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Electrical microstimulation of the nucleus incertus induces forward locomotion and rotation in rats

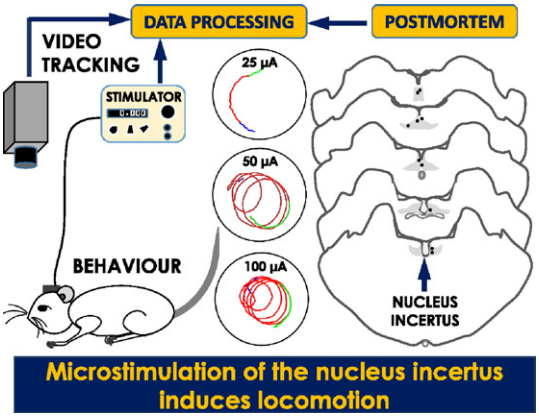
Usman Farooq [a,b,c,*,1], Jigna Rajesh Kumar [a,b,c,1], Ramamoorthy Rajkumar [a,b,c], Gavin S. Dawe [a,b,c,**]

[a] Department of Pharmacology, Yong Loo Lin School of Medicine, National University Health System, 117600, Singapore
[b] Neurobiology and Ageing Programme, Life Sciences Institute, National University of Singapore, 117456, Singapore
[c] Singapore Institute for Neurotechnology (SINAPSE), 117456, Singapore

HIGHLIGHTS

- Nucleus incertus modulates hippocampal theta which is associated with locomotion.
- Microstimulation of nucleus incertus in rats was sufficient to induce locomotion.
- Nucleus incertus plays a role in behavioral activation and locomotion.

GRAPHICAL ABSTRACT



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ABSTRACT

Locomotion is essential for goal-oriented behavior. Theta frequency oscillations in the hippocampus have been associated with behavioral activation and initiation of movement. Recently, the nucleus incertus, a brainstem nucleus with widespread cortical and subcortical projections, has been reported to modulate the septo-hippocampal axis triggering theta activity in the hippocampus. This suggests that activation of the nucleus incertus would induce movement. In this study, we investigated the effects of electrical microstimulation of the nucleus incertus on locomotion in conscious rats. Rats chronically implanted with microelectrodes targeting the nucleus incertus were electrically stimulated while their behavior was tracked. High frequency electrical microstimulation of the nucleus incertus was sufficient to induce forward locomotion and rotation. The latencies of evoked locomotion were consistent with a role of the nucleus incertus in modulating premotor areas, possibly the septo-hippocampal axis. Electrical microstimulation of the nucleus incertus increased velocity, mobility and rotations during stimulation and post-stimulation. These results suggest that the nucleus incertus plays a role in behavioral activation and locomotion.

* Correspondence to: U. Farooq, Interdepartmental Neuroscience Program, Yale University, PO Box 208001, New Haven, Connecticut 06520-8001, USA.
** Correspondence to: G.S. Dawe, Yong Loo Lin School of Medicine, #04-01Y, Level 4, MD3, 16 Medical Drive, National University of Singapore, 117600, Singapore.
E-mail addresses: usman.farooq@yale.edu (U. Farooq), gavin_dawe@nuhs.edu.sg (G.S. Dawe).
[1] These two authors contributed equally.

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1. Introduction

The nucleus incertus (NI) has long been hypothesized to be involved in behavioral activation [1]. Locomotion to execute behaviors is an inevitable consequence of behavioral activation. Locomotion and hippocampal theta oscillations are very tightly coupled in an awake animal [2–5]. Although the role of the NI in generation of hippocampal theta oscillations is well-established [6,7], evidence for a causal role for the NI in locomotion is lacking.

While the motor cortex and associated areas are directly involved in the initiation and maintenance of locomotion, many other regions such as the septo-hippocampal circuitry have been implicated in the premotor control of locomotion and the transition of the brain into a state appropriate for processing inputs during locomotion. For instance, theta oscillations are observed in the hippocampus of rodents hundreds of milliseconds before the onset of locomotion and throughout the movement period representing a brain state specialized for encoding spatially-related sensory input [1–4]. Optogenetic stimulation (at theta frequencies) of the glumatergic neurons in the medial septum and diagonal band of Broca (MSDB), the theta pacemaker, has been shown to evoke locomotion [5–6]. Similarly, high frequency (50–100 Hz) stimulation of certain hypothalamic nuclei, supramammillary nucleus, rostral pontine oralis nucleus (RPO) and raphe nuclei have been shown to evoke or inhibit hippocampal theta oscillations and, respectively, initiate or reduce locomotion [7–12].

The NI has dense projections to and from the MSDB. In addition, its projections span many of the other regions involved in modulation of hippocampal theta oscillations including the midbrain raphe nuclei, lateral habenula, RPO, certain hypothalamic nuclei such as the supramamillary nucleus, and the hippocampus itself. It also receives inputs from some of these regions [1,8,9]. These initial anatomical findings suggest that the NI is in a position to modulate hippocampal theta oscillations and pre-motor control of locomotion. Subsequently, a study demonstrated that high-frequency stimulation of the NI, evokes theta oscillations in the hippocampus [10]. Similar to the RPO and DRN, the NI also generates theta which is coupled with hippocampal theta activity [7]. In addition, the NI acts as a mediator between the RPO and medial septum-hippocampus system. Acute inactivation of the NI prevented RPO stimulation-induced hippocampal theta in the anesthetized rat [10]. The NI is a chief source of the highly conserved neuropeptide, relaxin-3, in the brain, which has been shown to be involved in modulating theta via the medial septum [11]. Relaxin-3 expressing neurons of the NI spontaneously fire in a phase-locked manner to the initial ascending phase of hippocampal theta [6]. Relaxin-3 signaling in the medial septum and hippocampus modulates theta activity and promotes spatial memory measured by the spontaneous alternation task [11]. Supporting the functional relevance of the NI in spatial memory, the reversible inactivation of the NI with lidocaine delayed the acquisition and retrieval phase in the Morris water maze [12]. Additionally, a host of studies has shown that neurons in the NI exhibit hippocampal theta-locked firing and theta-rhythmicity [6,10,13]. Therefore, as hippocampal theta is associated with locomotion, and the role of NI in modulating hippocampal theta is well-established, in the present study we hypothesized that activation of the NI would trigger locomotion and tested this hypothesis by direct microstimulation of the NI in freely-moving rats.

