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Original Research

Intracortical microstimulation parameters modulate flight behavior in pigeon

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Pigeons have a natural affinity for travel by flight. Researchers have recently achieved modulation of pigeon locomotor behaviour by intracortical microstimulation. However, there is a lack of research focused on the analysis of microstimulations parameters in the control of pigeon flight. Here, chronic microelectrode implantation technology is employed to establish a model for evaluation of the effects of pigeon flight modulation. Furthermore, three stimulation parameters are compared (amplitude, frequency, and duty ratio) and analyzed as to how they and their interactions affect the flight of pigeons. Results show that microstimulation of the pigeon formation reticularis medialis mesencephali area has significant effects on modulation of pigeon flight and there is a significant non-linear correlation between the stimulation parameters employed and modulation of the flight trajectory. Additionally, we found that the amplitude interacts with both frequency and duty ratio. These results indicate that the flight trajectory of a pigeon can be modulated by alterations made to microstimulation parameters.

Keywords

Intracortical microstimulation; flight; stimulation parameters; pigeon

Abbreviations

ICMS	Intracortical microstimulation
FRM	Formation reticularis medialis mesencephali
DIVA	Dorsalis intermedius ventralis anterior
ICo	Intercollicularis
PLC	Programmable logic controller
DC	Direct current
AVI	Audio Video Interleaved

1. Introduction

Using microstimulation to stimulate specific areas in the brain of animals to evoke movement has become an intense area of research in an interdisciplinary frontier that involves automatic control science and neuroscience. Intracortical microstimulation (ICMS) has proved an effective method for modification of neural circuitry (Meghan et al., 2016) and has been broadly applied in many fields (Devecioglu and Güçlü, 2017; Fisher et al., 2010; Little et al., 2013; Lyketsos et al., 2012; Sato and Maharbiz, 2010; Schmidt et al., 1996; Tabot et al., 2013; Torab et al., 2016). The biological robot in which brains are stimulated with micro-current to control the locomotor behavior of an animal has great advantages regarding flexibility, stability, and energy efficiency (Dickinson et al., 2000) because design is based on the animal's existing movement mechanisms. How to modify an animal's behaviour by activating brain areas with microstimulation has significant meaning for both theoretical study and practical application.

Much research (Talwar et al., 2002; Kobayashi et al., 2009; Wang et al., 2009; Cai et al., 2015; Wang et al., 2018) has shown that ICMS can achieve control of various specific movements of an animal. Talwar et al. (2002) have manipulated rat directional behaviour by stimulating the somatosensory cortex and reward centre with electrical current. By stimulating the medial longitudinal fasciculus of goldfish Kobayashi et al. (2009) controlled left/right turning movements. Similar results have also been achieved with the gecko (Wang et al., 2009).

Although Ferrier (1877) induced rotation of the head by stimulating the cerebellum of a bird, there is currently still a lack of study of how intracortical microstimulation influences the modulation of avian movement. The extraordinary flying abilities and clear neuroanatomical structure of pigeons (Karten and Hodos, 1967; Güntürkün et al., 2013) make them ideal experimental subjects for research into how stimulation may manipulate flying behaviour. Su et al. (2012) utilized microstimulation in the ventral nucleus of the thalamus (dorsalis intermedius ventralis anterior-DIVA) and striatum to induce pigeons to take flight. The DIVA area and striatum correlate with the feelings of fear and pain. By stimulating the nucleus intercollicularis (ICo), formation reticularis medialis mesencephali (FRM), and other related brain areas, Cai et al. (2015) induced pigeons to make actions, such as winging and turning, on the ground. They recently developed a stimulator to control pigeon flight outdoors (Wang et al., 2018).

Those previous studies typically applied strong electrical stimulation to ensure a response was produced (Meghan et al., 2016).

