

Cyborg Beetle Achieves Efficient Autonomous Navigation Using Feedback Control

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Abstract: Terrestrial cyborg insects were long discussed as potential complements for insect-scale mobile robots. These cyborgs inherit the insects' outstanding locomotory skills, orchestrated by a sophisticated central nervous system and various sensory organs, favoring their maneuvers in complex terrains. However, the autonomous navigation of these cyborgs was not yet comprehensively studied. The struggle to select optimal stimuli for individual insects hinders reliable and accurate navigations. This study overcomes this problem and provides a detailed look at the terrestrial navigation of cyborg insects (darkling beetle) by implementing a feedback control system. Via a thrust controller for acceleration and a proportional controller for turning, the system regulates the stimulation parameters depending on the beetle's instantaneous status. Adjusting the system's control parameters allows reliable and precise path-following navigations (i.e., up to ~94% success rate, ~1/2 body length accuracy). Also, the system's performance can be tuned, providing flexibility to navigation applications of terrestrial cyborg insects.

INTRODUCTION

Terrestrial insect-scale mobile robots have become prominent candidates for post-disaster search-and-rescue missions. Their tiny size and lightweight would help them easily penetrate deep into the rubbles of collapsed buildings without causing additional collapses. While there are growing efforts to achieve insect-level autonomy in these robots, it is still a challenge to match their natural-born counterparts, i.e., living ambulatory insects. While control autonomy was achieved in various insect-scale mobile robots [1-5], power autonomy was demonstrated only in a few platforms, like HAMR-F [2] or Robeetle [5]. Furthermore, although inverted and vertical climbing was demonstrated [1, 4], maneuvering across complex terrains is still a conundrum for these artificial robots. However, the answers to these challenges might be found in the emerging ideas of biohybrid systems, which utilize the advantages of organic components in robotics applications [6-10]. While bio-inspired and biomimetic systems seek insights from living materials, biohybrid systems incorporate them with synthetic devices [6, 8]. This approach allows the hybrid systems to exploit both organic and artificial benefits from their two facets [6, 8]. These systems were successfully and widely demonstrated in the form of millimeter-scale actuators or aquatic/airborne miniature robots [7-10]. In the case of insect-scale mobile robots, the biohybrid systems might be built by integrating living terrestrial insects (as robotics platforms) with synthetic electronic devices (as central control units). This integration would allow the inheritance of the insects' autonomy, including their perfect locomotory capabilities to crossover complex terrains, regulated by a top-notch inner control system (i.e., their central nervous system, CNS) [11, 12]. Besides, the insects' fascinating biological body structure, which is made of natural soft actuators (muscles), durable exoskeleton, complex joints, integrated proprioceptors, and environmental sensors, will provide these insect-platformed robots with both flexibility and robustness [6].

Such biohybrid systems are also known as cyborg insects, insect-machine hybrid systems, or insect biobots [13, 14]. These cyborg insects are the fusion of a living insect platform and a miniature electronic device (or a backpack), which is employed to regulate the insect's locomotion via electrical stimulation [13, 14]. These hybrid systems will possess the potential to outperform the artificial insect-scale mobile robots in insect-like autonomy. More specifically, with a simple add-on electronic circuit, these systems can retain the outstanding locomotory skills of the insect platform to favor the complex and unpredictable post-calamity terrains [15]. At the same time, they still can provide various controllability with low power consumption (i.e., a few 100 μ W [16-18]). Moreover, due to the biodegradable characteristic

and the natural reproduction ability, cyborg insects are environmentally friendly, have a low-cost assembly, and guarantee a sustainable supply.

Locomotion control of cyborg insects can be realized via the electrical stimulation of their (peripheral or central) nervous or neuromuscular systems [19-23]. This stimulation-based control brings simplicity by freeing the electronic backpack from sophisticated tasks conventionally required for legged robots (e.g., coordinating multiple legs and joints [24]). The computational resource thus is saved for other essential tasks (e.g., victim detection [25] or localization [26]). Besides, the well-established stimulation methods, which are greatly diverse in both regulated movements and insect species, provide flexible choices to cope with the desired locomotion control from the diverse insect kingdom. For example, a variety of motions (e.g., directional turns, forward/backward/sideways walks) in darkling beetles (*Zophobas morio*) and cockroaches (*Periplaneta americana*, *Gromphadorhina portentosa*) can be induced by stimulating their sensory systems like antennae, elytra, and cerci [17, 18, 21, 25, 27]. These induced motions were discussed as reassembling the insects' behavioral responses when the same sensory organs were naturally evoked [28, 29]. Electrical stimulation of muscular systems can also be employed. For instance, stimulating a giant beetle's leg and flight muscles (*Mecynorrhina torquata*) reportedly regulated both its terrestrial and inflight motions [23, 30]. Similarly, the stimulation of neural sites also involves maneuvering cyborg insects. For example, the flight initiation and cessation of giant beetles and the turning motion of cockroaches (*Blaberus discoidalis*) were obtained by stimulating their optic lobes [20] and prothoracic ganglion [22], respectively.

In the recent decade, the development of locomotion control gradually enabled the navigation control of cyborg insects, especially in terrestrial species. The pioneer demonstrations were manual navigations, directing the insects along predetermined paths [21, 31]. Automatic navigation systems were then presented to evaluate the insects' response to their stimulation [32] or exhibited functionalities of the backpack [33]. However, neither the systems' performances nor their control algorithms were introduced and analyzed in detail. Recently, a control system for autonomous navigation of terrestrial cyborg insects exploring unknown and obstructed environments was demonstrated [25]. Together with its pioneers, this system gradually brings cyborg insects closer to their practical applications in search-and-rescue missions.

Despite such a progressive development, almost all contemporary navigations of terrestrial cyborg insects encountered an identical issue, the high variation in the effect of electrical

stimulation across individual insects and species. Several studies employed a fixed stimulus, commonly interpreted as an effective one, across different insect individuals [21, 32, 34]. In addition, such an effective stimulus was altered between studies [21, 32, 34]. However, as each insect is physiologically unique, one's response to an identical stimulus will be unavoidably different from others. Thus, the fixity of electrical stimulation might cause a great deviation in the insects' response, consequently losing their controllability and worsening the navigation outcomes. This causality might explain the struggle of previous studies, in which the rate of successfully directing the cyborg insects along the assigned paths was ~10-30% [21, 32]. Although these numbers did not represent the studies' significance (as navigation was not the focus), they undoubtedly imply an urge to resolve the issue of electrical stimulation. While adjusting the stimulus individually for each insect is possible, this procedure is tedious. The search for an optimal stimulus to produce a convergent locomotory response across different individuals was attempted [34]. Despite its moderate outcome (i.e., ~50% of the experimented insects reacting similarly), this approach was limited to one species, *Gromphadorhina portentosa*, and yet tested in navigation control [34].