2. Materials and methods

Adult male Sprague–Dawley rats (300–350 g), obtained from InVivos Pte Ltd., Singapore, were utilized in this investigation. The procedures were conducted under a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the National University of Singapore and in compliance with the guidelines of the National Institutes of Health Guide for Care and Use of Animals. Rats were housed in pairs in individually ventilated cages that were maintained in a temperature controlled room (22 °C–24 °C) with a 12 h light–dark cycle. The animals were given ad libitum access to food and water and were acclimatized to the housing conditions for at least 5 days. All behavioral assessments were conducted during the light phase of the 12 h light-dark cycle.

2.1. Surgery

Each rat was anaesthetized with an intra-peritoneal injection of a ketamine (75 mg/kg body weight) and xylazine (10 mg/kg body weight) cocktail. Subsequently, it was mounted on a stereotaxic frame and body temperature was maintained at 37 ± 0.5 °C with a homoeothermic heating blanket. A trephine hole was drilled above the NI (AP: −9.7 mm; ML = 0.1 mm from the Bregma) [14]. A twisted bipolar nickel chromium stimulation electrode (custom-made in the laboratory from 125 μm diameter 80% nickel, 20% chromium wire) was tested for connectivity before implantation into the NI. The electrode was held in place with dental cement and anchoring screws fitted to the skull. The rats were then allowed a rehabilitation period of 1 week, with analgesic (carprofen) and antibiotic (enrofloxacin) treatments injected subcutaneously for the first 5 days.

2.2. Drugs

Ketamine (Parnell Manufacturing Pty Ltd.; Alexandria, NSW, Australia), xylazine (Ilium Xylazil, Troy Laboratories Pty Ltd.; Glendenning, NSW, Australia), enrofloxacin (Baytril 5%, Bayer Health Care; Seoul, Korea) and carprofen (Carprieve, Norbrook Laboratories (GB), Ltd.; Carlisle, UK) were freshly prepared in sterile isotonic saline (B. Braun, Germany) before use. Pentobarbital (Valabarb) was purchased from Jurox Pty Ltd., Australia.

2.3. Behavior

On complete recovery of the rat, it was exposed to a circular open arena (diameter: 120 cm), and connected to a tethered stimulation head mount. A dual output square pulse stimulator (S88X, GRASS Technologies, U.S.A.) and a photoelectric stimulus isolation unit (PSIU6X, GRASS Technologies, U.S.A.) were used for electrical stimulation. The stimulation protocols employed included a (1) 100 Hz stimulation train (with pulse width: 0.25 or 0.5 ms) lasting for 4 or 10 s with an inter-train interval of 10 s or a (2) 1 pulse stimulation given every 10 s in a subset of animals. The current intensity was progressively increased from 25 μA in 25–50 μA steps. No further increases in current intensity were used when a maximal response (locomotion) indicated that a saturation current was reached, as judged by 3 observers, or 700 μA was reached. The stimulation at each current intensity was repeated a minimum of 6 times (and not > 10 times). A minimum of 3 progressively increasing current intensity values were used for each animal. These stimulation protocols have been previously successfully employed in our laboratory and have been found to produce maximal NI-induced effects in target regions [15].

The behavior of the rat was recorded with Ethovision XT10 (Noldus, Netherlands). Raw position information of the animal (center point) was acquired using a 25 Hz sampling rate. Using this information, linear velocity of the animal, number of rotations and mobility were calculated. Linear velocity of the animal was defined as the composite differences between x and y coordinates of the center point of the animal between samples. The number of rotations were counted by measuring the direction of motion of an animal about external points. The animal had to rotate 180° to complete a rotation. A threshold of 50° was set to count rotations. That is, if the animal initially while rotating one way, rotated for 50° in the opposite way, resulted in the initial rotation reading being discarded and a rotation in the opposite direction being initiated. Once the cumulative number of rotations were counted, using the aforementioned methodology, for various epochs, the results

were divided by 2, to give number of 360-degree rotations. Mobility was defined as the change of pixels occupied by the animal between consecutive video frames. This parameter accounted for displacement of the center point of the animal from one location to another and also the general activity with a constant center point. Therefore, we believe this parameter is more sensitive to detecting increases in general activity of the animals. The data was subsequently exported to MATLAB 2014b, where custom-written MATLAB scripts were used to preprocess and analyze the data.

In cases where the software failed to detect the correct position of the animal, interpolation was performed to estimate the position of the animal.

2.4. Confirmation of electrode positions

Transcardial perfusion was performed with isotonic saline followed by 4% paraformaldehyde in phosphate buffer (0.1 M). The brain was post-fixed in 4% paraformaldehyde for a few days, and then transferred to 30% sucrose solution at 4 °C. Sections (40 μm) were collected on glass slides with a cryostat (CM 3050, Leica Biosystems, Germany). The slides were viewed and photographed under a microscope (BX-51, Olympus, Japan) to determine the position of the electrode (Fig. 1). Rats with electrodes which were found not to be in the NI were separately analyzed (Supplementary Fig. 1).

