward swimming, represented by the slip value, was slightly higher for DR worms than AL-fed worms (Table 1). The change in motor activity was not caused by the bactopeptone-free DR culture conditions, as higher *Escherichia coli* OP50 food concentrations (bacterial OD_{600}=12) on bactopeptone-free plates completely abolished the effect (see condition OD12 in Fig. 1A,C). Furthermore, the BBSF response to DR was not influenced by the presence or absence of food bacteria (OP50 at OD_{600}=1.5) in the assay medium (Fig. 1A,B), nor was it affected by the temperature or low osmolarity [distilled water compared with the physiological buffers PBS and M9 (285–300 mosmol)] of the assay medium (Fig. 2A,B). In M9 buffer with higher osmolarity (>370 mosmol), AL and DR worms tended to coil and swam in an uncoordinated manner (data not shown).

Swimming of AL-fed and DR wild-type worms was also determined as a function of the mechanical load using viscous liquids, in which the worms require greater muscle strength to maintain their locomotory rate (Sznitman et al., 2010). Taking into account that DR-fed *C. elegans* display an ~60% lower protein content, most likely a proxy for muscle mass, than AL-fed worms (Fig. 2C), it is remarkable that these worms were able to swim with an accelerated BBSF and speed even in liquids with higher viscosity (Fig. 2D,E). Thus, DR-induced acceleration of the BBSF is a robust phenotype that is observable under various experimental conditions.

Previous studies in *C. elegans* (Sawin et al., 2000) and some other free-living rhabditids (Rivard et al., 2010) have shown that the presence or absence of bacterial food as well as the feeding status affect the rate of locomotion. *Caenorhabditis elegans* worms starved for short periods (30 min) crawled with the same body bending frequency as AL-fed worms when transferred to assay plates lacking food bacteria. However, after transfer to assay plates with an OP50 bacterial lawn, the starved worms slowed their body bending frequency more drastically when crossing from a region of the plate without bacteria into the bacterial lawn than AL-fed worms, representing the enhanced versus the basal slowing response of starved and AL-fed *C. elegans*, respectively. Similar crawling responses were observed in our DR worms (Fig. 1A,B). In contrast to the results obtained for the swimming gait, our results collectively demonstrated that the crawling gait activity on assay plates was reduced in response to DR and was influenced by food availability during the locomotion assay. Hence, the DR-induced acceleration of locomotor activity is specific for the swimming gait.

To characterise the swimming response in greater detail, we investigated wild-type worms at different life stages. Compared with AL-fed worms, L4 larvae (age 48 h), young adults (age 3 days) and older adults (age 4–7 days) showed a 31 to 69% higher BBSF in response to DR (Fig. 3A). We next examined the effect of re-feeding on locomotor activity. Worms were first grown under standard DR conditions for 72 h and then transferred to AL agar plates (re-feeding

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Table 1. Swimming-related parameters in young adult N2 wild-type *Caenorhabditis elegans* worms (age 72 h) under *ad libitum* (AL) and dietary restriction (DR) conditions

Condition	Body length (mm)	Speed of progression (V_x) (mm s^{-1})	Speed of waves (V_w) (mm s^{-1})	Speed ratio (V_x/V_w)	Slip (%)
AL	1.03±0.07	0.29±0.03	2.02±0.16	0.14±0.01	85.8±1.1
DR	0.83±0.11	0.45±0.04	2.26±0.33	0.20±0.04	79.6±4.1

The parameters were analysed with the Multi-Worm Tracker system. Values are means ± s.d. 1 slip=100[1–(V_x/V_w)].

condition) for an additional 24–96 h, before being assayed for swimming activity. Interestingly, the DR-induced acceleration of the BBSF was not completely abolished after 72 h of AL re-feeding (Fig. 3A). As it has been reported that different DR regimes lead to overlapping, but non-identical, responses in *C. elegans* (Hara et al., 1998), the effects of starvation on the BBSF were also tested. For L4 larvae and adult worms, we observed an almost linear increase in the BBSF during the first 8 h of starvation (Fig. 3B). As depicted in Fig. 3C, the starvation response was slightly greater than the DR response. Taken together, our results demonstrate that L4 larvae and adult worms exposed to different food restriction regimens respond with increased BBSF.

Genetic models of DR and protein restriction confirm the effect of DR on the swimming gait of *C. elegans*

To further demonstrate that DR accelerates the BBSF, we used the *eat-2(ad465)* mutant strain. In *eat-2* mutant worms, the pharyngeal pumping rate and ingestion of food are reduced, and this strain therefore serves as a genetic model of DR (Thomas, 1990; Avery, 1993). Compared with N2 wild-type worms, adult *eat-2(ad465)* animals exhibited a 28% higher BBSF (2.10±0.15 Hz; Fig. 3D). Dietary restriction further increased the BBSF of the *eat-2(ad465)* worms. Interestingly, the BBSF values of DR-fed wild-type and *eat-2(ad465)* mutant worms were very similar (2.62±0.13 versus 2.65±0.16 Hz), suggesting an upper limit of the BBSF (Fig. 3D). In