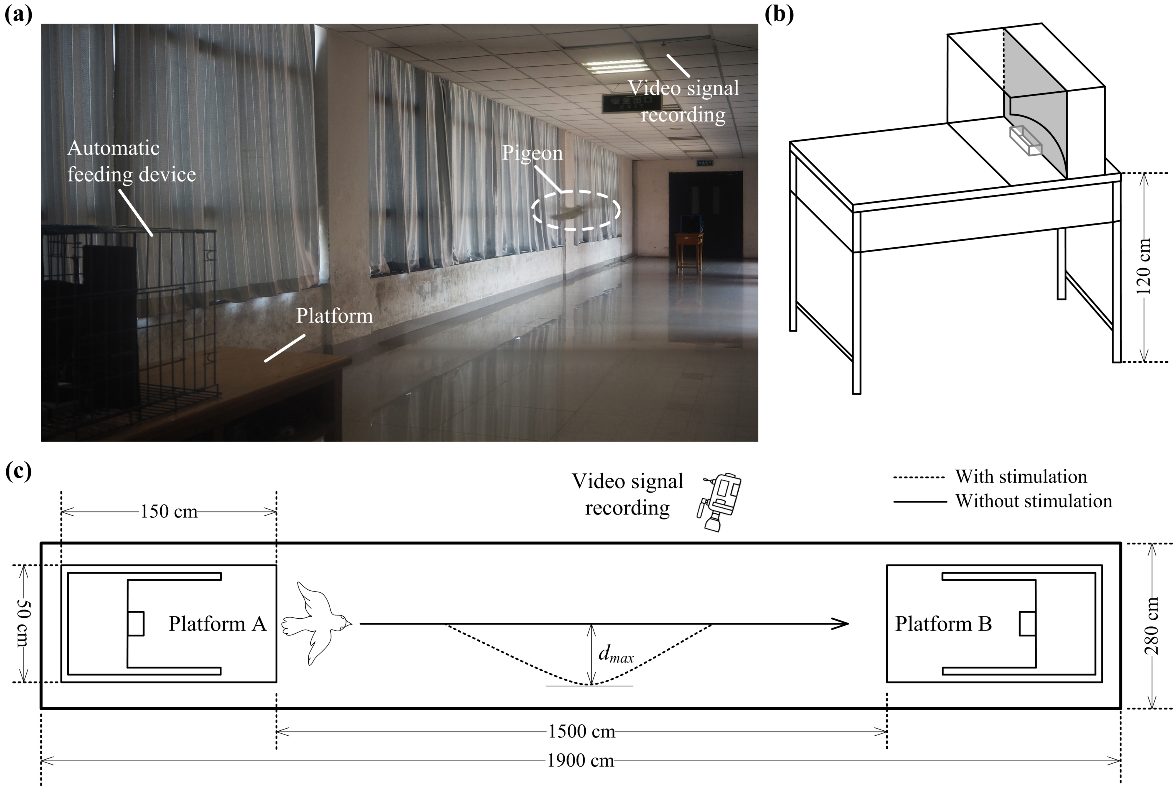

Figure 1. Behavioural task and experiment procedures. (a) Experimental site. Two 1.2 meter high platforms separated by 15 meters were located at either end of the experimental area. Cameras were placed on the ceiling to collect video of pigeon flight. (b) Schematic of automatic feeding platform. The automatic feeding equipment was installed on the platform to induce pigeon flight. It removed human involvement from the experiment. (c) Diagram showing flight task. Pigeons were trained to reach a platform (A) and get food from a food container. After food was consumed, pigeons flew to the other platform (B) and obtained food again. During the pigeon flight period, stimulation was applied to the FRM area.

By contrast the relationship between microstimulation parameters and induced flight behaviour of pigeons are still unclear.

Here, the aim is to modulate pigeon flight. It has been shown experimentally that applying microstimulation to the FRM area in the brain allows a lightly anesthetized or grounded pigeon to appear to turn in the same direction as the stimulated side. For this reason, the FRM arae was chosen for microstimulation. By studying how pigeons react to microstimulation during flight, the role of FRM can be explored. Further, the effect of stimulation parameters on modulating pigeon flight is evaluated to increase the precision of flight control. To the authors knowledge, this is the first research to focus on the control of pigeon flight behaviour by ICMS parameters.

2. Materials and methods

Six adult pigeons (Columba livia, unknown sex, 450-550 g) were used in this study. They were numbered by P030, P035, P042, P075, P078, P080. The pigeons were housed in individual wire mesh cages under a 12: 12 hour light-dark cycle. During the experiment, on work days food was restricted to the period of daily testing. All experiments were conducted in accordance with the Animals Act, 2006 (China) for the care and use of laboratory animals and approved by the Life Science Ethical Review Committee of Zhengzhou University.

2.1 Experimental Design

An experiment was designed to allow pigeons to fly back and forth to fetch food so that study of how stimulation of the FRM area with electrical signals modulated pigeon flight. Fig. 1 shows the experimental area. Two 1.2 meter high platforms separated by 15 meters were located at either end of the experimental area. Automatic feeding equipment was installed on the platform to induce flight between them. Pigeons were trained to reach a platform (A) and get food from a food container. After food was consumed, pigeons flew to the other platform (B) and obtained food again. This procedure continued throughout an experiment. During the pigeon flight period, stimulation was applied to the FRM area. Data were collected as video signals by cameras placed on the ceiling of the experimental site. Data were analyzed to investigate the effect of stimulating FRM on modulation of pigeon flight.

The automatic feeding devices were composed of an infrared sensor, a programmable logic controller (PLC), and a direct current (DC) motor. When the infrared sensor was triggered, the PLC that controlled the DC motor pushed a food container so that pigeons were able to collect food from it. After 15 seconds, the PLC reversed the motor and the food container was retracted, thus limiting pigeon feeding amount. The design removed human involvement from the experiment.

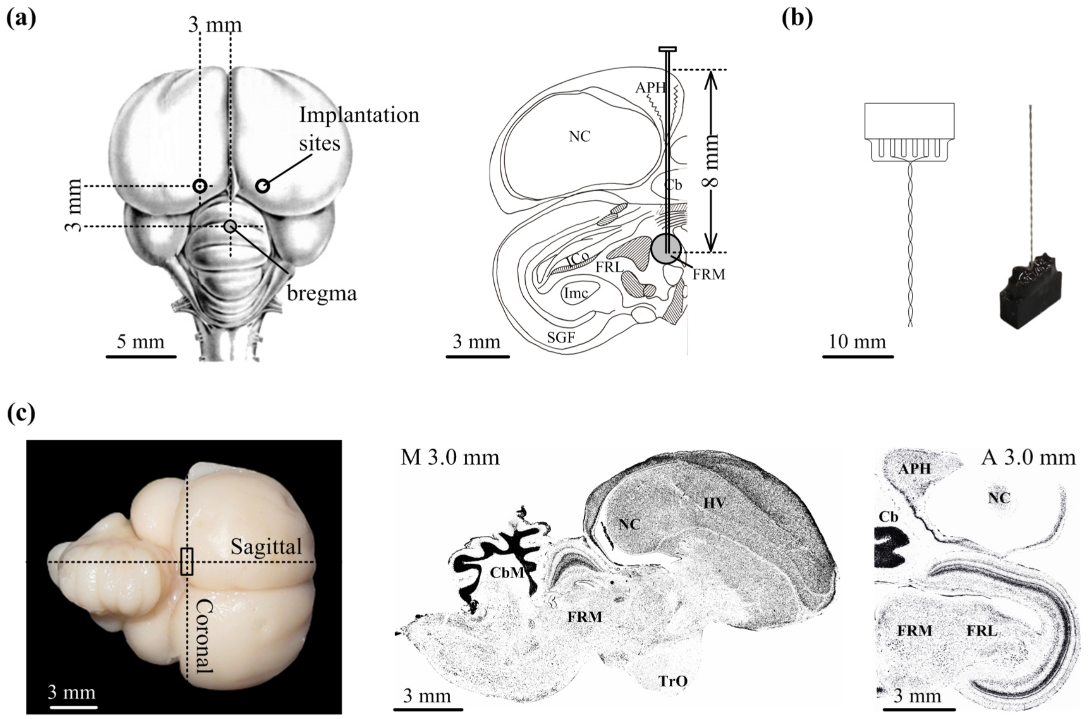

Figure 2. Location of FRM and its organizational structure. (a) Schematic of electrode implantation location in FRM area of a pigeon brain. The location of the FRM area is AP: 3.00 mm, ML: 3.00 mm, DP: 7.5-8.5 mm. (b) Stimulating electrodes, constructed from two twisted-pair electrodes. The electrodes are diameter 100 mm Ni-chrome stainless steel wire, Teflon insulation. The electrodes were implanted in left and right FRM areas simultaneously. (c) Slice schematic of electrode implantation. Left: Dorsal view of the pigeon's brain. The horizontal dashed line indicates section level AP 3.0. The vertical dashed line indicates section level ML 3.0. Middle: Vertical section of the electrode implantation location. Right: Transverse section of the electrode implantation location.