2.5. Data analysis

Statistical analyses were performed using MATLAB 2014b and the statistical language R. Based on inspection of our results, a 4 s pre-stimulation, *peri*-stimulation period (4 s or 10 s) and 4 s post stimulation period was used for analysis. The average velocity, mobility and number of rotations in these epochs were calculated for each animal, at each current intensity. To determine the optimal stimulation current intensity, a ratio of the mean value for each parameter during stimulation to pre-stimulation (*peri*/pre-stimulation ratio) for each current intensity was calculated (for instance, mean velocity during stimulation divided by mean velocity pre-stimulation for a certain current). For each animal, the current intensity which produced the maximal *peri*/pre-stimulation ratio for each parameter was used for further analysis. These values were tested for normality (with a Kolgomorov-Smirnov test) and subsequently analyzed using an appropriate statistical test: paired *t*-test or Wilcoxon rank test for two pairs of data and one-way ANOVA with post hoc t tests with Bonferroni corrections or Friedman with post hoc Dunn's tests with Bonferroni corrections for multiple comparisons. Similar analysis was performed on animals with electrode positions not in the nucleus incertus.

To quantify the delay in effects on NI stimulation, the latency to increase in velocity and mobility were calculated. These latencies for these parameters were defined as the delay at which the velocity and mobility of the animal during stimulation were three standard deviations above the mean pre-stimulation velocity and mobility, respectively, for 3 consecutive bins (75 ms). This latency was averaged for each current for each animal. For each animal, the average latency for the current which produced maximum *peri*- to pre-stimulation ratio of that parameter was chosen for across animal comparisons depicted in Fig. 5.

To study the relationship of current intensity to the various parameters of evoked behavioral activity (velocity, mobility and number of rotations), simple linear regression analysis was performed as follows: to account for across animal differences in the currents used, each current and the parameter of interest (mean value during stimulation for that parameter; for instance, mean velocity during stimulation for a certain current) was normalized for every animal individually by dividing them by the maximum current and the associated value for the parameter of interest, respectively, for that animal and then multiplying by a 100%, following which the simple regression analysis was performed for that parameter and the coefficient of determination (R-squared) calculated.

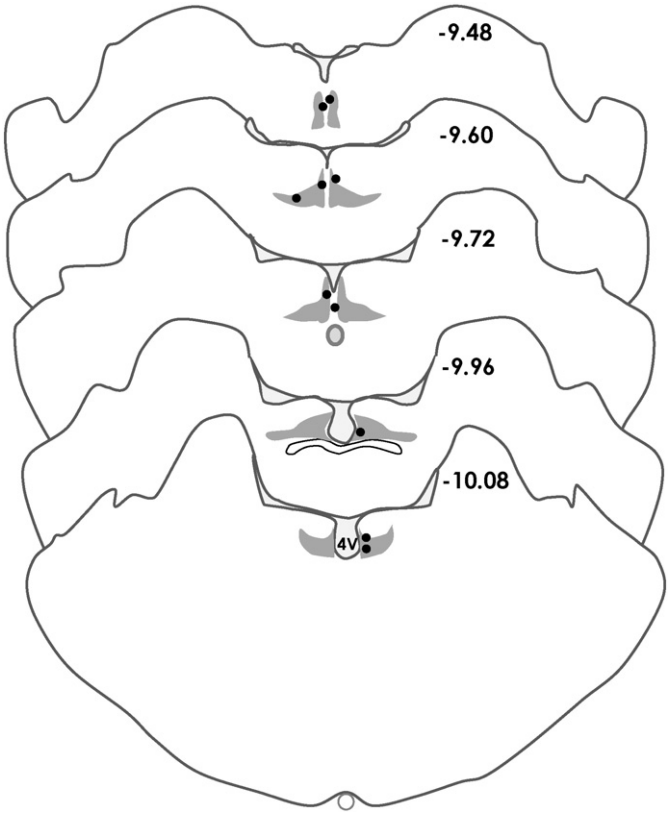

Fig. 1. Schematic representation of the positions of stimulation electrode tracks terminating in the NI (represented by grey shaded areas) across the anterior-posterior axis.

3. Results

Post-mortem analysis revealed that 10 out of the 14 implanted rats had the stimulation electrode in the NI (Fig. 1). The remaining animals, with electrode positions not in the NI, were pooled and analyzed separately (Supplementary Fig. 1).

3.1. Activity-associated parameters

High-frequency stimulation of the NI (100 Hz) was sufficient to induce forward locomotion increasing velocity (Fig. 2), mobility (Fig. 3) and rotation (Fig. 4). Increasing stimulation intensities resulted in increased locomotion responses in representative animals (Figs. 2A, C, E; 3A, C, E; and 4A, B, C, 25 μA, 50 μA and 100 μA, respectively) Furthermore, the increase in activity-associated parameters occurred during the stimulation and stopped rapidly after cessation of stimulation of the NI (Figs. 2B, D, F and 3B, D, F).

Stimulation of the NI, using a 100 Hz stimulation paradigm, increased velocity during and post stimulation (Friedman test: p value = 0.0074; post hoc Dunn's tests with Bonferroni corrections: pre versus *peri*: p value = 0.0006; pre versus post p value = 0.0357; *peri* versus post p value = 0.3061; Fig. 5A). Similarly, stimulation of the NI increased mobility during and post stimulation (Friedman test: p value = 0.0017; post hoc Dunn's tests with Bonferroni corrections: pre versus *peri*: p value = 0.0001; pre versus post p value = 0.0155; *peri* versus post p value = 0.2674; Fig. 5B).