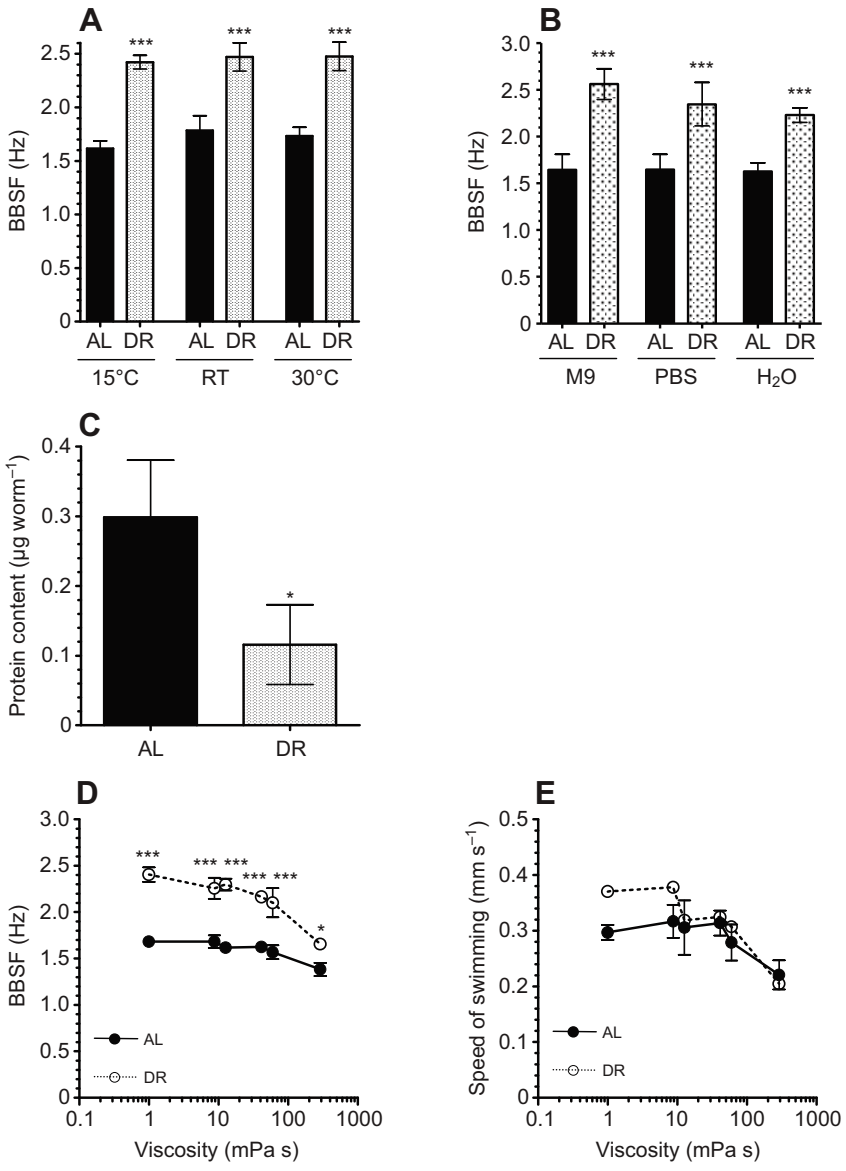

Fig. 2. Effect of assay medium on the DR-induced increase of BBSF in *C. elegans*. (A) The assay medium (M9 buffer) was cooled to ~15°C or heated to ~30°C. RT, room temperature. BBSF results (in Hz) are means (±s.d.) of *N*=3 independent experiments, with *n*=10–26 worms counted per condition. (B) Influence of different assay media on the BBSF of AL-fed and DR adult wild-type worms. Adult N2 worms were placed in M9 buffer, PBS buffer or water. *N*=3 independent experiments were performed with *n*=14–26 worms per condition. (C) Protein contents (µg protein per worm, as analysed via the bicinchoninic acid protein assay) of AL-fed (black bar) and DR (grey bar) adult wild-type worms. Means (±s.d.) of *N*=3 experiments are shown. (D,E) Influence of the viscosity of the assay medium on (D) BBSF and (E) swimming velocity of N2 worms. A comparison of the BBSF and velocity of AL-fed (black circles, straight line) and DR (white circles, dotted line) adult wild-type worms swimming in M9 buffer (1 mPa s) and viscous CMC buffer is presented. BBSF is expressed as body bends per second in Hz. Swimming velocity (mm s^{-1}) was analysed using the multi-worm tracker program. Mean (±s.d.) values were calculated for *N*=3 experiments and *n*=15 worms per condition. **P*<0.05; ****P*<0.001.