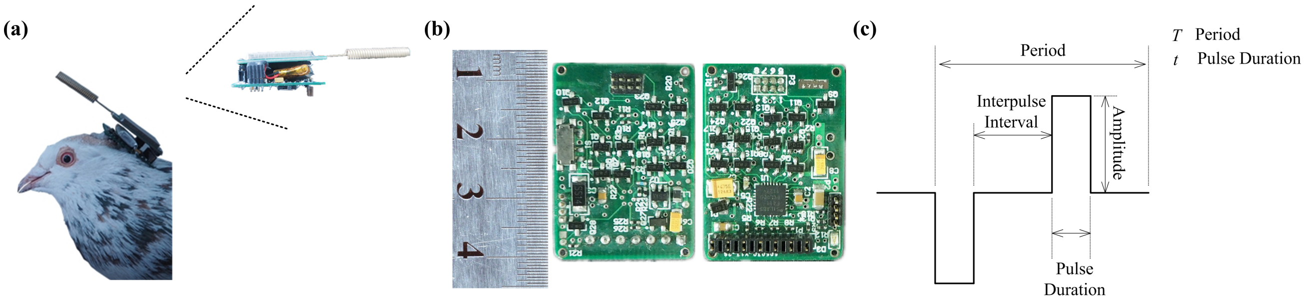

Figure 3. Wireless current stimulator and stimulation signal output waveform. (a) Pigeon with the wireless stimulator. The size of the stimulator is 3.3×2.4 cm, and weight 8.5g. (b) Control module and constant-current source stimulation module, next to a ruler as a size reference. The stimulator comprised a wireless communication module, control module, constant-current source stimulation module. (c) Parameters of the constant current biphasic stimulation waveform. "T" denoted period and "t" denotes the pulse duration as employed here.

2.2 Surgery

Male and female pigeons selected for the experiment weighed between 450-550 g. They were anesthetized by raperitoneal injection with 10% chloral hydrate (0.4 ml/100 g). After an anaesthetic took effect, head feathers were shaved and 2% lidocaine (0.2-0.3 ml) was injected subcutaneously as a further local anaesthetic. The pigeon was immobilized in a specially designed brain stereotaxic device. The location of the FRM area (AP: 3.00 mm, ML: 3.00 mm, DP: 7.5-8.5 mm) was determined based on a brain image (Karten and Hodos, 1967). Part of the skull was removed (3 mm $\times$ 3 mm) to expose the brain tissue. Following removal of the dura mater, an electrode was implanted (diameter 100 μm Ni-chrome stainless steel wire, Teflon insulation). The electrode was held in place by dental cement, and ear-brain glue was used as a buffer between the brain and the dental cement. Enrofloxacin solution (5%) was used in the surgical area to help recovery. The surgery area was cleaned regularly. The location of the FRM area and its organizational structure is illustrated in Fig. 2.

2.3 Stimulators and Their Parameters

The wireless current stimulator (size 3.3 $\times$ 2.4 cm, weight 8.5 g) was designed by the authors and comprised a wireless communication module, control module, constant-current source stimulation module, and a battery. The software application to set up

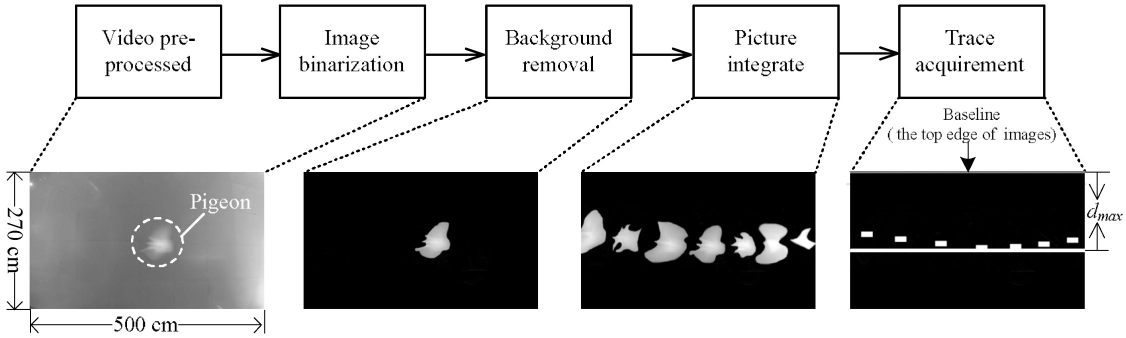

Figure 4. Video signal processing flow chart and effects schematic. Videos recorded flight during the experiment and the transmitted information of the video signals was stored in an Audio Video Interleaved (AVI) format (resolution 1280×720 px). Each image frame was then binarized and the background removed with an inter-frame difference method. The pigeon outline in each frame image was integrated and the graphic centre of gravity of the pigeon was obtained.

the stimulation parameters was also developed by the authors. Parameters were transmitted to the stimulator's wireless communication module by radio frequency. The control module enabled the constant current source stimulation module to generate stimulation signals corresponding to parameters received by the wireless communication module. The stimulator and its waveform parameters are illustrated in Fig. 3.

The stimulation waveform (constant-current, cathode leading, biphasic square waveform) of the stimulator is illustrated in Fig. 3(c). This square wave reduces irritative damage to cortical tissue by the charge balance existing in the polarity of alternating pulses (Meghan et al., 2016). Stimulation parameters included amplitude, frequency, pulse duration, interphase interval, and duration. As pigeons flew so fast and there was a correlation between the duration of stimulation-induced reaction and duration of stimulation, stimulation duration was uniformly set to 300 ms based on multiple previous experimental observations on the effect of pigeon response to stimulation. Since pulse duration and interphase interval were both affected by frequency, these parameters were correlated. Consequently, the duty ratio was used instead of pulse width and pulse interval. The duty ratio was defined as the ratio of the pulse width to the entire cycle:

$$DR = \frac{t}{T} \tag{1}$$

where DR is the duty ratio, t the pulse duration, and T the period of the stimulation square wave.

