

James Madison University
JMU Scholarly Commons

Senior Honors Projects, 2010-current

Honors College

Fall 2013

Influence of initial position and stimulus location of the paw on the nociceptive withdrawal reflex in intact rats

Christopher Anthony Chrzan II
James Madison University

Follow this and additional works at: <https://commons.lib.jmu.edu/honors201019>

Recommended Citation

Chrzan, Christopher Anthony II, "Influence of initial position and stimulus location of the paw on the nociceptive withdrawal reflex in intact rats" (2013). *Senior Honors Projects, 2010-current*. 401.
<https://commons.lib.jmu.edu/honors201019/401>

This Thesis is brought to you for free and open access by the Honors College at JMU Scholarly Commons. It has been accepted for inclusion in Senior Honors Projects, 2010-current by an authorized administrator of JMU Scholarly Commons. For more information, please contact dc_admin@jmu.edu.

Influence of Initial Position and Stimulus Location of the Paw on the Nociceptive Withdrawal
Reflex in Intact Rats

A Project Presented to
the Faculty of the Undergraduate
College of Science and Mathematics
James Madison University

In Partial Fulfillment of the Requirements
for the Degree of Bachelor of Science

by Christopher Anthony Chrzan II

December 2013

Accepted by the faculty of the Department of Biology, James Madison University, in partial fulfillment of the requirements for the Degree of Bachelor of Science

FACULTY COMMITTEE:

HONORS PROGRAM APPROVAL:

Project Advisor: Corey L. Cleland, Ph.D.,
Associate Professor, Biology

Barry Falk, Ph.D.,
Director, Honors Program

Reader: Mark Gabriele, Ph.D.,
Professor, Biology

Reader: Patrice Ludwig, Ph.D.,
Assistant Professor, Biology

Table of Contents

I. List of Figures	3
II. Acknowledgements	4
III. Abstract	5
IV. Introduction	7
A. Mammalian Reflexes	
B. Nociceptive Withdrawal Reflex	
C. Dependence of the NWR on Stimulus Location	
D. Dependence of the NWR on Posture	
E. Specific Aims	
V. Methodology	15
A. Rat Preparation	
B. Experimental Apparatus	
C. Experimental Protocol	
VI. Results	23
A. Dependence of Stimulus Location	
B. Dependence of Initial Position	
VII. Discussion	36
A. Summary	
B. Comparison to Previous Results	
C. Mechanisms	
D. Significance	
VIII. Literature Cited	43

List of Figures

Figure 1: The five stimulus locations on the bottom of the left hind left foot.....	17
Figure 2: Diagram of experimental apparatus.....	20
Figure 3: Axis of measurement.....	22
Figure 4: Translational and rotational movement of the foot.....	25
Figure 5: Changes in response direction and foot angle.....	26
Figure 6: Frequency of direction and magnitude of movement.....	27
Figure 7: The frequency of change in foot angle.....	28
Figure 8: The movement of the foot for each stimulus location.....	29
Figure 9: Dependence of mean direction and magnitude of response on stimulus location.....	30
Figure 10: The change in foot angle between the initial and final positions for each stimulus..	31
Figure 11: Initial locations of the foot prior to stimulation.....	33
Figure 12: The effect of initial foot location on movement.....	34
Figure 13: Final foot angle after movement as a function of original foot angle.....	35

Acknowledgements

I would first like to thank Dr. Corey Cleland, for without him this thesis would not have been possible. He was a vital asset in supplementing the learning process throughout the design, experimentation, analytical, and writing portions of this project. Dr. Cleland worked tirelessly to not only ensure that this document was finished, but also to ensure that a thorough understanding of every aspect of the process. In addition to his academic value, Dr. Cleland was a great friend and wonderful mentor throughout the process, as he was able to provide the advice and moral support that was needed in stride. I would also like to thank my readers, Dr. Patrice Ludwig, and Dr. Mark Gabriele for their time and guidance in the process of editing the document.

Abstract

Animals have the ability react to noxious stimuli from the environment via a reflex pathway known as the nociceptive withdrawal reflex (NWR). The NWR is a spinal reflex that protects the body from stimuli that may be tissue damaging. In addition, the NWR is known to be supraspinally modulated, which alters the strength of response.

Previous studies on the NWR have shown an influence of both stimulus location and initial posture on the NWR. The studies regarding the influence of initial position on the NWR, however, are more limited. Most of the research has been conducted on humans, and in no case has there been studies using intact, unanesthetized rats. The fundamental question this experiment aims to address is whether the *initial posture* can affect the NWR in *intact, unanesthetized rats*.