In tandem with these insect-centric approaches, researchers are also looking for solutions under engineering perspectives, for example, the control algorithm for cyborg insects. Many insect species reportedly exhibited various graded locomotion responses driven by electrical stimulation [16-18, 35]. Such graded reactions suggest a potential solution to overcome the demand of an optimal stimulus by utilizing a feedback control system, in which the controllers can fine-tune the insects' desired responses [16, 36, 37]. For example, the elicited angular speed of darkling beetles (via their antennae stimulation) and the provoked thrust force of giant beetles (via the activation of their subalar muscles) were reportedly in direct and inverse proportional relationships, respectively, with the frequency of the electrical stimulation [17, 38]. These relationships could be implemented to build a controller for regulating the insects' induced locomotory reactions and thus close the control loop for their navigation. Consequently, such a feedback control-based process would potentially resolve the issue of greatly deviated responses without knowing each insect's exact optimal parameters. The applicability of this approach was successfully demonstrated in the precise regulation of legs' motions [16] and straight-line flight control of giant flower beetles (*Mecynorrhina torquata*) [36], or the accurate orientation adjustment of migratory locusts (*Locusta migratoria manilensis*) [37]. The success of these works yields demands for further, more comprehensive

investigations on the applicability of this feedback control approach in more practical applications of cyborg insects, specifically their automatic terrestrial navigations.

Herein, this study demonstrates an ambulatory cyborg insect's feedback control-based navigation by implementing simple proportional controllers for its turning and forward motions to achieve its arbitrary path-following control. The cyborg insect (Fig. 1) was assembled from a living darkling beetle (*Zophobas morio*, ~0.6 g) and a miniature wireless backpack (~0.25 g). A feedback control system was developed to automatically navigate it along a predetermined sine curve. The cores of this system included a proportional controller to steer the beetle precisely and a thrust controller to accelerate it forward rapidly. The former was derived from the beetle's graded response to its antennae stimulation, whereas the latter was inspired by its reaction to the elytra stimulation [17, 18]. The performance of the designed system was investigated by adjusting its control parameters. The adjustment provided the navigation with a high success rate of more than 80% and a low error of less than ~10 mm, suggesting these engineering control factors engulfed the biological differences between individual beetles. Also, the feedback control-based navigation exhibited variations depending on the control parameters, providing flexibility for the application of terrestrial cyborg insects. This study thus strengthens the potential application of feedback controls (more generally, engineering viewpoints) in navigating (terrestrial) cyborg insects. Besides, the result displays the attainment of highly successful and precise automatic navigations, thus placing another steppingstone toward practical applications of cyborg insects.

RESULTS AND DISCUSSION

Locomotion Control of The Terrestrial Cyborg Beetle

Walking control of the terrestrial cyborg beetle was attained via electrical stimulation of its sensory organs [17, 18, 21]. Particularly, the stimulation of an antenna steered the beetle contralaterally, i.e., electrically stimulating the right antenna induced left turns, and vice versa (Fig. 1-B, 1-D) [17, 21]. This locomotory reaction resembles the beetle's natural response when one of its antennae is suddenly disturbed, e.g., being abruptly touched [29]. In specific, such a sudden disturbance excites the mechanoreceptors distributed around the antennal appendages, which signal the beetle's CNS [39, 40]. The CNS could then interpret the disturbance as an alert of potential dangers or obstructions and drive the beetle toward the opposite direction to escape the threat or avoid the obstacles [39, 40].

In addition, the beetle's turning motion was graded by adjusting the frequency of the electrical stimulation (Fig. 1-E). Higher frequencies induced faster turning speeds (Extended Data Fig. 2) [17]. As a result, the induced turning angles became more prominent as the stimulation frequencies were raised (Fig. 1-E, Supplementary Video 1). For example, increasing the stimulation frequencies from 10-16 Hz to 33-40 Hz doubled the elicited turning angle from ~ 12.5 deg to more than ~ 25 deg (Extended Data Table 1). Such graded locomotory response was implemented in the feedback control-based navigation to accurately steer the terrestrial cyborg beetle (Fig. 2-A).

In addition to the steerability (i.e., angular control), the electrical stimulation provided the control of translational motions for the cyborg beetle, e.g., forward/backward movements, left/right sideways walks [17, 18]. In specific, the elytra stimulation of the beetle accelerated it forward [18] (Fig. 1-B, 1-F). Once the electrical signal was transferred to both elytra simultaneously, the beetle rapidly accelerated forward with its linear speed increasing ~ 40 mm/s averagely (Fig. 1-F). Thus, such response was exploited as a thrust controller by the feedback control system to quickly direct the beetle toward frontal destinations (Fig. 2-A). Although the graded control of this motion was reported to be inversely proportional to the stimulation frequency [18], it was not implemented in the thrust controller for the sake of simplicity (Fig. 2-A).

Feedback Control-Based Automatic Navigation of The Cyborg Beetle

The feedback control system was designed to automatically navigate the cyborg beetle along a defined path (Fig. 2-A, 2-C). The system first computed the orientation error between the beetle and its assigned target (i.e., the angle θ , Fig. 2-B). This computation was updated periodically at every t_{update} (s). Afterward, a navigation command (i.e., left/right turn or acceleration) was established. The beetle was steered toward the destination when the angle θ was significant (i.e., $\theta > 25$ deg [32]). Otherwise, it was accelerated forward. Due to the graded response in turning (Fig. 1-E), the antennal stimulation frequency was regulated via a proportional controller, expecting to accurately and quickly reorient the cyborg beetle (Fig. 2-A). The controller's proportional gain (K_p) and the update interval (t_{update}) were altered to evaluate the system's performance. The automatic navigation was executed along a predetermined sine curve (Fig. 2-C). This path-following process was accomplished via a carrot-chasing fashion that the beetle was directed toward sequentially generated temporal targets (Fig. 2-B). Once the beetle nearly arrived at a target, a novel one approaching the end

of the curve was established. This process repeated until the beetle reached its ultimate destination.

Besides this artificially designed system, the beetle intrinsically possesses a complex and naturally evolved feedback control system (Fig. 2-A). During its locomotion, the beetle constantly acquires not only internal feedback from proprioceptors (for precise motor control) but also external information via a rich collection of sensory organs distributed over its body, e.g., tactile feedback from its legs, antennae, and elytra, as well as visual input from its eyes [12, 41]. The collected information interacting with the internal locomotory neural circuits (e.g., central rhythm generators) allows the beetle to adjust its movements accurately [42] and thus adapt to the dynamic surrounding terrains. Cockroaches, for example, reportedly used antennal and visual feedback to detect and negotiate with frontal obstructions (*Blaberus discoidalis*) [43], or adjusted their walking gaits according to the slipperiness of surfaces underneath (*Nauphoeta cinerea*) [44]. This intrinsic feedback control system potentially provides the cyborg beetle high robustness and adaptivity, allowing its automatic navigation to be vigorous under external disturbances (e.g., obstacles [25]). Thus, combining this inner natural control system with the external artificial controllers would enable robust navigation with high precision and reliability in cyborg beetles. In other words, these two systems could work in tandem and complement each other (Fig. 2-A) and thus express the beneficial fusion of engineering and biological perspectives in (terrestrial) cyborg beetles [25].