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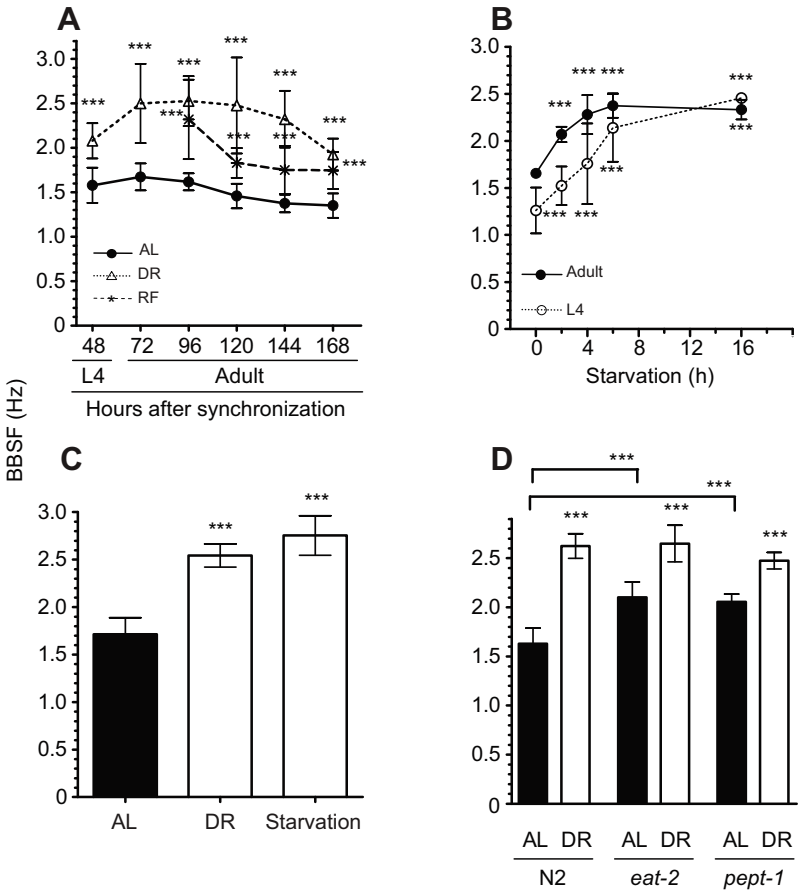

Fig. 3. Influence of the DR paradigm on the BBSF of wild-type larvae and adults of *C. elegans*. (A) BBSF values for synchronised wild-type L4 larvae (48 h) and reproductive hermaphrodites (72–168 h) under AL, DR and re-feeding (RF) regimes. The AL and DR regimes were applied beginning in eggs (0 h) up to the reproductive stage (168 h). In the re-feeding experiment, the worms were maintained under the DR regime for 72 h and then switched to the AL condition. Analysis of BBSF was carried out every 24 h. *N*=3 independent experiments were performed, with *n*=18 worms being tested per condition. The results of the AL feeding experiment were compared to those for DR and RF worms of the same age. (B) Acceleration of BBSF due to starvation. AL-fed L4 (white circles, dotted line) and adult (black circles) wild-type worms were starved for 2–16 h prior to analysis of their BBSF. *N*=2 independent experiments were performed with *n*=12 worms per condition. The AL worms were compared to the starved group at the same time point. (C) BBSF of synchronised adult wild-type worms exposed to AL (black), DR (grey) or starvation (white). For the starvation condition, AL-fed L4 larvae were placed on bacteria-free plates for 18–24 h. *N*=49 independent experiments were performed for AL and DR, with *n*=297 worms being tested for AL and *n*=283 worms tested for DR. For the starvation condition, *N*=13 independent experiments were performed with *n*=90 worms. (D) BBSF of *eat-2(ad465)* and *pept-1(lg1601)* mutants under DR and protein restriction. Wild-type worms were analysed 72 h after synchronisation (adult stage) (*N*=3 independent experiments performed on *n*=27 worms), while *eat-2(ad465)* worms were analysed after 96 h (adult) (*N*=3 independent experiments performed on *n*=21 worms) and on *pept-1(lg1601)* worms after 80–90 h (adult) (*N*=2 independent experiments performed on *n*=12 worms). Comparisons between the AL and DR conditions for the same strain and between AL-fed wild-type and AL-fed mutant strains are presented. **P*<0.05; ***P*<0.01; ****P*<0.001.

addition, we analysed *pept-1(lg1601)* worms, which serve as a genetic model of protein restriction (Meissner et al., 2004). The BBSF of AL-fed adult *pept-1(lg1601)* worms was 26% higher compared with AL-fed adult wild-type worms (Fig. 3D). RNAi knockdown of *pept-1* (K04E7.2) produced similar results (data not shown). As observed in the *eat-2(ad465)* worms, DR increased the BBSF values of *pept-1(lg1601)* worms to levels found in DR wild-type animals. This demonstrates that the BBSF of adult worms increases in genetic models of DR and protein restriction.