Based on the literature (Sholomenko et al., 1991; Goodman, 1958; Uematsu and Todo, 1997; Noga et al., 1991; Seki et al., 1997; Goodman and Simpson, 1960) and preliminary experimental analysis, the amplitude range was set between 60-450 μA, signal frequency range at 100-300 Hz, and the duty ratio ranged between 20-40%. The stimulation square waves that were generated by these parameters significantly altered the pigeon flight paths while the stimulus do not appear to damage pigeon brain function. Stimulation parameters were quantified to study differences in pigeon flight under different parameters (Table 1).

Pigeon reaction to microstimulation varies, particularly the amplitude parameter. Thus, when pigeon responses were evoked by stimulation, it was thought that the stimulus amplitude reached a threshold which was denoted by "T". It was gradually increased by a step size of 100 μA. A base frequency of 100 Hz was set. This frequency was gradually increased by steps of 100 Hz. The duty ratio started at 20% and was gradually increased by 10% steps.

Table 1. Parameter test values

Parameter	Unit	Range	Test levels
Amplitude	μA	60-450	T, + 100, + 200
Frequency	Hz	100-300	100, 200, 300
Duty Ratio	%	20-40	20, 30, 40

2.4 Acquiring and Analysing flight trajectories

The difference of flight trajectories between pigeons with and without stimulation were compared and analysed to evaluate the effect of stimulation on flight control. Videos recorded flight during the experiment and the video signals were transmitted to a PC through the Universal Serial Bus at a rate of 60 frames per second. The transmitted information was stored in an Audio Video Interleaved (AVI) format (resolution 1280 × 720 px). During the experiment, all devices for recording videos were immobile. For analysis, video signals were transferred into image signals via a frame-by-frame process. Each image frame was then binarized and the background removed with an inter-frame difference method. Images were processed pictures with only the outline of the flying pigeons shown on a black background. The pigeon outline in each frame image was integrated and the graphic centre of gravity of the pigeon was obtained (Fig. 4).

The maximum difference between the recorded flight trajectory and the baseline was adopted to quantify the influence of stimulation to analyze how parameters altered pigeon flight. Video images, recording pigeon flight trajectories, were converted into a matrix to record pigeon location changes, with a baseline set at the top edge of each image frame. All recorded flight trajectory images were converted to a 720 × 1280 matrix. The actual recording coverage was 270 cm × 500 cm. Based on these location changes in the matrix, the maximum difference between a flight trajectory and the baseline was calculated as:

$$d_{\max} = \frac{p_{\max} - p_0}{P} \times D, \tag{2}$$

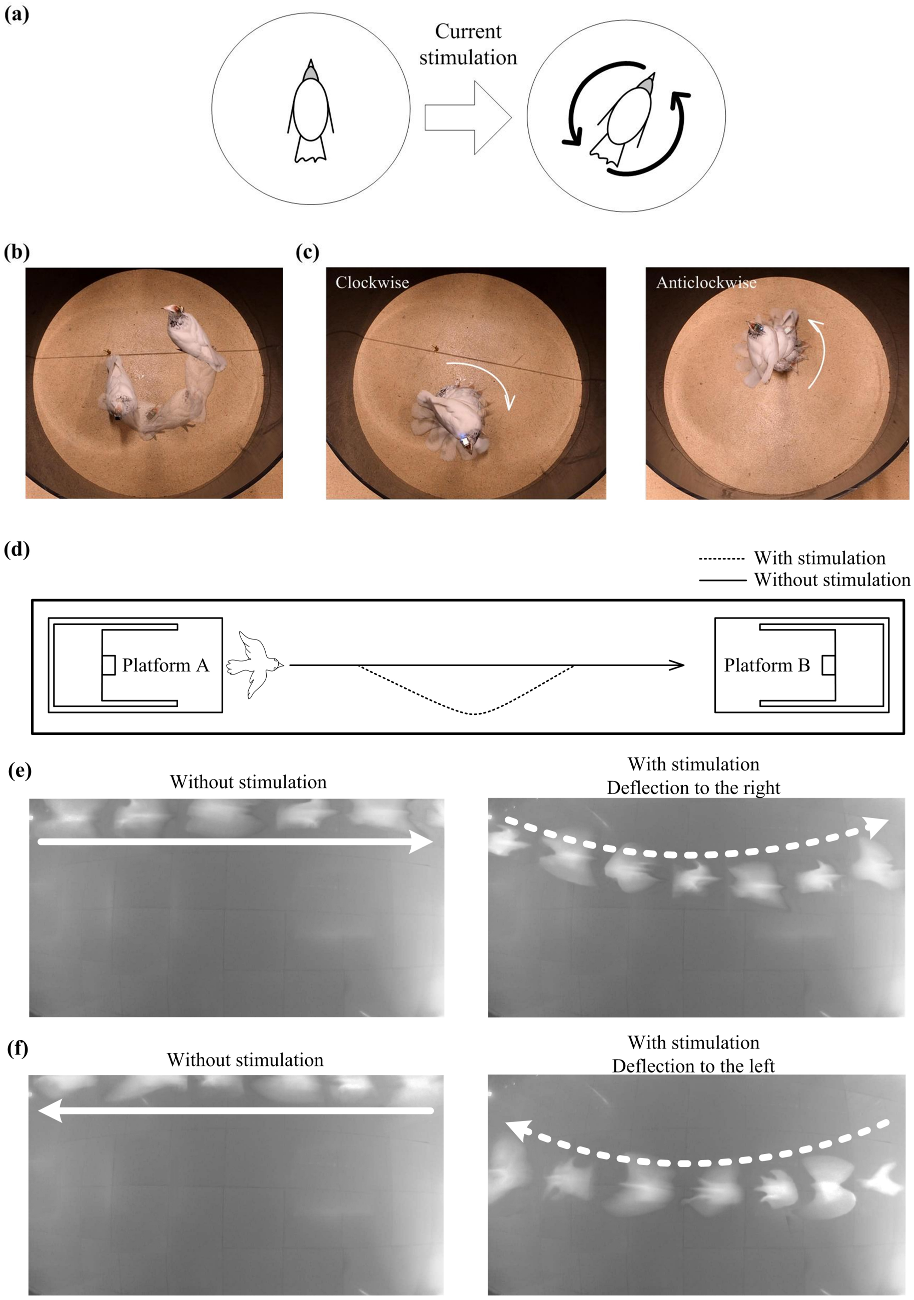

Figure 5. Reaction to electrical stimulation of pigeons either on the ground or in the air. (a) Schematic for pigeon movement on the ground with current stimulation. (b) Movement of pigeon in absence of current stimulation. (c) Movement of pigeon induced by current stimulation. (d) Schematic for pigeon flight trajectories with/without current stimulation. (e) Flight trajectories of pigeon without/with current stimulation in left side FRM. (f) Flight trajectories of pigeon without/with current stimulation in right side FRM.

where d_{max} was the maximum difference between each the flight trajectory and the baseline, p_{max} was the matrix row of the maximum difference location in a given trial, p_0 was the matrix row of the baseline, and p_0 was set to zero. P was the total number of rows in the matrix and was set to 720. D was the actual distance corresponding to P and was set to 270 cm.