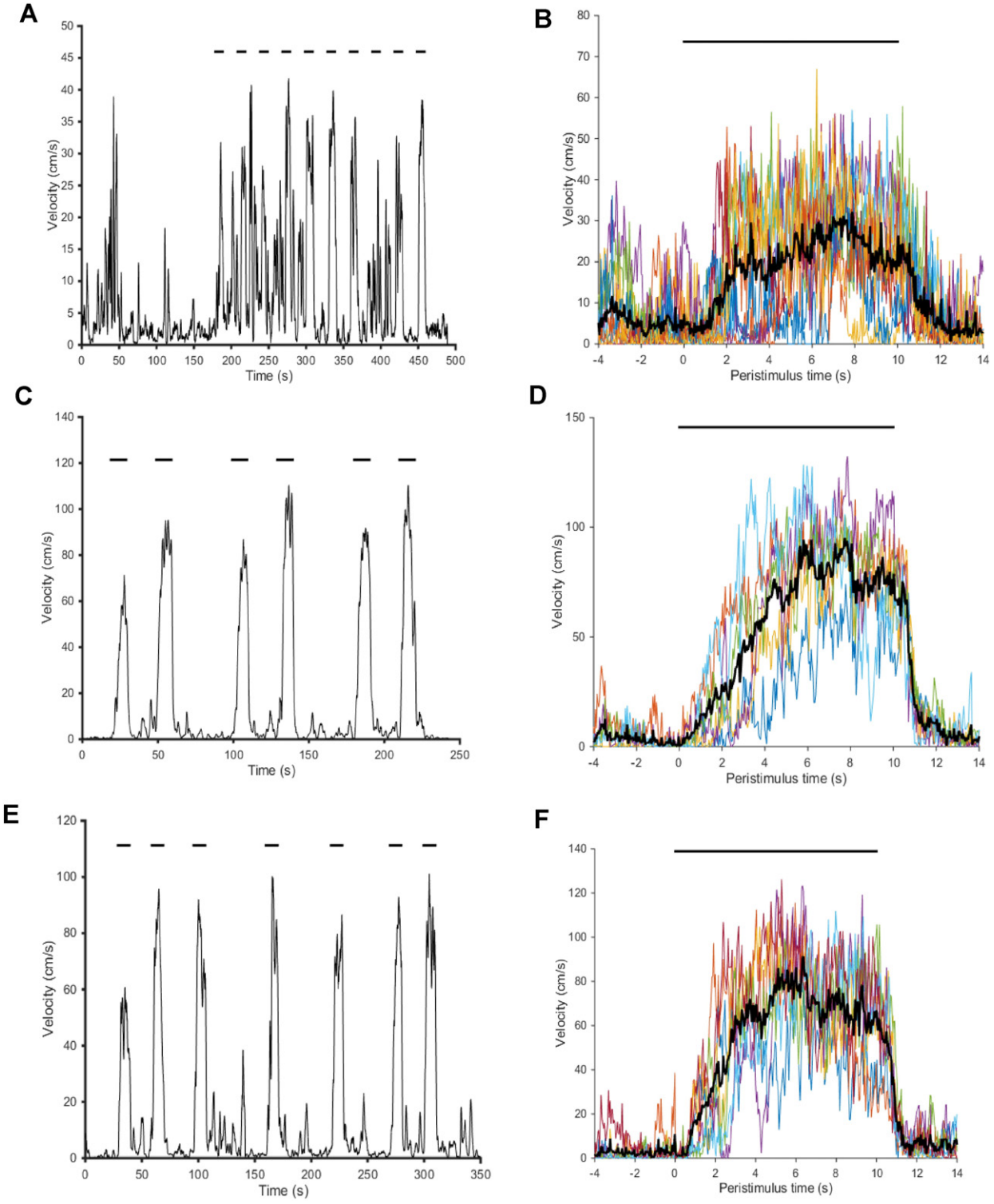

Fig. 2. Plots from one representative animal demonstrating an increase in velocity on stimulation of the NI. (A) Time versus velocity plot with the NI being stimulated at 100 Hz at a current of 25 μA (0.25 ms pulse width). (B) Peri-stimulus time versus velocity plot of (A) with the black curve depicting the mean response, while the other colors showing the responses to each stimulation train. (C–D) Similar time-velocity and peri-stimulus plots when the stimulation current was 50 μA (0.25 ms pulse width). (E–F) Similar time-velocity and peri-stimulus plots when the stimulation current was 100 μA (0.25 ms pulse width). The black horizontal bars in all plots represent the stimulation train.

Finally, stimulation of the NI also increased the total number of rotations during and post stimulation (Friedman test: p value = 0.0031; post hoc Dunn's tests with Bonferroni corrections: pre versus *peri*: p value = 0.0001; pre versus post p value = 0.0174; *peri* versus post p value = 0.1805; Fig. 5C).

However, in sharp contrast to the aforementioned results in the animals with electrode positions not in the NI (Supplementary Fig. 1), stimulation (at 100 Hz) had no significant effect on velocity (Friedman test: p value > 0.05; Supplementary Fig. 2A; Supplementary Fig. 3), mobility (Friedman test: p value > 0.05; Supplementary Fig. 2B;