Body size and body elasticity do not affect the DR swimming gait

Adult worms exposed to DR displayed shorter body lengths and smaller body volumes compared with AL-fed worms (Fig. 4A,B,D). Both AL and DR worms swim in M9 assay buffer (density: ρ=1 g cm^3; viscosity: μ=1 mPa s) at low Reynolds numbers (*Re*=0.32 versus 0.45) based on their aforementioned swimming speed and body lengths of *L*=1.11±0.3 and 1.01±0.3 mm, respectively. However, the altered *Re* value of DR worms indicates that they experience a slightly higher friction drag. A previous biomechanical analysis of worms showed that muscle power during swimming is primarily devoted to maintaining body elasticity (Park et al., 2007). To test whether body elasticity and body size influence the swimming activity in response to DR, we employed the mutant strains *dpy-5(e61)*, *lon-2(e678)* and *egl-4(n478)*. Despite their altered body lengths, widths and/or elasticities (Park et al., 2007), these mutants showed BBSF values similar to those of N2 wild-type worms under AL and DR feeding conditions (Fig. 4C). In addition, larvae that were approximately 50% smaller (L4 larvae) exhibited a similar BBSF to adult worms (Fig. 3A). Furthermore, we found that wild-type worms fed heat-inactivated bacteria at a concentration similar to our standard AL condition were smaller than AL-fed control worms grown on living OP50 (Fig. 4D). However, their reduced body length did not affect their BBSF (Fig. 4E). Remarkably, when the concentration of heat-inactivated bacteria was reduced to a value comparable to that of the DR condition, no further decrease in body length was observed (Fig. 4D). Nevertheless, these worms responded with a significant acceleration of the BBSF (Fig. 4E), indicating that body size and the BBSF are not linked. In conclusion, the DR-induced acceleration of the BBSF appears to be a biochemical response that is not influenced by the biomechanical constraints of the animals.

DR mitigates swimming-induced paralysis in *dat-1*-deficient mutants

Finally, we chose *dat-1* mutant worms deficient in the plasma membrane dopamine transporter DAT-1 in order to link DR to synaptic processes that control the swimming activity of *C. elegans*. In *dat-1* mutants, dopamine accumulates within the synaptic gap owing to a lack of re-uptake of the biogenic amine. As a consequence, the elevated dopamine concentration induces a paralysis in L4-stage animals under swimming conditions known as swimming-induced paralysis (SWIP) (Hardaway et al., 2012) that is associated with reduced acetylcholine signalling in cholinergic motor neurons (Allen et al., 2011). Accordingly, L4 *dat-1(ok157)* mutant worms became paralysed within 10 min when swimming in water, while N2 wild-type worms exhibited sustained thrashing (Hardaway et al., 2012) (Fig. 5A). It has been previously demonstrated that a decreased dopamine synthesis/release or an increased acetylcholine signalling at neuromuscular junctions

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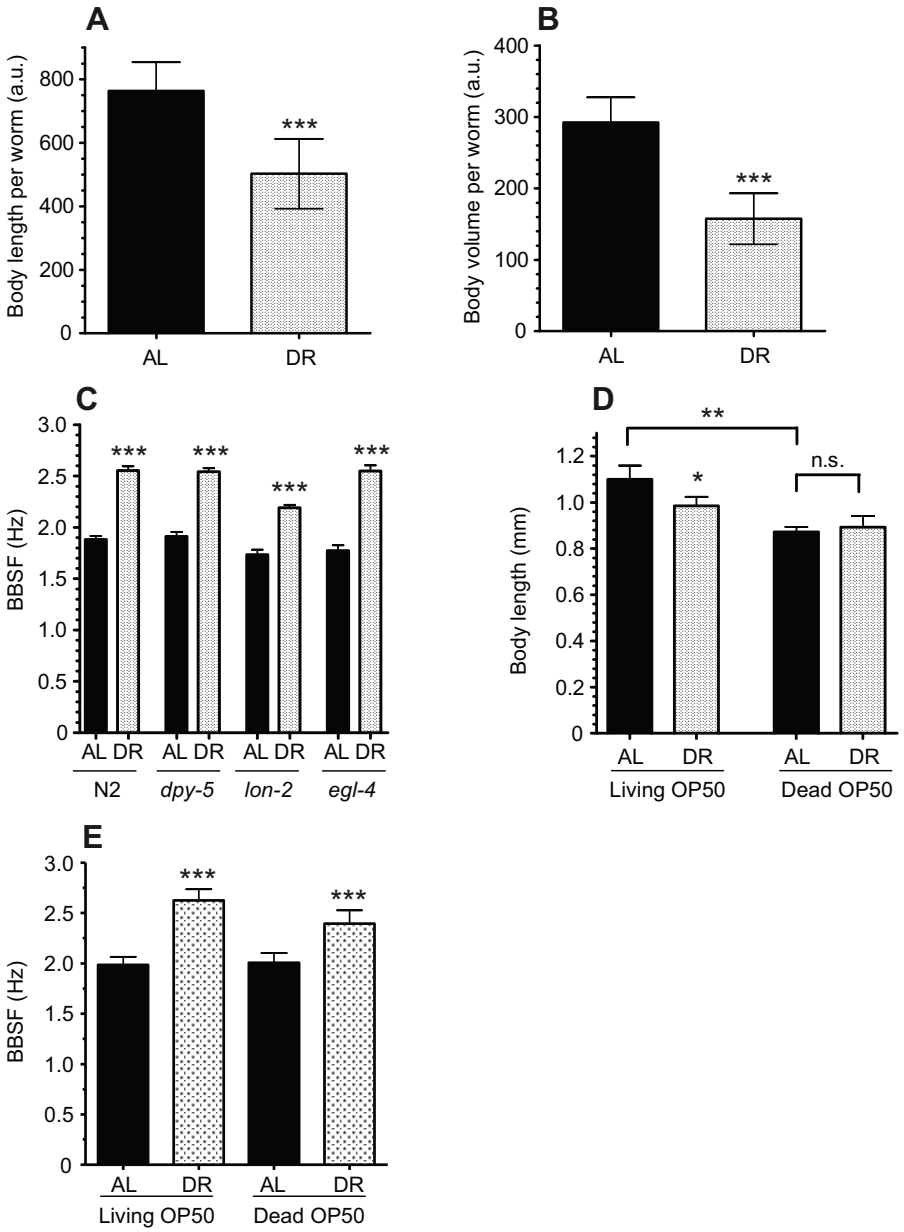