The experiment was repeated six times for each group of stimulation parameters to obtain six maximum difference values between flight trajectories and the baseline linear flight path. The two values with the largest difference from the mean were removed from the dataset. The mean of the remaining four values was calculated to obtain a value for d_{max}.

2.5 Statistical analysis

Three parameters of the microstimulation (amplitude, frequency, duty ratio) were chosen for analysis of the effect of stimulation on flight. Each parameter was tested at each of the three levels and three amplitude levels were tested at each of the rest parameter levels. The tests were repeated six times for each group of stimulation parameters on all pigeons. The number of trials for P030, P035, P042, and P075 was 162 and for P078 and P080, was

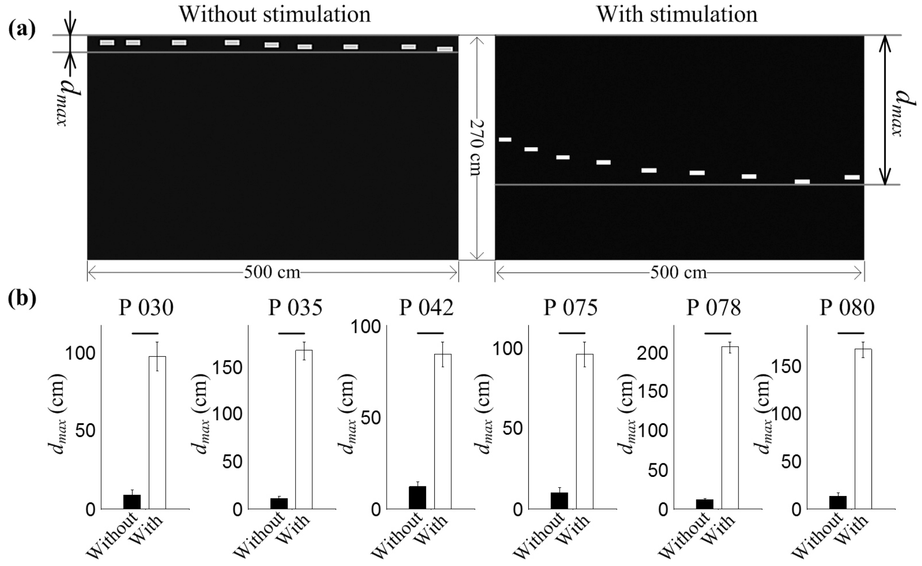

Figure 6. Results for pigeons without/with stimulation during flight. (a) Flight trajectories for pigeon No. P080 (left: without stimulation, right: with stimulation). (b) Statistical summary of results for all six pigeons' $d_{\max}$ (mean $\pm$ standard error-SE).

respectively 102 and 42. The $d_{\max}$ value in the text and figures was reporteded as mean $\pm$ standard error (SE).

Statistical differences were evaliated with a Wilcoxon rank-sum test (Wilcoxon, 1945), the confidence interval was set to $\alpha < 0.05$. The p-value was considered statistically significant at $p < 0.05$. Statistical calculation was carried out with the ranksum function (MATLAB 2014a (MATLAB, Inc.).

3. Results

Four scenarios were designed to study how stimulating the FRM area affects pigeon movements on the ground and in the air. These scenarios were utilized to quantitatively analyse the effects of pigeons reacting to stimulation parameters. The four scenarios were:

i. Stimulation during pigeon movements on the ground and in flight.
ii. Control of pigeon flight.
iii. Stimulation with a single parameter.
iv. Stimulation with multiple parameters to examine interactive effects between them.

3.1 Stimulation during pigeon movements on the ground and in flight

After pigeons had recovered from surgery, they were used in two experiments: First, pigeons were stimulated when they moved on the ground; in the second experiment, pigeons were stimulated in flight. Pigeons in the first experiment (on the ground) were put into a round pail radius 50 cm, height 80 cm. Pigeons were then stimulated with an electrical stimulus of amplitude 150 μA, frequency 100 Hz, duty ratio 20%, and three seconds duration (Fig. 5(a)).

Six pigeons received microstimulation in this experiment. All pigeons exhibited stepping of both feet, and turning to the side they were stimulated on in their cerebral hemisphere. For example, if they were stimulated on left side of the brain, they would turn towards the left (counter-clockwise).; timulated on the right, they would turn towards the right (clockwise). Fig. 5(b) and 5(c) show the response of pigeon No. P035 exhibited with or without stimulation. From the figure it can be seen that when there was no electrical stimulation, the pigeon's movements were random and its movements were irregular. After stimulus application, the pigeon would rapidly spin either clockwise or counter-clockwise. Moreover, these movements went around the centre of a circle. The the angle of rotation was found to be correlated with stimulus duration. The shorter the stimulus duration, the smaller the angle of rotation that was observed.

Pigeon flight was studied in the experimental site (Fig. 5(d)). Unstimulated pigeons flew straight from platform A to platform B and the return trajectory (platform B to platform A) was also a straight line. The left side of Fig. 5(e) and 5(f) are the flight trajectories of pigeon No. P087 when unstimulated. Pigeons were then stimulated with a microstimulation amplitude of 150 μA, frequency 100 Hz, and 20% duty ratio. Results for six pigeons showed that all stimulated pigeons deviated from the original straight line trajectories and turned toward the direction of the stimulated cerebral hemisphere As just described, if they were stimulated in the left side of the brain, then their trajectories would turn towards the left; and vice versa. The right side of Fig. 5(e) and 5(f) show flight trajectories of pigeon No. P078 with stimulation in the right and left brain, respectively. After stimulation was stopped, pigeons gradually returned to their original unstimulated straight line trajectories.