The cyborg beetle was navigated successfully to follow the predetermined sine curve and arrive at the destination (Fig. 2-C, 2-D, Extended Data Fig. 3, Supplementary Video 2). In specific, such navigations occurred for ~71% of the time ($N = 19$ beetles, $n = 112/157$ trials), combining all sets of K_p and t_{update} . Furthermore, the successful navigation trajectories distributed spatially in the form of sine curves around the predetermined one (Fig. 2-D), relatively reflecting the accuracy and efficiency of this feedback control system. The navigation of contemporary terrestrial cyborg insects was relatively challenging, with the insects being successfully navigated along predefined paths for only ~10-30% of the time [21, 32]. Despite being just a relative comparison (as navigation was not in these studies' interest), the success rate difference provides a glimpse at a beneficial attribute of the designed system, i.e., the regulation of electrical stimulation. As introduced, the previous works implemented an identical stimulus for different insect individuals despite their physiological distinctions [21, 32, 34]. This open-loop control greatly diverged the insects' reactions, leading to a significant variation in the controllability and thus lessening the navigation outcomes. Herein, as such a

variation was compensated via the feedback regulation of the stimulation frequency (Fig. 2-C), the successful automatic navigation of the cyborg beetle was more likely to be attained (i.e., ~71%, Fig. 2-D, Extended Data Fig. 3).

Evaluation and Optimization of the Automatic Navigation

Besides its general success discussed above, tuning the two control parameters (i.e., K_p and t_{update}) altered the performance of the feedback system (Fig. 3). For instance, with $t_{update} = 1.0$ s, the beetle stayed closer to the defined path when K_p was set as 0.5 (Fig. 3-A). More specifically, adjusting these two parameters impacted four different aspects of the system, including “success rate,” “tracking error,” “navigation time,” and “control effort” (Fig. 3-B to 3-E). As an indicator of its reliability, the system’s success rate was judged as the fraction of successful navigations (Fig. 3-B). Similarly, the system’s accuracy was expressed via its tracking error. This factor was calculated by dividing the region formed by the beetle’s actual trajectory and the predetermined sine curve by the latter’s length [32] (Fig. 2-C, 3-C). The third factor, navigation time, depicted the time efficiency of the system and was computed as the elapsed time the beetle needed to reach the destination (Fig. 3-D). Finally, the system’s cost or its control effort was the average number of stimuli it delivered to obtain a successful navigation (Fig. 3-E). The variations of these four aspects corresponding to the two control parameters would provide the cyborg beetle’s automatic navigation the flexibility to be tuned or optimized depending on requirements.

When a highly successful navigation is favored over the other three aspects, either a frequent update interval or a large proportional gain should be set (Fig. 3-B, Extended Data Table 2). For example, when the beetle was under a frequent control (i.e., $t_{update} = 1.0$ s), a high success rate of more than 75% was attained regardless of K_p (Fig. 3-B). However, when the update interval was increased (i.e., 1.5 s and 2.0 s), leading to a growth in the beetle’s control-free motion, the success rate was more significant with the large K_p of 0.75 compared to the other two values (i.e., 0.25 and 0.5) (Fig. 3-B). At $t_{update} = 1.5$ s, this K_p resulted in a 20% higher success rate than its two counterparts. The distribution of stimulation frequencies generated under each K_p accounted for these success rate differences. As the proportional gain was large (i.e., $K_p = 0.75$), the frequency was biased toward its high values (Fig. 3-F). This tendency steered the beetle with higher angular displacements (Fig. 1-E, Extended Data Fig. 4-A) and thus compensated the control-free motion.

Unlike the success rate, when t_{update} was 1.0 s, the tracking error was found to be significantly affected by altering K_p (Fig. 3-A, 3-C, ANOVA test, $P = 0.032$, $df = 45$, Extended Data Table 2). Setting K_p to 0.50 provided an error of 4.99 ± 3.90 mm, whereas that of the other two gains was ~ 4 mm higher. A different aspect of the navigation accuracy was the beetle's distance to the sine route during its journey (Extended Data Fig. 4-B, Extended Data Table 2). When K_p was set as 0.50, this distance averaged as 9.82 ± 6.34 mm. It, however, became farther when the other two K_p was used (Extended Data Fig. 4-B, t-test, $P < 0.001$, $df > 2000$). The superiority of $K_p = 0.50$ might relate to the diversity of its stimulation frequencies (Fig. 3-F), which almost evenly spread from 10 Hz to 40 Hz. Meanwhile, the frequencies employed by the other two gains was slanted toward either end of the spectrum. The lean toward low frequencies (i.e., $K_p = 0.25$) might result in less effects on correcting the beetle's orientation, whereas the other extreme (i.e., $K_p = 0.75$) possibly overshoot the beetle, fluctuated it along the path, and thus worsen the navigation (Fig. 3-A, Extended Data Fig. 4-A). Unlike the success rate, the impact of K_p on the tracking error no longer presented when the beetle's control-free motion became dominant (i.e., $t_{update} = 1.5$ s and 2.0 s, Fig. 3-C, ANOVA test, $P > 0.1$, $df > 30$, Extended Data Table 2).

Despite its high success rate and low tracking error, the combination of $K_p = 0.50$ and $t_{update} = 1.0$ s did not benefit the navigation program in terms of control effort. The program applied ~ 37 stimuli averagely to successfully direct the beetle using this combination, which was not an advance compared to the other two gains (Fig. 3-E, t-test, $P > 0.6$, $df > 27$, Extended Data Table 2). As all three K_p used the same update interval of 1.0 s, their similarity in control effort was consistent with their insignificant effect on the navigation time (Fig. 3-D, ANOVA test, $P > 0.1$, $df > 30$, Extended Data Table 2). However, when t_{update} was raised, large proportional gains such as 0.50 and 0.75 tended to favor these two navigation factors (Fig. 3-D, 3-E, t-test, $P < 0.04$, $df > 18$). In the instance of $t_{update} = 2.0$ s, the necessary numbers of stimuli for a successful trial increased from ~ 30 to ~ 51 when K_p was reduced from 0.75 to 0.25. This tendency was possibly associated with the stimulation frequency, in which the beetle was more energized under high frequencies (established by $K_p = 0.50$ and 0.75) leading it to reach the destination earlier. Although these stimulations were meant to steer the beetle, this argument was not invalid. The beetle's reactions are not pure rotations but accompanying forward runs, which is a common escape strategy of terrestrial insects [45]. In addition, a closer look at the beetle's instantaneous linear speed throughout its navigation also supported the argument (Extended Data Fig. 4-C, Extended Data Table 2). At $t_{update} = 1.5$ s, for example, the speed was

~11 mm/s with $K_p = 0.25$, and then increased to ~24 mm/s and ~16 mm/s with $K_p = 0.5$ and 0.75 , respectively (Extended Data Fig. 4-C, t-test, $P < 0.001$, $df > 2000$).

Herein, the experimental outcomes consolidate a trending perspective on controlling living insects. Despite of high variation in responses as biological subjects, the (terrestrial) cyborg insects can be well controlled from engineering viewpoint [16, 36, 37], e.g., being navigated via feedback control systems. The insects' navigation, thus, will depend on not only their physiology but also the implemented controller and its parameters. The parameters can be adjusted to serve different purposes. As indicated, for example, accurate path-following navigations would need a fast t_{update} and a medium K_p , whereas navigations expecting only a high success rate would be attained with a large K_p disregarding t_{update} . Similarly, different controllers will satisfy different goals. For instance, in this study, the proportional controller was deployed to favor the tracking error, making it mostly irrelevant to the navigation time. Thus, an additional proportional controller for the forward acceleration (i.e., the elytra stimulation) might be necessary for compensation. Besides, more advanced controllers, e.g., PI controller, Fuzzy Logic, or Deep Learning can be considered to reduce the error and improve the navigation performance [36, 46, 47].