Fig. 4. Influence of DR on body proportions and BBSF of adult wild-type and mutant *C. elegans*. (A) Body length and (B) body volume per worm, analysed using the flow cytometry-based COPAS Biosort system. Means (±s.d.) were calculated for *N*=3 independent experiments performed in *n*=8654 AL worms and *n*=23,124 DR worms. ***P<0.001, Student's *t*-test. (C) BBSF values for AL-fed (black) and DR (grey) mutants, showing altered body sizes and elasticities. Values are means (±s.d.) of *N*=3–5 independent experiments involving *n*=6–12 worms per experiment. (D) Body lengths and (E) corresponding BBSF values for N2 animals fed on living (AL, DR) and heat-inactivated [dead; OD25 (=AL), OD2 (=DR)] *Escherichia coli* OP50. Values are means (±s.d.) of *N*=4–6 independent experiments involving *n*=5–12 worms per condition. *P<0.05; **P<0.01; ***P<0.001; n.s., not significant.

suppresses SWIP (Allen et al., 2011). Similarly, we found that paralysis was significantly mitigated when *dat-1(ok157)* worms were grown under DR conditions. After 10 min, approximately 70% of the DR-fed *dat-1(ok157)* worms were still swimming, whereas less than 10% of the AL-fed *dat-1(ok157)* worms exhibited swimming activity (Fig. 5A,B). These data allude to a link between DR and synaptic processes (namely, increased acetylcholine and/or decreased dopamine signalling) and support our hypothesis that the accelerated BBSF of DR-fed worms represents a biochemical response.

The DR-induced rescue of the SWIP phenotype resembles the reported suppressor effect of reduced dopaminergic signalling in *dat-1(ok157)* caused, for example, by introducing *cat-2* or *dop-3* loss-of function alleles (*cat-2* encodes the dopamine synthesis enzyme tyrosine hydroxylase, *dop-3* the D2-like dopamine receptor DOP-3) (Allen et al., 2011; Hardaway et al., 2012). Hence, we next examined whether a reduced dopamine signalling is involved in the DR-induced alterations of swimming activity. As shown in Fig. 5C, *cat-2(e1112)* and *dop-3(vs106)* worms that had been grown under AL and DR conditions exhibited BBSF similar to that of N2 wild-type worms, indicating that a reduced dopaminergic signalling does not affect the swimming activity of *C. elegans*. We conclude that dopamine signalling is most probably not involved in the observed DR effects on swimming activity in *C. elegans*.

DISCUSSION

Our results revealed that *C. elegans* responds to DR by showing a markedly accelerated swimming gait. In contrast, crawling gait locomotor activity was reduced under DR compared with the AL condition. The latter is consistent with reported data from worms exposed to short-term starvation (Sawin et al., 2000; Rivard et al., 2010). Because nematode swimming is less efficient than crawling (Gray and Lissmann, 1964), the swimming response might be important for maintaining, or even increasing, the swimming speed during nutrient limitation so that they can exit unfavourable conditions. In this regard, the observation made by Vidal-Gadea et al. (Vidal-Gadea et al., 2012) that *C. elegans* when entering liquid began swimming and at the same time terminated feeding behaviour

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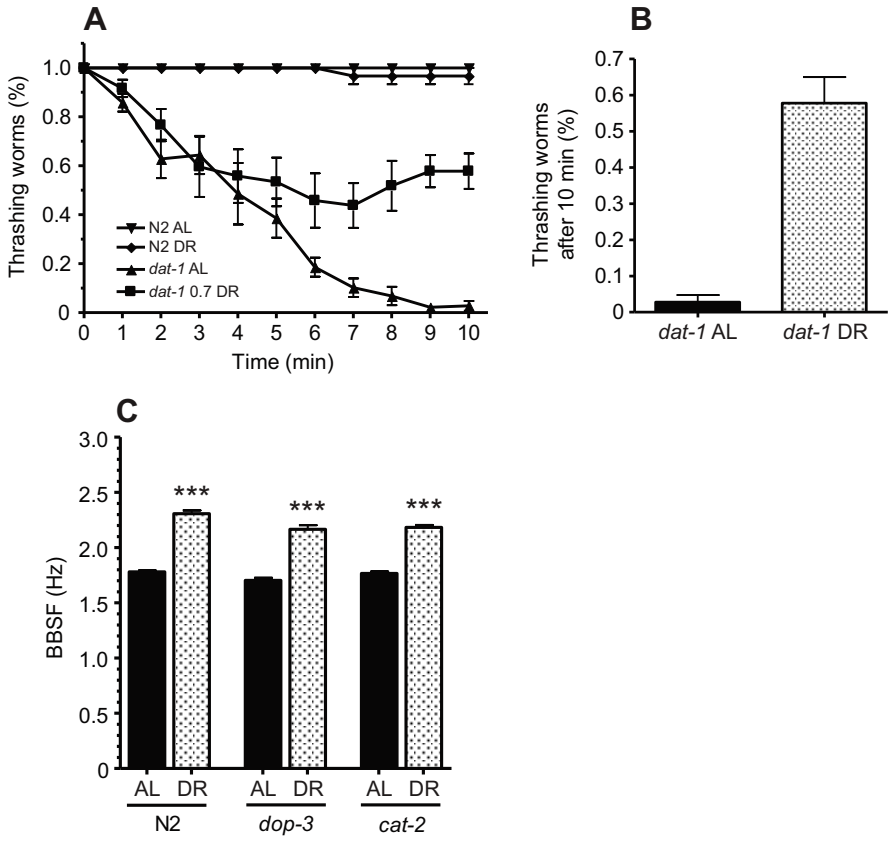