3.2 Control of pigeon flight

This scenario mainly focused on studying how stimulation in the FRM region affected pigeon flight. In this section, only the analysis of how to control pigeon flight by stimulating in the left side FRM area of pigeons is reported as stimulation effects in this side are consistent with stimulation in the right side FRM area. In this experiment, stimulation was applied in the left side of the FRM area of six pigeons. The stimulation parameters were: amplitude 350 μA, frequency 100 Hz, and duty ratio 20%.

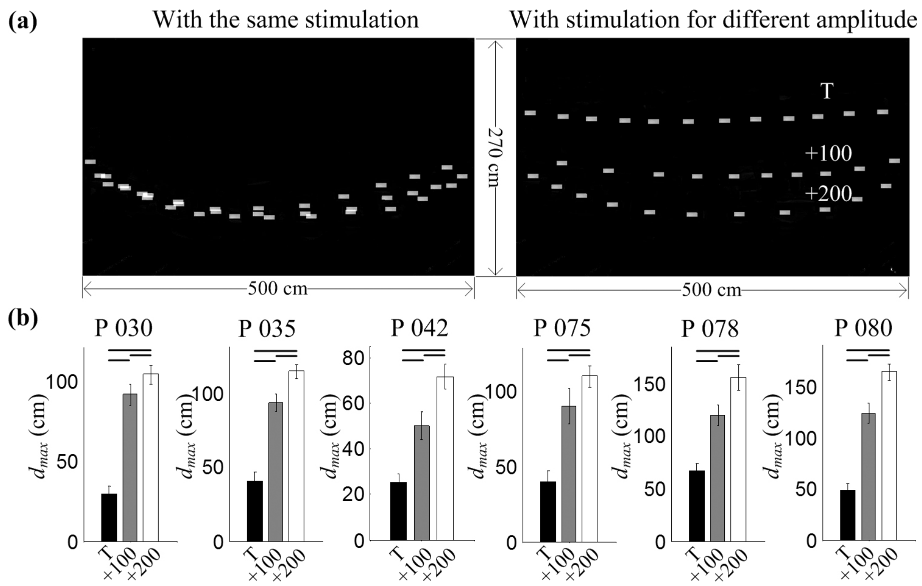

Figure 7. Regulating pigeon's flight with different stimulation parameters. (a) Flight trajectories of pigeon No. P075 with the same set of parameters applied three times. (b) Statistical summary of six pigeons (mean $d_{\max} \pm$ standard error, SE) with different parameters.

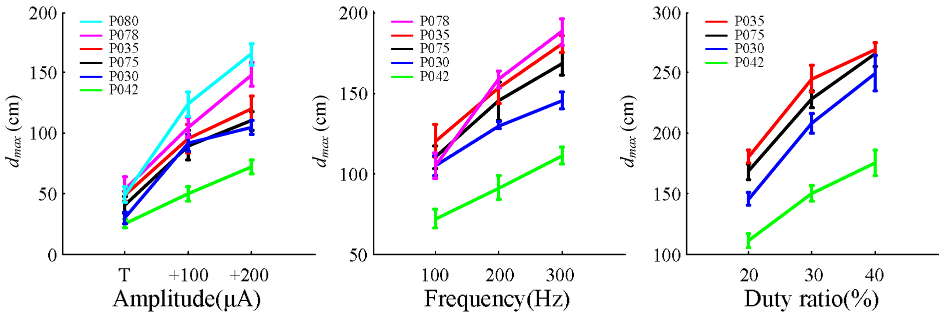

Figure 8. The effect on $d_{\max}$ (mean $\pm$ SE) of changing a single stimulation parameter. Left: Amplitude. Frequency was set to 100 Hz and the duty ratio set to 20% when the effect of stimulus amplitude was analysed. The amplitude was gradually increased by a step size of 100 microamp. Middle: Frequency. To determine the effect of frequency, amplitude and the duty ratio were held constant at T + 200 microamp and 20%, respectively. The frequency was gradually increased by a step size of 100 Hz. Right: Duty ratio. When the duty ratio was analyzed, amplitude and frequency were held constant at T + 200 microamp and 300 Hz, respectively. The duty ratio was gradually increased by a step size of 10%.

Results show that without stimulation, pigeon flight was close to the top edge of captured images and formed linear trajectories. There are no significant changesin flight trajectory from trial to trial for each pigeon, neither was there differences when different pigeons were compared. With electrical stimulation, all pigeons were biased against the original linear flight trajectory. Fig. 6(a) shows the $d_{\max}$ value of pigeon No. P080 without/with stimulation. There are significant differences in the $d_{\max}$ value of all pigeons with stimulation and without stimulation (rank sum-test, $p < 0.001$). The $d_{\max}$ value of all pigeons without stimulation (P030 9 $\pm$ 2.7, P035 10.5 $\pm$ 2.5, P042 9.8 $\pm$ 2.8, P075 10.7 $\pm$ 2.7, P078 10.5 $\pm$ 2.3, P080 11.8 $\pm$ 3.0), and the $d_{\max}$ value of all pigeons with stimulation (P030 98.2 $\pm$ 9.3, P035 167.5 $\pm$ 9.8, P042 95.8 $\pm$ 8.0, P075 206.5 $\pm$ 7. 6, P078 177.5 $\pm$ 10.8, P080 229 $\pm$ 7.4) are summarized in Fig. 6(b).

From this result, it can be concluded that stimulating the pigeon FRM region will affect pigeon flight trajectory. Moreover, with the same set of stimulation parameters, pigeons fly consistent trajectories. However, the $d_{\max}$ value will change as stimulation parameters change. The $d_{\max}$ value rises as stimulation amplitude is increased. The left side of Fig. 7(a) illustrates the flight trajectories of pigeon No. P075 when the same stimulus was applied three times with the same parameters (amplitude $T+$ 200 μA, frequency 200 Hz, and 30% duty ratio). The right side of the figure gives three flight trajectories of pigeon No. P075 with stimulation of a different amplitude (T μA, $T+$ 100 μA, and $T+$ 200 μA) and the same frequency and duty ratio (200 Hz, and 30% duty ratio).