The presented outcomes also provide a different look at studying electrical stimulation for the locomotion control of cyborg insects. In particular, the quest to search for an optimal stimulus working across various individual insects [34] should not be exclusive but complemented with investigating possible relationships between the electrical stimulation and the induced locomotory reaction. Those relationships will potentially inspire the development of appropriate feedback control systems, which automatically alter the stimulation parameters based on the insects' behavioral responses. For example, the presented proportional controller was motivated by the graded turning response of the beetle to its antennae stimulation. As similar graded responses are popular in various insect species [23, 37], it is practically ambitious to achieve precise navigation across the diversity of the insect kingdom by implementing feedback control-based approaches.

CONCLUSION

This study evaluates and solidifies the role of feedback control in the automatic navigation of terrestrial cyborg insects. This approach helps to overcome the issue of optimal stimulation parameters, which long obstructed the navigation of ambulatory insects. In addition, the

feedback control-based navigation is shown to be precise, reliable, as well as flexibly adjustable. Although there are still many technical obstacles to overcome to bring the ideas of cyborg insects from laboratory environments to practical applications, e.g., the need for an onboard localization system and well-planned power management, the results of this study contribute another essential step to this challenging but intriguing journey.

Acknowledgments

The authors offer their appreciation to Mr. Chew Hock See, Ms. Kerh Geok Hong, Mr. Tan Kiat Seng, Mr. Roger Tan Kay Chia at School of MAE, NTU, and Prof. Andrew Straw at University of Freiburg for their continuous support in setting up and maintaining the research facilities. This work has been supported by the Singapore Ministry of Education (RG140/20). T.T. Vo-Doan is currently supported by Human Frontier Science Program Cross-disciplinary Fellowship.

Author contributions

HS, TTVD, and HDN conceived and designed the research. HDN, TTVD established the insect control protocol and navigation program. DVT developed hardware and software for the backpack. HDN, TTVD conducted the experiment and analysis. HDN, TTVD wrote and edited the manuscript. HS, TTVD supervised the research. All authors read and edited the manuscript.

Competing interests

Authors declare no competing interests.

Online content

Any methods, additional references, extended data, supplementary information, and code are available online.

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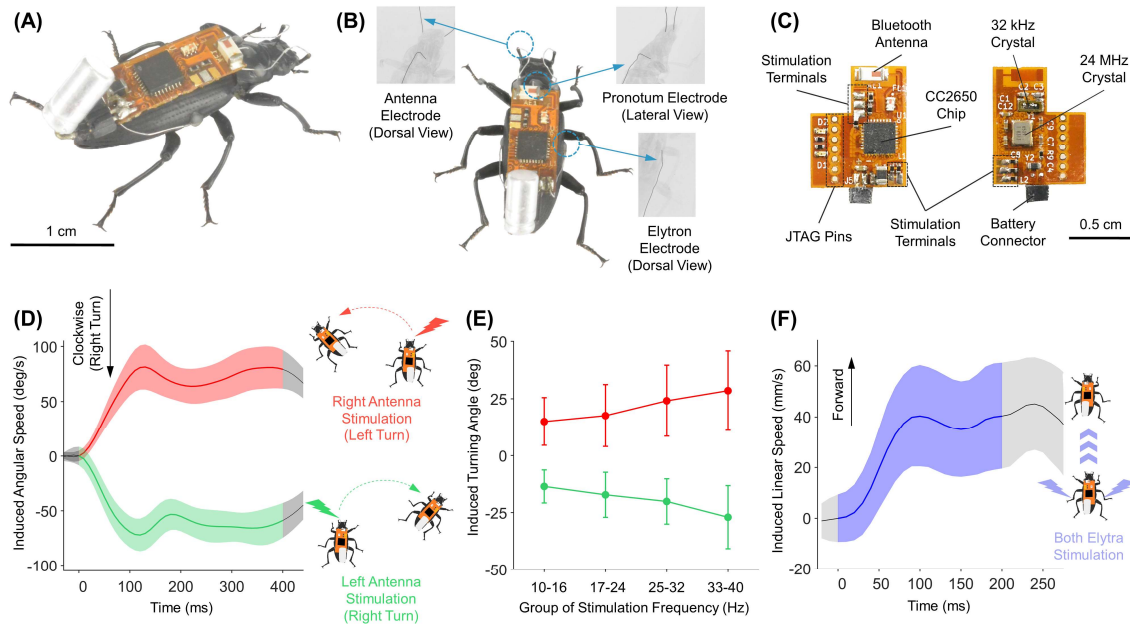


Fig. 1. Overview of the terrestrial cyborg beetle and its locomotion control. (A) The cyborg beetle is the fusion of a living ambulatory insect, *Zophobas morio* (~0.6 g), and a wireless stimulator (i.e., the backpack, ~0.25 g) mounted on its elytra. The backpack is powered by a rechargeable Lithium Titanate battery (1.5 V, 8 mAh, ~0.2 g). (B) Two pairs of working electrodes are implanted into the beetle's antennae and elytra to induce its turning motions and acceleration. The common electrode is inserted into its pronotum. (C) The wireless backpack uses a Bluetooth Low Energy microcontroller as its core and provides up to eight programmable stimulation terminals. (D) Representative data of the beetle's turning response. Once the stimulation is applied, the beetle turns contralaterally. Its angular speed rapidly ramps up within the first 100 ms and then fluctuates around a saturated level. Black and colored lines indicate the mean angular speed during stimulation-free and stimulation periods. The shaded regions display standard deviation. (E) Graded control of the beetle's turning response via the stimulation frequencies. Higher frequencies tend to elicit faster angular speeds and larger turning angles. Red and green colors represent the stimulation of the right and left antenna, respectively. Circular markers denote the mean of the induced turning angle, whereas error bars show its standard deviation (N = 16 beetles, n = 859 stimuli). (F) The cyborg beetle's forward acceleration is induced by stimulating its two elytra simultaneously. The beetle's forward speed quickly raises when the stimulation is applied. Black and blue lines represent the mean forward speed in stimulation-free and stimulation periods, respectively. The shaded regions show the standard deviation.