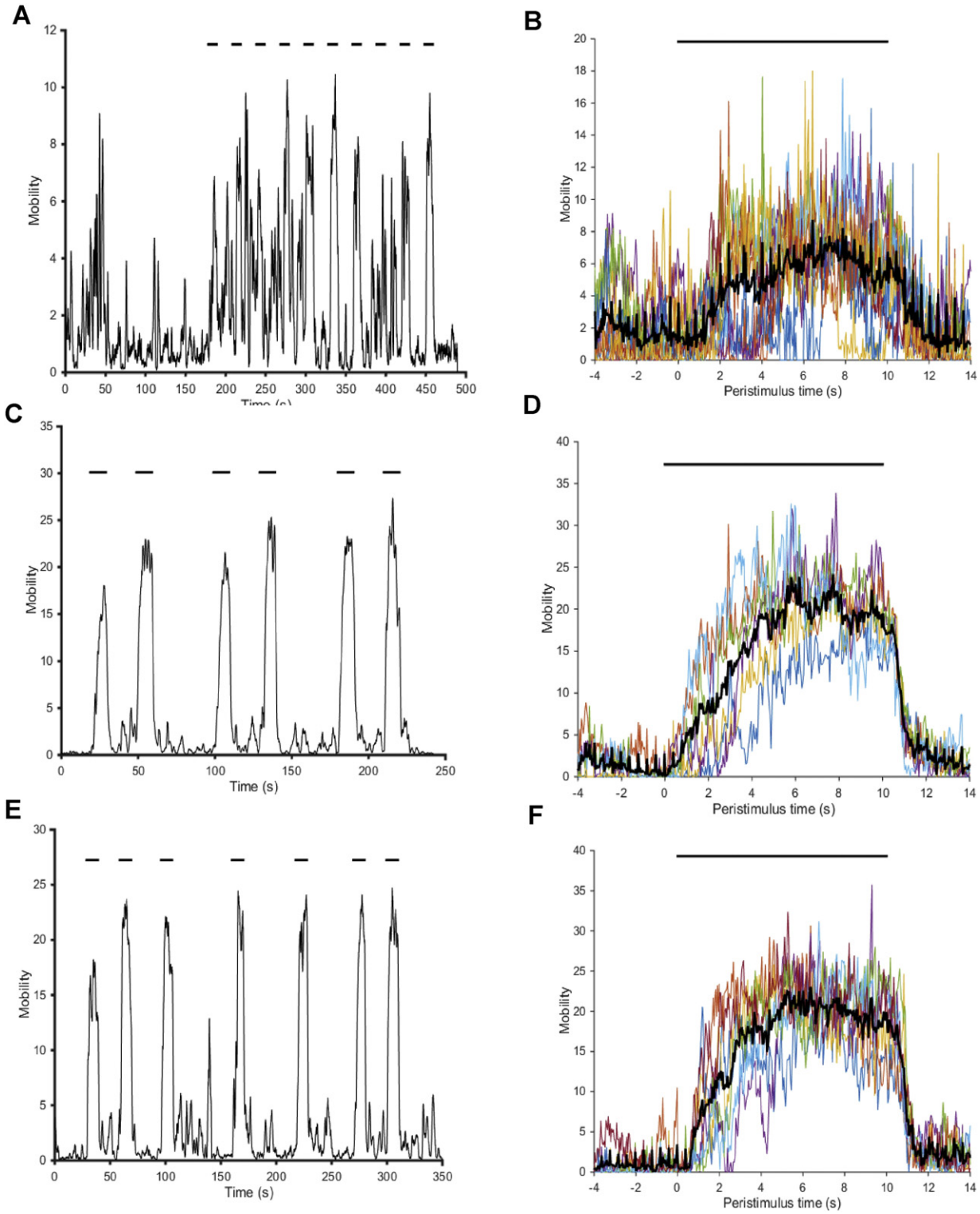

Fig. 3. Plots from the representative animal demonstrating an increase in mobility on stimulation of the NI. (A) Time versus mobility plot with the NI being stimulated at 100 Hz at a current of 25 μA (0.25 ms pulse width). (B) Peri-stimulus time versus mobility plot of (A) with the black curve depicting the mean response, while the other colors showing the responses to each stimulation train. (C–D) Similar time-mobility and peri-stimulus plots when the stimulation current was 50 μA (0.25 ms pulse width). (E–F) Time-mobility and peri-stimulus plots when the stimulation current was 100 μA (0.25 ms pulse width). The black horizontal bars in all plots represent the stimulation train. This is the same animal as Fig. 2.

Supplementary Fig. 4) and total number of rotations (Friedman test: p value > 0.05; Supplementary Fig. 2C). There was no current intensity which produced a significant increase in the activity associated parameters.

Unlike the 100 Hz stimulation paradigm, single pulse stimulation of the NI did not elicit changes in any of the parameters of interest (Freidman test: p value > 0.05 for velocity, mobility and number of rotations).

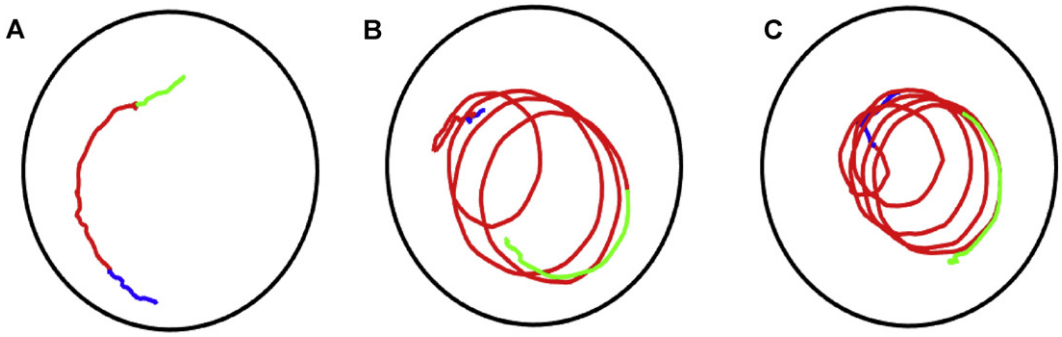

Fig. 4. Trajectory of the representative animal (same animal as Figs. 2 and 3) in real space (maze represented by black circle) during pre- (represented in blue), peri (represented in red)- and post- stimulation (represented in green) of the NI. All plots are on the same scale. (A) is at the 25 μA current, (B) at the 50 μA current while (C) at the 100 μA current.