All six pigeons are spurred with three different kinds of stimulation. Based on the threshold of each pigeon, the amplitude is increased by 100 μA every step, and other parameters keep the same (100 Hz frequency and 20% duty ratio). The results demonstrate that increasing the amplitude parameter result in a significant gain in the $d_{\max}$ values of pigeons (rank sum-test, $p < 0.05$). The $d_{\max}$ values of all pigeons with stimulation for different amplitude (T μA, $T+$ 100 μA, and $T+$ 200 μA), P030 (30 $\pm$ 4.5, 92 $\pm$ 6.9, 105 $\pm$ 5.9), P035 (48 $\pm$ 4.3, 95 $\pm$ 12, 120 $\pm$ 10.0), P042 (25 $\pm$ 3.8, 50

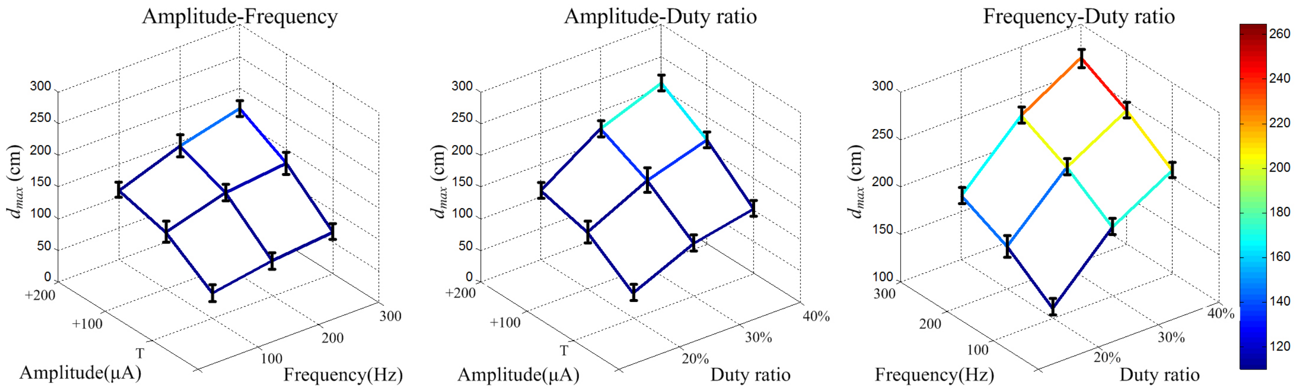

Figure 9. Effect on $d_{\max}$ (mean $\pm$ SE) of changed stimulation pair parameters. The dmax values of pigeon No. 075. Left: Amplitude-frequency. When interactive effects between an amplitude parameter and a frequency parameter was analysed, the duty ratio was set to 20%. Middle: Amplitude-duty ratio. The frequency is set to 100 Hz when the effect of the amplitude-duty ratio pair was examined. Right: Frequency-duty ratio. The amplitude was set to T + 200 microamp when the effect of the frequency-duty ratio pair was examined.

$\pm$ 6.0, 72 $\pm$ 5.7), P075 (40 $\pm$ 7.1, 90 $\pm$ 12, 110 $\pm$ 7.2), P078 (53 $\pm$ 10.2, 105 $\pm$ 8.1, 148 $\pm$ 9.9), P080 (49 $\pm$ 6.3, 124 $\pm$ 10.0, 165 $\pm$ 8.5) is illustrated in Fig. 7(b).

3.3 Single stimulation parameter experiment

In this section the effect on pigeon behaviour of stimulation amplitude, frequency, and the duty ratio were individually analyzed. All stimulations in this scenario were performed on the left side of the pigeons FRM area. Results are given in Fig. 8. Here, a repeated measure linear model is used to evaluate the effects of parameters. Analysis of variance was utilized to determine the effects of single parameter and interaction between parameter pairs.

When the effect of each specific parameter of pigeon behaviour was analyzed, the other parameters were held constant. Frequency was set to 100 *Hz* and the duty ratio set to 20% when the effect of stimulus amplitude was analysed. To determine the effect of frequency, amplitudeand the duty ratio were held constant at $T + 200$ μA and 20%, respectively. When the duty ratio was analyzed, amplitude and frequency were held constant at $T + 200$ μA and 300 *Hz*, respectively. Single factor variance showed that the stimulation amplitude (one-way ANOVA, F (2, 15) $= 16.1$, $p < 0.001$), frequency (one-way ANOVA, F (2, 12) $= 5.88$, $p < 0.05$), and duty ratio (one-way ANOVA, F (2, 9) $= 6.36$, $p < 0.05$)were significantly correlated with pigeon flight. Moreover, as the magnitude of stimulation parameters was increased, the $d_{\max}$ value of pigeon flight trajectories also increased.

It was also found that as the magnitude of the stimulation parameter increased, the increase in the value of $d_{\max}$ was reduced. Furthermore, the overall trend in change of different pigeon $d_{\max}$ values was consistent. For the same increase in stimulation magnitude, when the stimulus amplitude parameter increased from T to $T + 100$, the average $d_{\max}$ value gain was 57 cm, whereas when the stimulus amplitude increased from $T + 100$ to $T + 200$, the average $d_{\max}$ value gain as only 27 cm. For the frequency, a change in frequency from 100 *Hz* to 200 *Hz* increased the average value of $d_{\max}$ by 30 cm. While when the frequency increased from 200 *Hz* to 300 *Hz*, the average increase of $d_{\max}$ was 23 cm. Similarly, an increase in the duty ratio from 20% to 30%, increased $d_{\max}$ by 53 cm. When the duty ratio increased from 30% to 40%, there was only a 30 cm increase in $d_{\max}$.

3.4 Stimulating with multiple parameters to examine the interactive effects of stimulation parameters

Experiments explored the interactive effects of stimulation parameters. Here, the three stimulation parameters, amplitude, frequency, and duty ratio were grouped into three pairs. When interactive effects between an amplitude parameter and a frequency parameter was analysed, the duty ratio was set to 20%. The frequency is set to 100 *Hz* when the effect of the amplitude-duty ratio pair was examined. The amplitude was set to $T + 200$ μA when the effect of the frequency-duty ratio pair was examined. The d_{max} values of pigeon No. 075 are illustrated in Fig. 9, when the pigeon was stimulated by different parameter pairs.