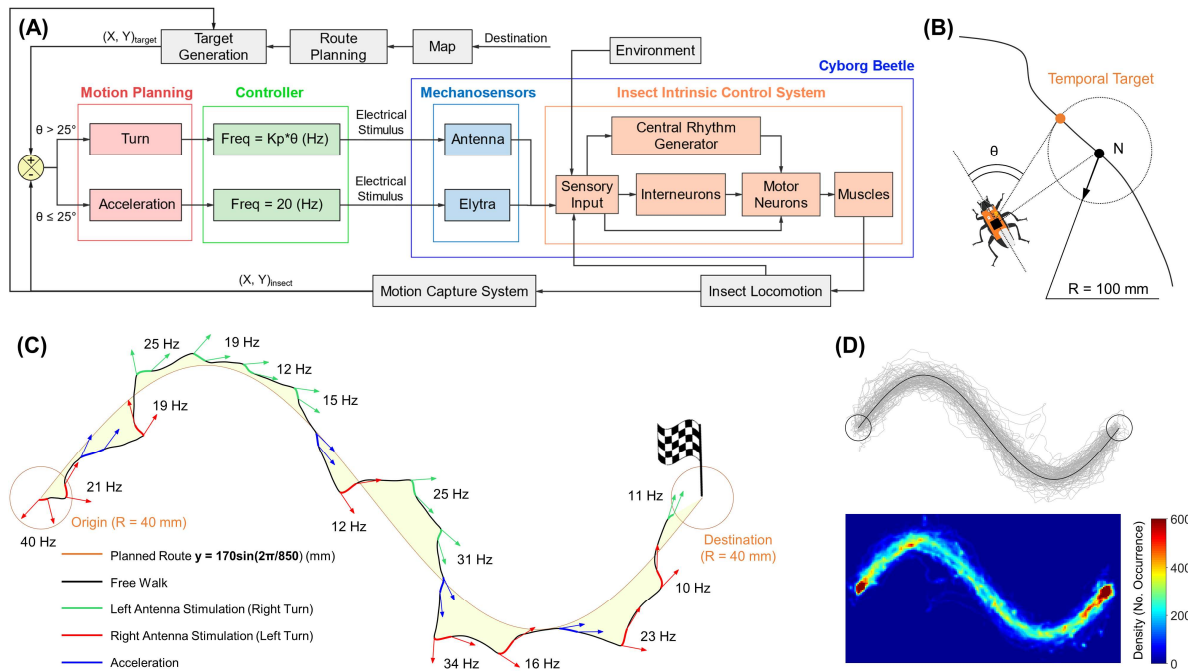


Fig. 2. The feedback control system for autonomous navigation of the cyborg beetle. (A) The system combines two artificial control units (i.e., the motion planning block and central controller) and the cyborg beetle's natural control system (i.e., its sensory organs and CNS). First, the motion planning block works out the navigation command (i.e., steering or accelerating) by comparing the beetle's location and its assigned path, which can be planned via the destination and a map of the surrounding terrain. Then, the central controller (i.e., the proportional and thrust controllers) issues an appropriate electrical stimulus to stimulate the beetle's corresponding sensory organs. Finally, the beetle's CNS perceives and uses this electrical stimulus together with other external cues to adaptively alter its motions. The induced locomotion is monitored and feedbacked to the system via a 3D motion capture unit (Extended Data Fig. 1). (B) The system attained the path-following control by sequentially generating temporal targets along the path and directing the beetle toward them. These targets are intersections between the circles centering at the beetle's projection onto path and the path itself (i.e., point N). (C) The system attempts to navigate the beetle toward these targets by adjusting the stimulation frequency according to its orientation error, the angle θ . This adjustment prevents the necessity of an optimal stimulus. The shaded yellow region is used to calculate the tracking error. (D) The system navigates the beetle successfully $\sim 71\%$ of the time ($N = 19$ beetles, $n = 112/157$ trials), marking a significant improvement (loosely compared to previous works). A successful navigation is counted if the beetle reaches the destination within 5 minutes (Extended Data Fig. 1). Gray curves represent the successful trials, whereas the black curve denotes the predetermined path. The heatmap shows the distribution of the beetles' positions in successful navigations.

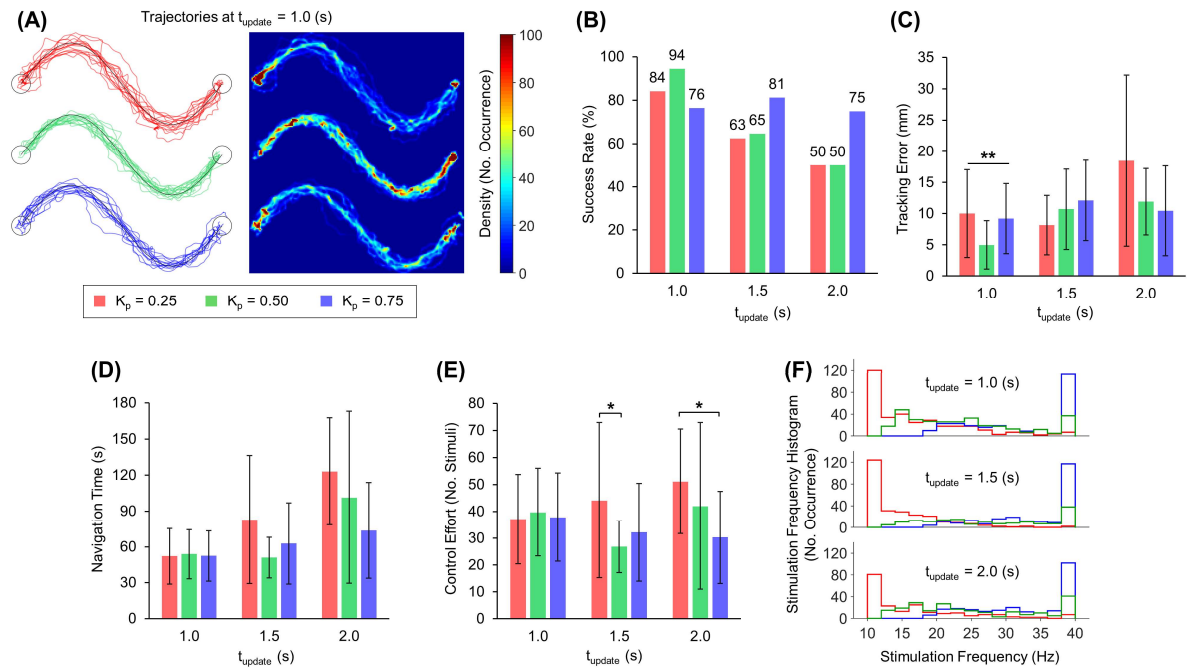


Fig. 3. Performances of the feedback control system when tuning t_{update} and K_p . (A) The adjustment of K_p and t_{update} enables a flexible regulation of the navigation's reliability (i.e., success rate) and precision (i.e., tracking error). As depicted, the moderate K_p of 0.5 is sufficiently large to correct the beetle's orientation but not too big to cause overshoots. Thus, the beetle's trajectories of this K_p are more spatially concentrated around the predetermined path, or more precise, than the other two gains. The effect of the two control parameters is apparently displayed via two factors: (B) success rate and (C) tracking error (**: $P < 0.05$, ANOVA test). The combination of a fast update interval and a moderate K_p allows the cyborg beetle to be navigated precisely (i.e., less than ~ 10 mm tracking error) and reliably (i.e., more than 90% success rate). However, the two parameters don't significantly affect (D) navigation time and (E) control effort (although slow t_{update} seems to request large K_p to favor these factors, *: $P < 0.05$, t-test). This insignificance is expected as the beetle's graded acceleration [18] is not integrated into the thrust controller. (F) Under the proportional controller, the stimulation frequencies steering the beetle are constantly adjusted. The distribution of these frequencies is skewed either toward the left or right end of the frequency spectrum under a minor (i.e., 0.25) or large (i.e., 0.75) K_p , respectively. Meanwhile, it is fairly spread when a moderate K_p is selected. These distribution differences help explain the variation in the system's navigation performance. Red, green, and blue represent three values of K_p , i.e., 0.25, 0.50, and 0.75, respectively. Error bars display standard deviations. $N = 19$ beetles, $14 \leq n \leq 20$ trials for each pair of the two parameters.