Fig. 5. Influence of DR on swimming-induced paralysis in *dat-1(ok157)* mutant *C. elegans*. N2 wild-type and *dat-1(ok157)* mutant worms were grown under AL and DR conditions. Five to 10 early- to mid-stage L4 animals were transferred in a drop of water, and their swimming behaviour was monitored over 10 min. (A) Percentage of thrashing worms determined each minute. (B) Percentage of thrashing worms at the 10 min time point. Values in A and B are means (±s.d.) of N=3–5 experiments, with n=10–30 animals being tested per condition. (C) BBSF of N2 wild-type, *cat-2(e1112)* and *dop-3(vs106)* mutant worms containing an impaired dopaminergic signalling. Worms were grown on AL and DR plates before they were assayed for their swimming activity. Values are means (±s.d.) of at least four independent experiments with eight to 12 animals tested per condition. ***P<0.001.

(foraging, pharyngeal pumping and feeding) is remarkable. Therefore, we propose that the DR-induced acceleration of the BBSF and swimming speed is of adaptive significance. Because several DR techniques have been established in *C. elegans* (Hara et al., 1998), it is important to note that our protocol is a modified solid medium-based DR protocol. This method (Palgunow et al., 2012) allows standardised reduction of bacterial food during development without starvation, dauer formation or growth arrest. As our method is based on the exclusion of peptone and serial dilution of living *E. coli*, possible side effects of antibiotics or heat treatment can be excluded. Furthermore, peptone effects can be excluded because higher food concentrations on peptone-free plates completely abolish increased motor activity (see Fig. 1A,C). Moreover, *eat-2* worms, which are a genetic model of DR, exhibited an enhanced BBSF in the presence of peptone.

Our data suggest that the DR-induced swimming gait motor activity is a biochemical response, rather than a biomechanical response. Our experiments involving mutants (see Fig. 4C) and worms grown on dead bacteria (see Fig. 4D,E) indicated that body elasticity and body size were not linked to the altered BBSF. In contrast, our SWIP experiments using *dat-1*-deficient mutants support a biochemical mechanism by linking DR to synaptic processes involved in swimming activity. DAT-1 deficiency results in the accumulation of dopamine in the synaptic cleft. When dopamine reaches a critical threshold, there are inhibitory effects via the dopamine receptor DOP-3 on acetylcholine release in the ventral-cord motor neurons, triggering paralysis (Allen et al., 2011). Because DR mitigated the SWIP phenotype, we hypothesised that DR either reduced neuronal dopamine release or increased acetylcholine release at neuromuscular junctions. Our data on the swimming activity of *cat-2* and *dop-3*-deficient mutants with an impaired dopaminergic signalling (see Fig. 5C) clearly point to a dopamine-independent mechanism. Hence, we suggest that the DR stimulation of acetylcholine release in motor neurons acts in parallel to dopamine signalling through the DOP-3 receptor. In line with this, dopamine-independent suppression of SWIP has been also found in mutant worms that are characterised by an increased acetylcholine signalling in the neuromuscular junction, such as the acetylcholine esterase loss-of-function mutant *ace-1(nd35)* or the AMPA-type glutamate receptor gain-of-function mutant *glr-1(nd38)* (Allen et al., 2011). Moreover, our SWIP results serve as an example of a dietary regime that has a beneficial effect on disturbed neuromuscular activity, consequently affecting behaviour. Interestingly, a low-carbohydrate, high-fat diet, known as the ketogenic diet, that mimics the effects of fasting is used to treat epilepsy (Kossoff et al., 2009). Although this diet is an established anticonvulsant treatment, its mode of action remains elusive.