The results of two-factor analysis of variance tests demonstrated that the amplitude parameter has a significant interaction with both the frequency parameter and the duty ratio parameter (two-way ANOVA, $F(4, 27) = 3.32$, $p < 0.05$ (P 030), $F(4, 27) = 5.12$, $p < 0.05$ (P 035), $F(4, 27) = 4.08$, $p < 0.05$ (P 042), $F(4, 27) = 3.56$, $p < 0.05$ (P 075), $F(4, 27) = 3.96$, p < 0.05 (P 078) for an amplitude-frequency pair, $F(4, 27) = 4.21$, $p < 0.05$ (P 030), $F(4, 27) = 3.75$, $p < 0.05$ (P 035), $F(4, 27) = 4.15$, $p < 0.05$ (P 042), $F(4, 27) = 4.01$, $p < 0.05$ (P 075) for an amplitude-duty ratio pair). The interactive effects of a frequency-duty ratio pair were not significant (two-way ANOVA, $F(4, 27) = 0.31$, $p > 0.05$ (P 030), $F(4, 27) = 0.02$, $p > 0.05$ (P 035), $F(4, 27) = 0.03$, $p > 0.05$ (P 042), $F(4, 27) = 0.03$, $p > 0.05$ (P 075)).

4. Discussion

This paper presents a study of pigeon flight modulated by microstimulation in the FRM brain area. Quantitative analysis showed how by adjusting the amplitude, frequency, and duty ratioparameter values of microstimulations, pigeon flight was affected. Interaction effects between stimulation parameters were also analysed. It was found that:

i. Stimulation of the pigeon FRM area affects pigeon behaviour, irrespective of whether pigeons were on the ground or flying.

ii. A $d_{\max}$ value increased non-linearly as the magnitude of stimulation parameters was increased.

iii. Stimulation amplitude had a significant interaction with both the stimulus frequency and a duty ratio parameter. However, no interaction was found between stimulus frequency and the duty ratio.

These results provide a theoretical foundation for research on how microstimulation modulates pigeon flight behaviour. This study also provides an effective method for a precise control of flight behaviour.

Neuromodulation studies of animal movement have successfully manipulated specific behavioral actions by microstimulation (for example linear movement and steering), ground movements (rodents, reptiles), and swimming (fish). Most of the neuromodulation research on pigeons has occurred either while subjects have been anaesthetised or during ground movement. There is little research on modulating avian flight behaviour. Su et al. (2012) stimulated the DIVA area and striatum of pigeons to evoke movements. The DIVA area and striatum correlate with feelings of fear and pain. The reason for pigeons to take flight, dodge, or escape, are that they attempt to avoid pain or fear. Therefore, such behaviors are random with no particular direction. It is hard to generate a stable response to this kind of stimulation. In contrast, in this study, microstimulation activates a brain area related to movement and a pigeon reacts reliably and stably to the stimulus. Cai et al. (2015) also applied stimulation to a movement-related brain area. But that research focused on pigeon movement on the ground and the control of pigeon flight outdoors (Wang et al., 2018). Here, this study establishes an evaluation model to explore the effect of stimulation parameters on avian flight behaviour.

Here, it is reported that irrespective of whether pigeons fly or move on the ground, when microstimulation occurs in the left FRM area, movement will tend to the left. Alternatively, if the stimulation is to the right FRM area, a pigeon will tend to turn right. Pigeon flight is much faster than their movement on the ground and even a very short stimulus in the air will generate amplified turning behaviour. Platforms are located at the edges of the experiment site to make full use of the available space.

By studying the effect of stimulation parameters on pigeon flight, the minimum amplitude that induces turning for different pigeons was found. At a stimulation frequency of 100 Hz and duty ratio of 20%, explicit turning behaviours for four pigeons started at a stimulus amplitude of 70-100 μA. Two other pigeons startede explicit turning behaviour at a stimulus amplitude of 150 μA. The reason for this diference is unclear, but it may be related to specific attributes of individual pigeons. Exploration of this phenomenon was beyond the scope of this study. Moreover, the influence of stimulation parameters on pigeon flight was also found to be related to the flight status of a pigeon during stimulation. It was discovered that some pigeons will learn to steer after a period of stimulation. If a stimuus is applied as a pigeon makes an active turn, the $d_{\max}$ value of the pigeon trajectory will significantly increase. Furthermore, if the turning caused by stimulation is different from the active turning of the pigeon, it will suppress the turning behaviour This reduces the turn range or even causes the pigeon to fly in the opposite direction.

The $d_{\max}$ value with and without stimulation was adopted to create an evaluation model to assess the effect of stimulation parameters on flight modulation. To obtain the $d_{\max}$ measure a video camera was employed to asist in recording pigeon flight into a two-dimensional space. Currently, the effect of microstimulation in the FRM area on pigeon flight has not been studied in 3-dimensional space.

The stimulation amplitude, frequency, and duty ratio are the three important parameters of stimulus signal. Through analysis of variance, it has been found that these three parameters are related in the modulation of pigeon flight. Moreover, stimulation amplitude has a significant interaction with both stimulation frequency and the duty ratio. Results also demonstrate that the duration of stimulation does not affect the $d_{\max}$ of flight trajectories. For example, when a pigeon flight trajectory arrives at the position of maximum deviation from the linear baseline, continued stimulation does not increase $d_{\max}$. The pigeon will continue to fly a parallel linear course, after deviating from the learned direct linear path, until the stimulation stops. The pigeon will then gradually revert back to the original trajectory. For this reason, $d_{\max}$ is considered to be related to the energy of the stimulation signal. Increasing amplitude, frequency, and duty ratio all increase signal energy per time unit. Therefore, they all cause the value of $d_{\max}$ to increase.

5. Conclusion

Microstimulation of the pigeon FRM brain area was undertaken to develop and explore control of pigeon flight. This differed from earlier studies which typically evoked pigeon responses on the ground. Furthermore, a model was established to evaluate the effect of modulating pigeon flight and to compare different stimulation parameters, such as stimulus amplitude, frequency, and duty ratio, and then analyse how these parameters and interactions among them might affect pigeon flight. Results illustrate that the $d_{\max}$ value increases non-linearly as the magnitude of microstimulation parameters are increased. It was found that the stimulation amplitude has a significant interaction with both a stimulation frequency parameter and a duty ratio parameter. This is considered meaningful for the analysis of pigeon flight mechanisms. Instead of using strong electrical stimulation on experimental animals to induce reactions, an appropriate set of stimulation parameters was employed to obtain the desired responses. This study provides an effective solution for reducing the damage of electrical stimulation, and provides a foundation for the control and further investigation of pigeon flight.

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Conflict of interest

The authors declare no competing interests.

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