3.2. Latency to increase in velocity and mobility

Across all animals, the median latency to increase in velocity on stimulation of the NI was 2.4 s (illustrated in Fig. 5D). In agreement with this finding, the median latency to increase in mobility was 2.2 s (illustrated in Fig. 5E).

3.3. Relationship of current to various activity-associated parameters

In order to study the relationship of current to the various parameters of locomotor activity, current and the parameter of interest were normalized for each animal, as described in Section 2.5. Following which a simple linear regression model was fit to the data of all animals.

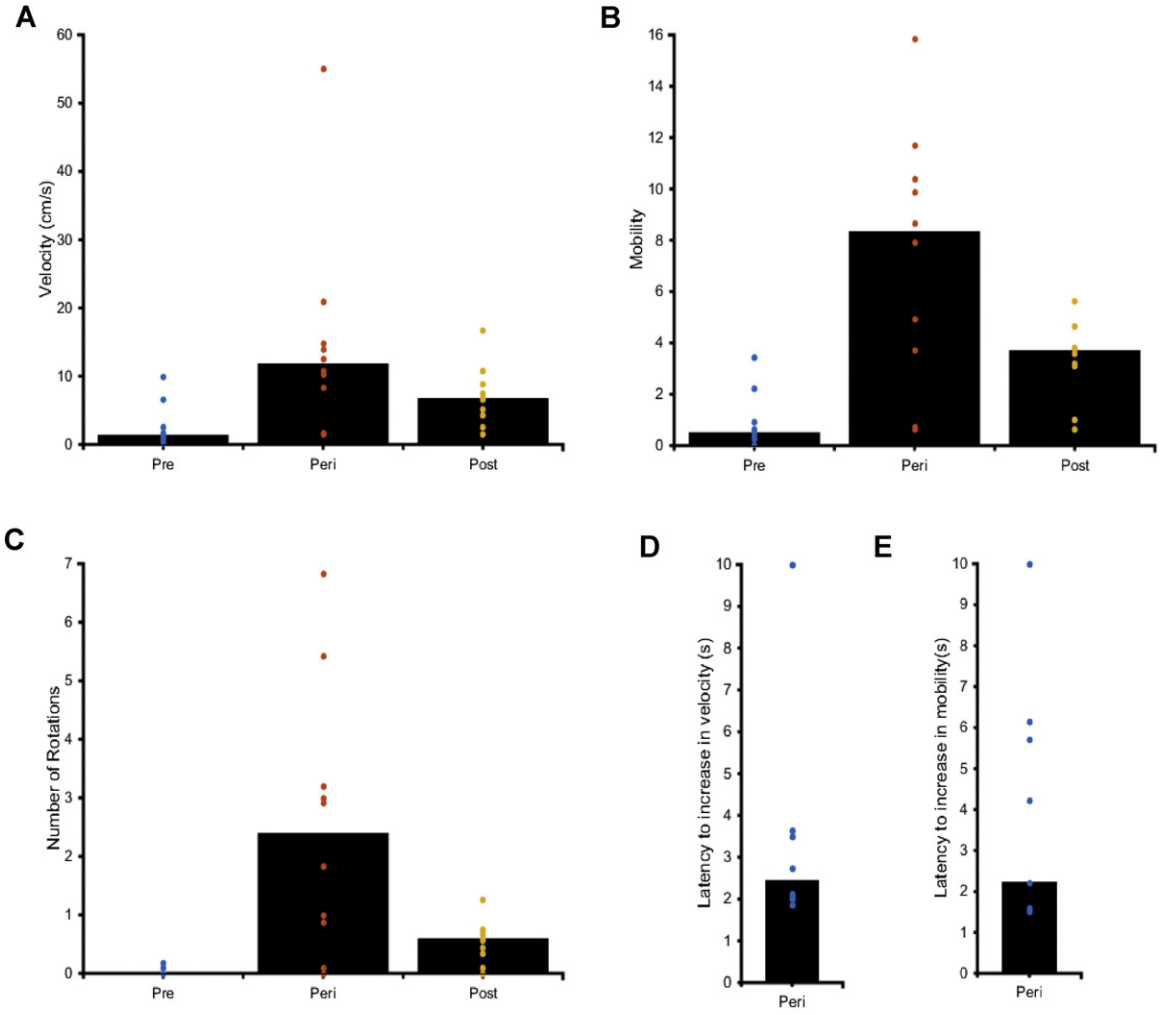

Fig. 5. Stimulation of the NI increases behavioral activity. (A) Velocity of individual animals pre-, peri- and post- stimulation. The bars represent the median velocities in each condition. (B) Mobility of individual animals pre-, peri and post- stimulation. The bars represent the median velocities in each condition. (C) Number of rotations of individual animals pre-, peri- and post stimulation. The bars represent the median velocities in each condition. (D) Latency to increase in velocity on NI stimulation in individual animals. The bar represents the median latency. (E) Latency to increase in mobility on NI stimulation in individual animals. The stimulus intensities were as described in Section 2.3. The bar represents the median latency.