Methods

Animals

Zophobas morio, also known as darkling beetle, was used as the living insect platform in this study. The beetle's relatively small size (~2–2.5 cm) and lightweight (~0.4–0.6 g) make it a potential mobile platform for cyborg insect development. Although ethical regulations for invertebrate research are still under debate [48], this study attempted to provide good living conditions for these insects. Their colonies were rear inside the compartments of a Mouse Housing System (NexGen® Mouse 500, Allen Town®), allowing clean air to be circulated. The density of each compartment was kept at 20 beetles/~9400 cm³ (i.e., 19 × 13 × 38 cm³), providing a spacious territory. The compartments were washed weekly to maintain their hygiene. Temperature and relative humidity were maintained at ~25 °C and 60%, respectively. Water and food were supplied to the territories twice per week via various vegetables (e.g., carrots and apples). These conditions were provided not only for the intact insects (those to be used for the study) but also for post-experiment ones (those no longer used for the study).

Wireless Backpack Stimulator

A miniature stimulator, or backpack (15 × 5 mm²), was designed to wirelessly control the beetle's locomotion (Fig. 1-C, Supplementary Video 1). The backpack utilized a microcontroller CC2650 as its main core (TI, 48 MHz, 128 KB of Flash, Bluetooth® Low Energy 4.2). The backpack allowed a transmission distance of 10 meters and provided up to eight independent stimulation terminals. It was powered by a rechargeable lithium-ion battery (1.5 V, 8 mAh). The total weight of the backpack and battery was ~0.5 g. The backpack was mounted onto the beetle's elytra using beeswax (Fig. 1-A, 1-B). Its communication with the main PC was mediated by a Bluetooth central station (TI, SmartRF06 Evaluation Board, Extended Data Fig. 1). The backpack was implemented to demonstrate the wireless locomotion control of the beetle via its antennae stimulation (Supplementary Video 1).

Implantation and Electrical Stimulation

Five electrodes made of coated copper wires (55 μm bare diameter, 44 AWG, Remington Industries) were implanted into the beetle's antennae and elytra to induce turning and accelerating motions, respectively (Fig. 1-B). The insulation layer at two ends of each electrode was first removed using flame (the de-insulated length was ~1 mm). Ones of these ends were

then inserted into the beetle, while the others were connected to an electrical stimulator. Prior to the implantation, the beetle was anesthetized using CO₂. The flagellums of its antennae were then trimmed off to insert two working electrodes. The insertion was ceased once reaching the antennal base, indicated by a small resistance. A beetle pin (No. 00, Indigo Instruments) was then used to pierce three tiny holes on the beetle's body, one was at the pronotum and two were at the leading edge's vein of each elytron. Three remaining electrodes were implanted into these opens, in which the common electrode was situated at the pronotum. The depth of these implants was at ~2 mm. All five electrodes were secured using beeswax. The beetle was then given ~1 hour for recovery.

The elytra stimulation was conducted using a fixed electrical stimulus, which had 2.5 V amplitude, 20 Hz frequency, 200 ms duration, and 50% duty cycle. These parameters were selected to attain significant responses from the beetle. The antennae stimulation was performed using a short electrical stimulus, whose amplitude, pulse-width, and duration were fixed as 2.5 V, 2 ms, and 400 ms, respectively. These parameters were observed providing apparent turning reactions. The stimulation frequency was adjusted by the navigation program. The adjustment range was from 10 Hz to 40 Hz so that the induced angular speed was proportionally graded (Fig. 1-E, Extended Data Fig. 2, Supplementary Video 1) [17].

Setup and Evaluation of Navigation Experiment

The automatic navigation of the beetle was evaluated under a loosened tethered condition to maintain the experiments for long periods. A long copper wire (i.e., 1.5 meters, 44 AWG Magnet Wire, Remington Industries 44SNSP) was used to transmit the electrical stimuli generated by the stimulator to the beetle. The stimulator was an Arduino Uno board (ATmega238, 16 MHz, 32 kB of Flash), associated with a resistor voltage divider to regulate the stimulus amplitude to 2.5V. This circuit was connected to the main PC via serial communication (460800 bps). The navigation program was written in MATLAB[®] and embedded into the main PC. The program computed the stimulation commands and transferred them to the stimulator to generate corresponding electrical pulse trains, thus maneuvering the beetle's motion. This computation was processed under a feedback control manner, meaning the program established the command based on the beetle's instantaneous position (Fig. 2-A, Extended Data Fig. 1). The position and orientation of the beetle were provided by a 3D motion capture system (Vicon[®], 100 fps, 120 × 120 × 120 cm³, Extended Data Fig. 1-A), which tracked a set of three retroreflective hemisphere markers affixing on a light carbon fiber frame mounted

on the beetle (3 mm radius, ~20 mg, Extended Data Fig. 1-B). Coordinates of the three markers were used as the program's feedback data (Extended Data Fig. 1-C). They were also synchronized with the stimulation commands and logged by the main PC for post-experimental analysis.

In each navigation trial, the navigation program automatically maneuvered the beetle to move from the origin to the destination, two circular areas with a radius of 40 mm (Fig. 2-C). The beetle's journey was expected to follow a predetermined sine curve (Fig. 2-C), whose equation was:

$$y = 170 \frac{2\pi x}{850} \text{ (mm)}.$$

A trial would be terminated if the beetle reached the destination or left the floor ($120 \times 60 \text{ cm}^2$) or the experimental time exceeded 5 minutes, whichever came first. The destination and origin were swapped between two consecutive trials to avoid potential biases. There were 12 trials conducted for each beetle. An interval of 5 minutes rest was applied after each trial. A trial was counted as successful if the beetle arrived at the destination before the termination conditions were met. Otherwise, it was recorded as a failed navigation.

As introduced, the navigation performance was evaluated under different combinations of the two control parameters K_p and t_{update} . The proportional gain was set to either 0.25, 0.50, or 0.75, whereas the update interval was selected at 1.0 s, 1.5 s, or 2.0 s. The nine combinations were randomized throughout the experiment to avoid biases. Herein, the adjustment of the two control parameters did not serve to find their optimal values but to evaluate the navigation across various conditions, e.g., from frequently monitoring the beetle (fast t_{update}) to maximizing its free motion (slow t_{update}).