Compared with AL-fed worms, the protein content of the DR nematodes was reduced by approximately 40%. In mammals, muscle proteins represent nearly half of the total protein pool (Rooyackers and Nair, 1997). Assuming similar proportions in *C. elegans*, we postulated that the DR worms exhibited less muscle mass than the AL-fed worms. Accordingly, previous studies have shown that starvation activates the proteolytic system and leads to the degradation of body wall muscles in *C. elegans* (Zdinak et al., 1997; Szewczyk et al., 2000; Szewczyk et al., 2002). The observed reduction of the protein content combined with higher locomotory activity is reminiscent of a phenomenon described in penguins and rats. Penguins end their long fasting period during breeding when their fat stores are depleted and protein utilisation increases. At this point, the starved birds begin walking and re-feeding (Robin et al., 1988; Koubi et al., 1991; Robin et al., 1998). In starved rats, there is also an increase in locomotor activity related to increased protein utilisation (Koubi et al., 1991). Therefore, utilisation of muscle

protein might be an internal signal that induces locomotor activity as an evolutionarily conserved mechanism present in animals from diverse taxa, including *C. elegans*. Accordingly, in *C. elegans*, deficiency in the intestinal peptide transporter PEPT-1 that mimics protein restriction attributable to a dramatically decreased uptake of nutritional di-/tripeptides (Meissner et al., 2004) leads to a higher BBSF, even under AL feeding conditions (see Fig. 3D). Thus, we predict that a reduced protein/amino acid level contributes to augmentation of the swimming rate.

Adaptation of locomotor activity in response to food deprivation has been observed in other species. For example, *E. coli* reduces its swimming velocity in response to starvation by adjusting the intracellular concentration of the second messenger cyclic di-GMP (Boehm et al., 2010). In addition, starved shrimp (Yu et al., 2010) and fish (Gingerich et al., 2010) decrease their swimming speed. In contrast, rats show hyperactive wheel running in response to starvation (Koubi et al., 1991; Pirke et al., 1993). Similarly, caloric restriction increases physical activity in rats (Geng et al., 2007; Teske and Kotz, 2009) and rhesus monkeys (Weed et al., 1997). It is of note that up to 80% of human patients with the eating disorder anorexia nervosa show excessive physical activity (Koubi et al., 1991; Hebebrand et al., 2003). This phenomenon was originally discussed in 1888 by Gull (Gull, 1888). However, it remains unclear whether the hyperactivity of these patients is just a strategy to lose weight or whether it is derived from an unconscious biological need to forage or even to regulate hypothermia (Hebebrand et al., 2003; Carrera et al., 2012). In summary, in response to food deprivation, either decreased or increased locomotor activity can occur depending on the species. These responses reflect passive and active modes of foraging, respectively. The present study revealed that in *C. elegans*, increased locomotor activity is exclusive to the swimming gait. We propose that this gait-specific adaptation might be a survival strategy for food-deprived nematodes allowing them to leave unfavourable conditions.

MATERIALS AND METHODS

Maintenance of *C. elegans* strains and viscous liquids

The nematodes were maintained at 20°C on NGM agar plates with OP50 *E. coli* as a food source, according to standard protocols (Brenner, 1973). The following strains were obtained from the *Caenorhabditis* Genetics Center (USA): Bristol N2 (used as wild-type), *cat-2(e1112)II*, *dop-3(vs106)X*, *dpy-5(e61)I*, *eat-2(ad465)II*, *dat-1(ok157)III*, *egl-4(n478)IV*, *lon-2(e678)X* and *pept-1(lg1601)X*. Viscous liquids were prepared from carboxymethylcellulose (CMC) diluted in M9 buffer as described previously (Sznitman et al., 2010). CMC was fully dissolved via heating in an autoclave, and the viscosity of the CMC solutions was measured using a rotation viscosimeter (MC 200, Fa. Paar, Austria).

Flow cytometry and protein determination

The Complex Object Parametric Analyser and Sorter (COPAS) Biosort system (Union Biometrica, Belgium) was employed to sort worms and to determine time of flight and extinction values for the worms. These values are proxies for the length and volume of the nematodes, respectively (Klapper et al., 2011; Miersch and Döring, 2012a; Miersch and Döring, 2012b). Standard instrument settings and thresholds were used, as described previously (Klapper et al., 2011; Miersch and Döring, 2012a; Miersch and Döring, 2012b). The sorting and gating regions were adapted for this study. To determine the total protein content of the worms, 1000 adult worms were collected using the COPAS Biosort system in M9 buffer. The worms were homogenised in a modified NET buffer (50 mmol l^{-1} Tris pH 7.5, 150 mmol l^{-1} NaCl, 1 mmol l^{-1} EDTA pH 8.0, 0.5% CHAPS, 1× protease inhibitor cocktail; Roche Diagnostics, Germany) with a Precellys®24 bead-beating homogeniser (Peqlab, Germany) as described previously (Miersch and Döring, 2012a; Miersch and Döring, 2012b). The resulting supernatant was stored at −20°C until analysis. The amount of protein was determined using the Pierce bicinchoninic acid protein assay kit (Thermo Fisher Scientific, USA).