The coefficient of determination, R^2, was calculated, to assess the goodness of fit. An ANOVA was performed comparing the fit to the overall mean, to determine if there was a significant relationship. Indeed, a linear relationship between normalized current and normalized velocity was observed ($R^2 = 0.575$; F-statistic: 47.4, p value $= 5.38e^{-08}$; Fig. 6A). Similarly, a linear relationship between normalized current and normalized mobility was observed ($R^2 = 0.753$; F-statistic: 106, p value $= 3.73e^{-12}$; Fig. 6B). Finally, a linear relationship between normalized current and normalized total number of rotations was also observed ($R^2 = 0.666$; F-statistic: 65.9, p value $= 2.29e^{-09}$; Fig. 6C).

4. Discussion

Our results demonstrate that high-frequency stimulation of the NI is sufficient to induce forward locomotion, rotation and general behavioral activity (as indicated by an increase in mobility). The sizeable increase in velocity, mobility and number of rotations during stimulation is evidence of the strong role of the NI in locomotor activity (Figs. 2–6). This increase in behavioral activity is consistently and reliably observed on stimulation on a single-trial basis — evident even during individual stimulation epochs (refer to Figs. 2 and 3 for examples of individual trial increase in velocity and mobility on stimulation). Furthermore, the increase in activity-associated parameters declines abruptly after cessation of stimulation of the NI (Figs. 2B, D, F and 3B, D, F). These findings indicate that sustained neuronal activity in the NI, like that induced by electrical microstimulation, is expected to increase locomotor activity.

We found that the latency of evoked locomotion was in the order of seconds (Fig. 5 D and E). Direct stimulation of the premotor and primary motor cortex has been shown to produce muscle twitching with short trains (<50 ms) and complex coordinated movements at longer trains (>500 ms) [16–18]. Thus it is unlikely that the NI directly induces movement in a manner similar to the motor cortices. This suggests that the NI induces locomotion via modulation of premotor circuits, possibly the septo-hippocampal axis.

As mentioned before, several complementary pieces of evidence point to the contribution of NI in theta-associated locomotion. The NI (1) has dense bidirectional connections with the medial septum and the diagonal band of Broca, regions critical for generating hippocampal theta oscillations [1,8], (2) is able to evoke hippocampal theta and also shows synchronized theta activity with the hippocampus when the RPO is stimulated [7,10], (3) is a mediator of RPO-induced hippocampal theta [9], (4) neurons expressing relaxin-3 fire in coherence with hippocampal theta and exhibit theta-rhythmicity [6], and finally (5) is able to modulate theta via the medial septum and this translates to changes in spatial memory [11]. With information that NI projects to glutamatergic MSDB neurons and optogenetic stimulation of glutamatergic neurons in the MSDB reliably evokes locomotion (latency in the order of hundreds of milliseconds) and hippocampal theta oscillations [19], it is tempting to speculate that NI projections to MSDB neurons might be involved in inducing locomotion. Future studies with optogenetic/DREADDs manipulation of NI projections to the medial septum might be able to parse the role of these projections in locomotion.

In the present study, locomotion was induced by stimulation of the NI at frequencies similar to those published earlier that have been

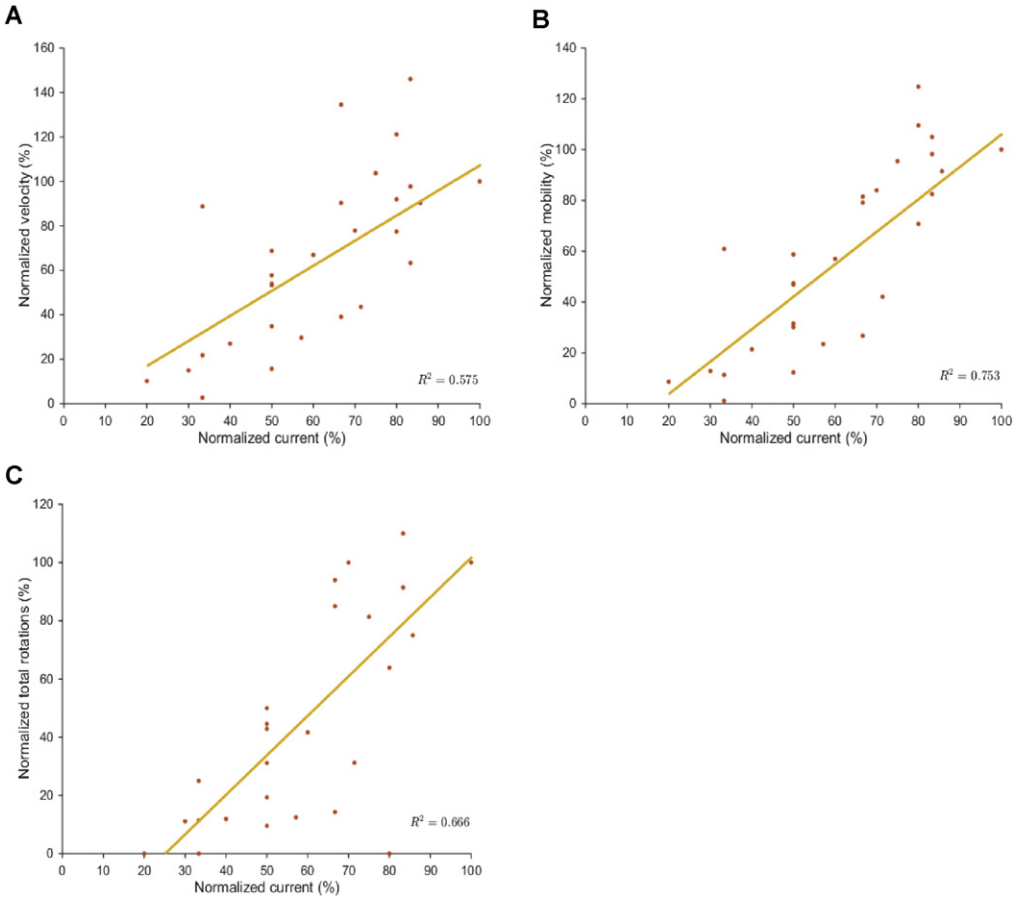

Fig. 6. Linear relationship between stimulation current at the NI and activity associated parameters. (A) Normalized velocity increases linearly with normalized stimulation current at the NI when all animals with electrode positions in the NI are pooled together. (B) Normalized mobility increases linearly with normalized stimulation current at the NI when all animals with electrode positions in the NI are pooled together. (C) Normalized number of rotations increases linearly with normalized stimulation current at the NI when all animals with electrode positions in the NI are pooled together. The coefficients of determination for each relationship are shown in the respective plots. All current intensities were used for this analysis.