Data Analysis

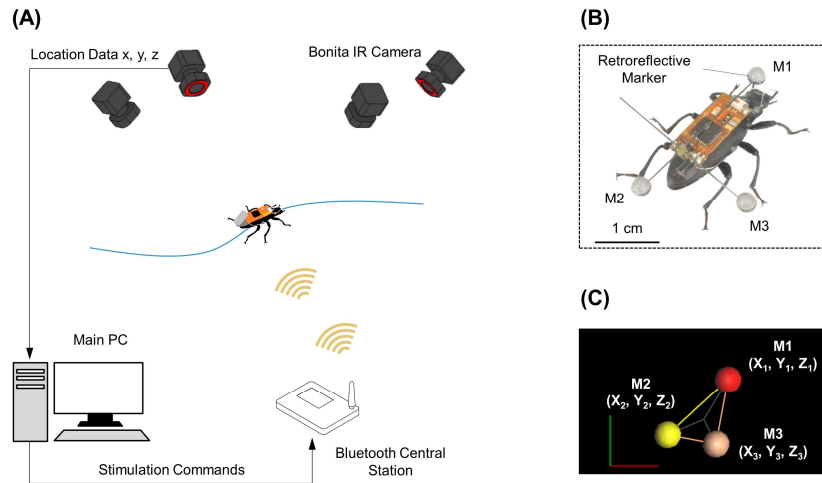
As introduced, the navigation performance was evaluated via the four factors, "success rate," "tracking error," "navigation time," and "control effort." One-way ANOVA test (0.05 significant level) was employed to study the two control parameters' effect on these factors, i.e., the impact of adjusting K_p under a given t_{update} . In addition, statistical comparisons in the study were examined via t-test (0.05 significant level).

The beetle was observed to become unresponsive when several consecutive stimuli were applied to only one of its antennae, which might be a consequence of habituation, implant impairments (caused by accumulated electrical charges), or a contribution of both [49, 50].

This bias was removed from the data analysis by excluding those trials containing 15 or more consecutive unilateral stimuli. These trials occupied 60/288 total experimented trials, i.e., ~21%. In addition, 71 other trials (~24%) were also excluded because the three retroreflective markers were miss-tracked more than 20% of the time.

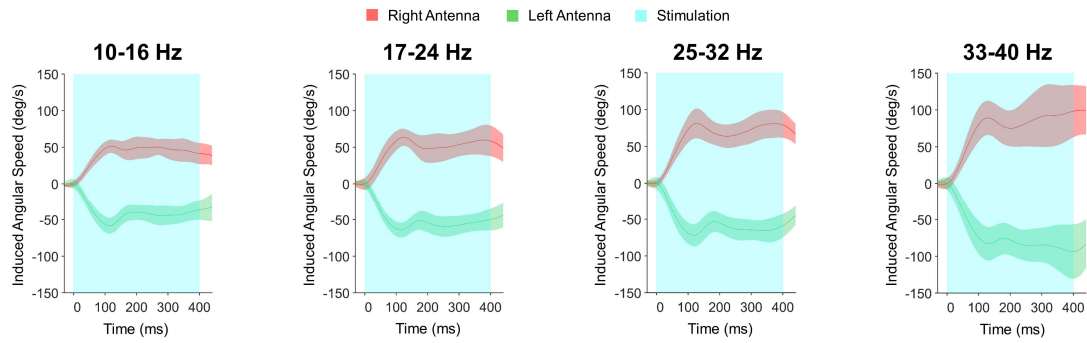
Location data of the beetle was passed through a moving average filter (0.1-seconds sliding window) for noise removal. The data was then implemented to calculate the four navigation factors and reconstruct the beetle's motion, i.e., its linear and angular speeds induced by the stimulation (Fig. 1-D to 1-F, Extended Data Fig. 2), its instantaneous linear speed and distance to the path during the navigation (Extended Data Fig. 4-B, 4-C). The last two elements were sampled at a rate of 500 ms. The instantaneous linear speed was defined as the average speed within a 100-ms window. The right (i.e., clockwise) turns were assigned negative values, and vice versa.

The beetle's turning response to its antennae stimulation (Fig. 1-D, 1-E, Extended Data Fig. 2) was reconstructed from the navigations attained with $K_p = 0.50$, which contained diverse stimulation frequencies. The data were then grouped into four ascending groups of the stimulation frequencies, including 10-16 Hz, 17-24 Hz, 25-32 Hz, and 33-40 Hz. Then, outliers were removed and determined as those data falling outside the range of $\pm 2.7\sigma$, where σ was the standard deviation of each group.



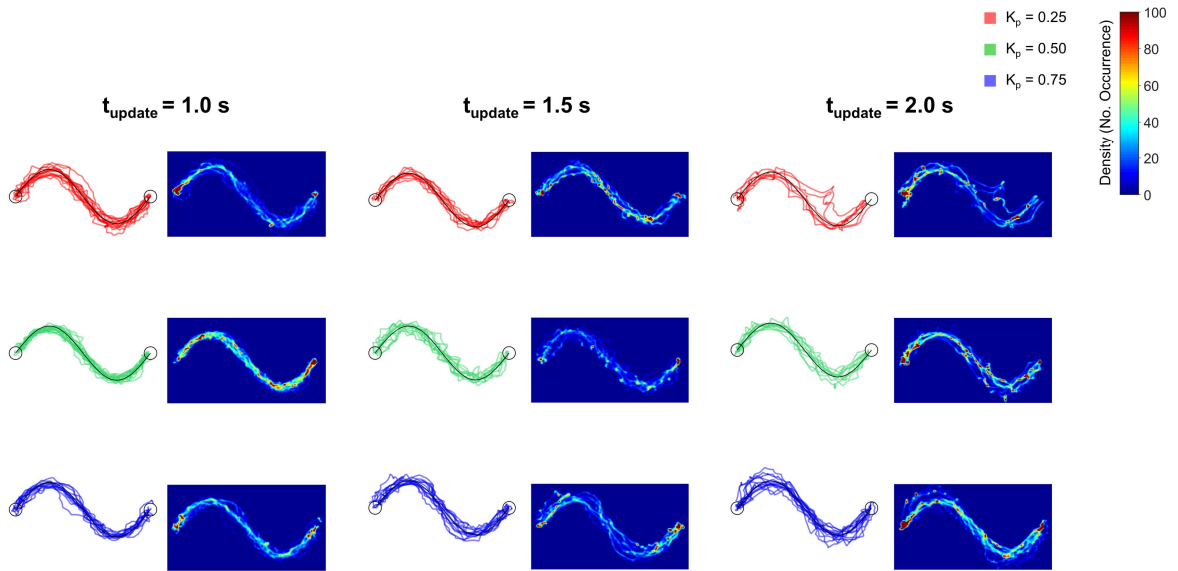
Extended Data Fig. 1

Setup of the navigation experiment. (A) The study is conducted within a 3D motion capture system (Vicon®), made of an aluminum frame and four infrared (IR) cameras (Bonita, 100 fps). The beetle is navigated on a Styrofoam sheet ($120 \times 60 \text{ cm}^2$), following a predetermined sine curve. Each navigation trial is terminated when the beetle is out of the sheet or not reaching the destination after 5 mins, and a failed navigation is recorded. The four cameras monitor the beetle's location and stream it to the main PC to work out the stimulation, which is then sent to the beetle to alter its motion and thus close the control loop. (B) Three retroreflective markers are mounted on the beetle's elytra to represent its position and orientation. (C) Coordinates of these markers are digitalized using Vicon Tracker® software and transferred to the navigation program embedded on the main PC.