Food restriction regimes

The applied DR protocol was recently developed in our laboratory and allows a standardised variation of the extent of DR without starvation, dauer formation or arrest of the animals during development (Palgunow et al., 2012). To analyse the effect of increasing DR conditions, photometrically adjusted optical densities (250 µl, OD_{600nm}=0.7 to 12.0, represents DR 0.7 to 12.0) of the *E. coli* strain OP50 were spread onto antibiotic-free NGM agar plates lacking bactopeptone as the sole carbon source for bacterial growth. For our standard DR regime, 3.75×10^8 cells (OD_{600nm}=1.5) were seeded on NGM agar plates lacking bactopeptone. AL-fed control animals were grown on 5 g l^{-1} bactopeptone (BD Biosciences, Germany) NGM agar plates (9 cm in diameter) spread with a lawn of 3.75×10^8 bacterial cells (250 µl of OP50 *E. coli* bacteria at an OD_{600nm}=1.5). The AL and DR plates were incubated at 37°C for approximately 16 h. The photometrically determined bacterial density on the AL and standard 1.5 DR plates was approximately 2.5×10^{10} and 5×10^8 bacterial cells per plate, respectively. In the starvation regime, nematodes were placed on 0 g l^{-1} peptone NGM agar plates lacking bacteria for a specified number of hours. At the beginning of each experiment, nematode cultures were synchronised using 5% sodium hypochlorite to retrieve their eggs, and a defined number of eggs (standard condition 600 eggs) were then placed on the AL and DR plates. To guarantee constant food conditions, the animals were transferred to freshly prepared plates after 54–58 h (≈L4 stage). To cultivate worms on dead bacteria, overnight cultures of *E. coli* OP50 cells were adjusted to cell densities of OD_{600nm}=25 and OD_{600nm}=2 for the AL and DR plates, respectively, before being heat inactivated either through autoclaving or boiling for 20 min. Suspensions of dead cells were spread on bactopeptone-free NGM plates that were treated as described for the culture plates containing living bacteria.

Body bending analysis

To quantify the number of body bends, three to 10 nematodes were transferred with a worm-picker (platinum wire) from AL or DR plates onto empty NGM plates to clean worms from bacteria. After approximately 1 min, worms were placed in a 50 µl droplet of M9 buffer (or any other liquid) onto a diagnostic slide (three wells, 14 mm diameter; Menzel). The worms were immediately filmed with a Canon Legria HF20E camera (Canon, Germany) attached to a Zeiss Stereo Discovery V8 microscope (Carl Zeiss AG, Germany). For the body bending analysis on NGM agar plates, the worms were picked from either 5 g l^{-1} peptone plates with a bacterial lawn (AL control plates) or 5 g l^{-1} peptone NGM agar plates lacking bacteria. The crawling worms were immediately recorded for 30 s using a Tucsen camera (S/N K3000176, 3.0 MD, Xintu Photonics Co., China) attached to a Zeiss Stemi 2000-C microscope. The body bends of swimming worms were counted for 30 s, whereas the body bends of crawling worms were counted as long as the worms crawled uniformly. Windows Media Player Classic (version 6.4.9.1) was used to play the recorded videos. One body bend corresponds to the movement of the head region thrashing from one side to the other and back to the starting position.

Quantification of the speed of movement and Reynolds number

The crawling and swimming speeds of the worms were analysed using the Multi-Worm Tracker (MWT) system, provided by Swierczek et al. (Swierczek et al., 2011). Video recordings were obtained (as described above), converted from MTS to AVI format and trimmed, when necessary, using Aiseesoft MTS Konverter Software, version 6.2.16. If necessary, the videos were cropped to a size of 1 cm for size adjustment and calculation of the exact pixel size per millimetre. The video recordings were usually performed at a resolution of 640×480 pixels for swimming worms and 1024×768 pixels for crawling worms at 25 and 30 frames s^{-1}, respectively. The speed of the worms was analysed over a time period of 10 s of constant swimming or crawling. Mean speed values (s in the MWT program) were calculated from every individual data point within the analysed 10 s video recording for each worm. The slip ratio was calculated using the following

equation: $100[1–(V_x/V_w)]$, where V_x describes the speed of progression measured in mm s^{-1}, and V_w is the speed of waves defined by the body bending frequency (f) per second and the length of the worm (λ), as analysed by the MWT system ($V_w=f\lambda$).

Reynolds numbers for AL and DR worms swimming in M9 were calculated using the equation $Re=\rho UL/\mu$, where ρ is the density of M9 (1 g cm^3), μ is the viscosity of M97 (1 mPa s), U is the swimming speed of *C. elegans* and L is the worm's body length (Sznitman et al., 2010).

Swimming-induced paralysis (SWIP) assay

The N2 wild-type and *dat-1(ok157)* populations were synchronised via hypochlorite treatment. For each strain, 600 eggs were transferred to AL and DR plates at an OD_{600nm}=0.7 (1.88×10^8 cells were spread). The worms were then cultured at 20°C under standard conditions. The SWIP assay was performed as described previously (Hardaway et al., 2012). Briefly, six to 10 early- to mid-stage L4 animals were transferred in 50 µl of water to a single well of a diagnostic slide (three wells, 14 mm diameter; Menzel). Their swimming behaviour was then recorded for 10 min. The video recordings (AVI format) were analysed by monitoring the fraction of thrashing worms over time.

Statistical analysis

Data are expressed as mean values with standard deviations (±s.d.). Statistical analyses were performed using Student's *t*-test and one-way ANOVA with Bonferroni multiple comparison as a *post hoc* test in GraphPad Prism 4.0 software.

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Competing interests

The authors declare no competing financial interests.

Author contributions

K.L., U.F. and D.-C.G. performed experiments and data analysis. F.D. developed the concepts. K.L. and F.D. interpreted the results, and prepared and edited the manuscript.

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Supplementary material

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