shown to generate hippocampal theta oscillations in anaesthetized animals [10], a local field potential which reliably precedes locomotion in behaving animals. We have previously shown that similar high frequency stimulation of the NI significantly reduces firing rate in the medial prefrontal cortex and impairs ventral hippocampal-medial prefrontal cortical long-term potentiation [15,20]. Additional evidence from our laboratory indicates that similar high frequency stimulation of the NI (to a much greater extent than single-pulse stimulation) evoked potent changes in firing rate in a variety of brain regions, including the ventral hippocampus, periaqueductal grey area, lateral habenula and midbrain serotonergic nuclei [21]. These findings taken together indicate that the NI fires at high frequencies during various behaviors, and high frequency stimulation of the NI is sufficient to evoke behaviors, including locomotion. Therefore, high frequency activity in the NI, might be required to optimally modulate downstream cortical and subcortical regions. Intriguingly, in the current study, all the activity-associated parameters (velocity, mobility and rotational motion) exhibited a significant linear relationship with the current intensity (Fig. 6). A higher stimulation current intensity, besides recruiting a wider swathe of neuronal tissue, increases the probability of depolarization and neuronal firing of the stimulated tissue. Therefore, our findings suggest that the stimulation-induced increase in behavioral activity is not an all-or-none phenomenon, rather is dependent linearly on the recruitment and firing of NI neurons.

Brainstem structures namely, raphe nuclei, RPO and ventral tegmental nucleus of Gudden that are proximal and strongly connected to the NI, also show theta rhythmicity [1,22]. The ventral tegmental nucleus of Gudden is also a strategically connected GABAergic nucleus that shows strong state-dependent synchrony to hippocampal theta and plays a role in locomotion [23,24]. Interestingly, microstimulation of the median raphe, which is proximal to- and has bidirectional connections with the NI has been found to inhibit hippocampal theta and concurrent motor behaviors such as walking, rearing and grooming [25]. Indeed, the strong connections between the NI, median raphe and interpeduncular nucleus appear to form a brainstem control of behavior [1].

As we used electrical stimulation to activate the nucleus incertus, we cannot rule out the possibility of stimulating passing fibers and surrounding areas. However, based on the extensively studied role of the NI in evoking hippocampal theta oscillations in anaesthetized animals, which reliably accompany locomotion in awake behaving animals, we believe this is not the case. In addition, the animals with electrode positions which missed the NI, did not exhibit a significant increase in locomotion associated parameters (velocity, mobility and rotations) on stimulation (Supplementary Figs. 2–4). Finally, in certain cases (e.g. Fig. 2–5), the evoked locomotion was evident at very low currents (25 μA). Furthermore, the median minimum current which evoked a significant increase in behavioral activity was 150 μA. It is important to note here that this significant minimum current was calculated for each animal individually. The minimum current which evokes a significant increase at the group level is likely to be even lower. These findings, taken together, strongly indicate that the NI itself is involved in inducing locomotion. Recently, we have shown that the NI strongly expresses D2 receptors, local activation of which consistently induced hypolocomotion [26]. Antipsychotics, which are D2 receptor antagonists, increased NI c-Fos activation [27], supporting the hypothesis that NI activation increases locomotion. The current study supports and extends these findings by demonstrating that the high frequency stimulation of the NI is sufficient to induce locomotion, and by elucidating the properties of the NI-evoked locomotion.

In addition to a marked increase in forward locomotion and general behavioral activity, we observed increased rotations on stimulation of the NI. As these experiments were performed in a circular open field, it is possible that the environmental constraints promoted motion in circles. Further studies in linear environments, such as linear tracks, will elucidate whether this is indeed the case. Another possibility is that our electrodes might have stimulated one side of the NI, more than the other, and therefore, resulted in rotational locomotion. However, given the small dimensions of the NI, it is not possible to accurately target the two halves of the NI for selective electrical microsimulation. Future studies, using other techniques, such as optogenetics, to selectively target labelled cell populations in one side of the NI might prove useful. However, stimulation of the NI at the high frequencies (~100 Hz) required to elicit locomotion may prove challenging with optogenetics.

5. Conclusions

Based on the anatomical connections of the nucleus incertus, it has been suggested that the NI is involved in behavioral activation [1]. Yet, despite its well-established role in generation of hippocampal theta oscillations, direct evidence for the role of NI in locomotion/behavioral activity remained lacking. Here, we demonstrate that activation of the nucleus incertus, via high frequency stimulation, reliably and potently induces forward locomotion, as assessed by various locomotion-associated parameters. The latencies of evoked locomotion is consistent with a role of the NI in modulating premotor areas, possibly the septo-hippocampal axis. Furthermore, this increase in locomotion is consistently and reliably locked to activation of the NI. Taken together with earlier findings [10,11], this indicates that the NI is involved in behavioral activation, including generation of hippocampal theta oscillations and locomotion.

Statement of interest

None.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.physbeh.2016.03.033.

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