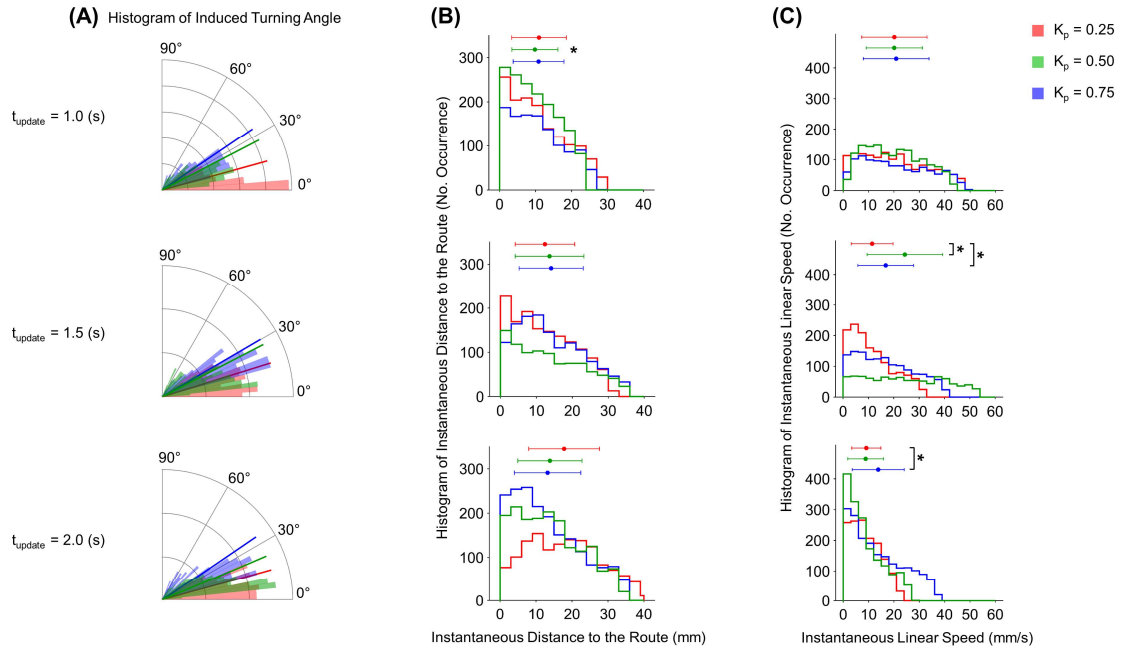
Extended Data Fig. 2

Graded response of the beetle to its antennae stimulation. The angular speed elicited by the electrical stimulation tends to increase as the stimulation frequency is incrementally adjusted ($N = 16$ beetles, $n = 859$ data points). In addition, this speed promptly raises upon the arrival of the electrical stimulus. It then peaks after ~ 100 ms and slightly fluctuates afterward. As introduced, this graded reaction inspires the implementation of a proportional controller to steer the cyborg beetle.



Extended Data Fig. 3

Successful navigations in each pair of the two control parameters. The adjustment of these parameters impacts the reliability (i.e., success rate) and precision (i.e., tracking error) of the cyborg beetle's navigation, implying the beetle operates similarly to a traditional engineering control system ($N = 19$ beetles, $14 \leq n \leq 20$ trials for each pair). For example, a fast update interval ($t_{\text{update}} = 1.0 \text{ s}$) can be paired with a moderate K_p of 0.5 to attain reliable and precise navigations. In contrast, slow update intervals (i.e., 1.5 s or 2 s) need a large K_p to guarantee a high success rate.



Extended Data Fig. 4

Different looks at the feedback-based control navigation. (A) In tandem with the frequency distribution (Fig. 3-F), the mean of induced turning angles in large and small K_p is either larger or smaller than a moderate K_p , helping to explain their imprecise navigations given a short update interval ($t_{update} = 1.0$ s, Fig. 3-C). (B) Consistent with the tracking error (Fig. 3-C) is the distance of the beetle to the predetermined path during its navigation. Under a frequent control ($t_{update} = 1.0$ s), a moderate K_p of 0.5 keeps the beetle staying near the path (* $P < 0.001$, t-test), resulting in a small tracking error. (C) In line with the navigation time and control effort (Fig. 3-D, 3-E), K_p has no significant effect on the beetle's instantaneous speed when t_{update} is fast. When t_{update} is slow, larger K_p tends to make the beetle move faster (* $P < 0.04$, t-test), reducing navigation time and control effort.

Extended Data Table 1

Induced turning angles under the antennae stimulation. Higher stimulation frequencies likely steer the beetle more profoundly.

Stimulation Frequency (Hz)	Induced Turning Angle (deg)	
	Left Antenna (Right Turns)	Right Antenna (Left Turns)
10 - 16	-13.55 ± 07.25	15.01 ± 10.46
17 - 24	-17.23 ± 09.88	17.60 ± 13.56
25 - 32	-20.12 ± 09.95	24.11 ± 15.51
33 - 40	-27.04 ± 13.84	28.50 ± 17.34

Extended Data Table 2

Variation of the feedback control-based navigation when adjusting the two control parameters. Owing to the feedback control system, the beetle's navigation outcome can be adjusted depending on applications.

t_{update} (s)	K_p	Success Rate (%)	Tracking Error (mm)	Navigation Time (s)	Control Effort (No. Stimuli)	Distance to Path (mm/s)	Linear Speed (mm/s)
1.0	0.25	84	10.00 ± 07.04	52 ± 23	37 ± 17	10.95 ± 07.60	20.16 ± 12.88
	0.50	94	04.99 ± 03.90	54 ± 21	40 ± 16	09.82 ± 06.34	20.12 ± 11.06
	0.75	76	09.22 ± 05.63	52 ± 21	38 ± 16	10.83 ± 07.07	20.88 ± 12.96
1.5	0.25	63	08.17 ± 04.75	83 ± 54	44 ± 29	12.40 ± 08.28	11.48 ± 08.26
	0.50	65	10.71 ± 06.45	51 ± 17	27 ± 10	13.67 ± 09.55	24.38 ± 14.89
	0.75	81	12.12 ± 06.44	63 ± 34	32 ± 18	14.11 ± 08.92	16.84 ± 11.00
2.0	0.25	50	18.50 ± 13.70	123 ± 44	51 ± 19	17.81 ± 09.85	09.11 ± 05.74
	0.50	50	11.91 ± 05.31	101 ± 72	42 ± 31	13.86 ± 08.92	08.85 ± 07.04
	0.75	75	10.46 ± 07.22	74 ± 40	30 ± 17	13.22 ± 09.24	13.80 ± 10.25

Supplementary Information for

Cyborg Beetle Achieves Efficient Autonomous Navigation Using Feedback Control

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Wirelessly graded locomotion control of the cyborg beetle.

Video 1. The electronic backpack allows the locomotion control to be executed wirelessly. In addition, the stimulation parameter (e.g., frequency) can be regulated, helping to realize the graded response, and thus the proportional control, of the beetle.

Feedback control-based automatic navigation of the cyborg beetle.

Video 2. Successful navigations of the beetle are digitally reconstructed from the collected data. For a convenient display, trajectories of these navigation are plotted as if the destination was not swapped between two ends of the predetermined sine path.