Noxious stimuli were provided in the form of heat delivered by a laser for each of the fifteen trials for each rat studied (n=7). The stimuli were aimed at five specific locations on the surface of the rat paw, and the response was recorded with a high speed videocamera positioned under the paw. The response magnitude and direction of the paw movement were quantified by identifying the initial (before NWR) and final (after NWR) position of the raw paw. Similarly, the change in foot angle was also quantified for each of the trials.

The results reveal that, unexpectedly, stimulus location did not significantly affect the magnitudes or direction of movement, or the change in foot angle. However, the initial location of the foot did have a significant effect on the withdrawal movement, as stimulation of the foot while it was positioned laterally from the body resulted in medial movement of the foot or stimuli of the foot while it was positioned medially from the body resulted in lateral movement of the foot. In a similar pattern, stimulating the foot while it was positioned rostrally in relation

to the body resulted in movement in the caudal direction and stimulating the foot while it was positioned caudally in relation to the body resulted in movement in the rostral direction. The initial angle of the foot was also shown to influence the final angle of the foot, as the angle of the foot changed very little throughout the withdrawal.

The results of this study on intact, unanesthetized rats demonstrate that there is evidence that initial position of the rat paw influences the NWR. However, this study was unable to demonstrate that stimulus location influences the NWR. This finding raises the question why stimulus location is not a factor in the rat paw, but has been shown to be a factor in the rat tail and other mammalian studies.

Introduction

The environment in which an animal lives contains many threats to its survival. For example, threats include environmental hazards such as dangerous weather, infection, predation from other animals, or any threat that could potentially hinder the ability of the animal to pass on its genetic information. The ability to sense predatory threats is especially important for survival, and thus natural selection has produced various mechanisms for animals to sense their surroundings and formulate responses such as attacking (Cooper and Stankowich, 2010), remaining still (Hassenstein and Hustert, 1999), or escaping (Eaton, 1984).

Escape responses have been studied in diverse invertebrates such as crayfish (Edwards et al., 1999), cockroaches (Camhi and Tom, 1978), leeches (Lewis and Kristan, 1998), earthworms (Drewes, 1984), *Caenorhabditis elegans* (Mohammadi et al., 2013) and mammals such as cats (Sherrington, 1910), rats (Schouenborg and Kalliomaki, 1990), rabbits (Clarke and Harris, 2002), and humans (Andersen and Sonnenborg, 1999). For both invertebrates (Domenici et al., 2011) and vertebrates (Andersen, 2007) there is evidence that the direction of the escape or withdrawal response depends on the location of the stimulus. For example, Edwards (1999) found that stimulating a crayfish in different locations resulted in a limited number of responses. The escape or withdrawal response has also been shown to depend on initial posture. For example, the escape response of *Drosophila melanogaster* (common fruit fly) is dependent on orienting the body position away from the stimulus before initiating movement. However, in mammals there is little research regarding how the initial posture affects the withdrawal response.

A. Mammalian Reflexes

Mammalian reflexes are involuntary movements in response to specific stimuli that are typically short latency and stereotyped (Prochazka, 1999). Sensory receptors transduce relevant sensory stimuli and transmit the information to the central nervous system where they can either synapse directly with motor neurons or indirectly via spinal interneurons. Once the motor neurons receive and process the input they transmit commands to the muscle, resulting in muscular force and movement. The resulting movement can also involve the coordination of multiple muscles (Windhorst 1996).

For example, an anatomically simple reflex, the stretch reflex, involves a primary spinal afferent synapsing directly onto a motor neuron in the ventral horn of the spinal cord (Jolly, 1910). Reflexes that show a monosynaptic pattern are often the quickest reflexes as they have less synapses due to the lack of intervening interneurons (Delcomyn, 1998). However, not all reflexes are monosynaptic. For example, the pupillary reflex, in which the iris responds to increased light by contracting and inhibiting muscles in eye, is a reflex that crosses more than one synapse (Roazanowski and Murawski, 2013). Another example is the arc of Ib afferents from the golgi tendon organs that are disynaptic and involve interneurons (Ellrich and Hopf, 1998).

Although most reflexes are confined to the spinal cord or brainstem, some reflexes include a long-loop pathway that passes through the brain (Matthews, 1991). As a result, long loop reflexes may have longer post-stimulus latency (Hammond, 1955). Unlike spinal reflexes, long loop reflexes are especially sensitive to “set” (initial conditions or intention of the subject) which both qualitatively and quantitatively alters the response (Matthews, 1991). Further, it has

been found that the motor response to a particular sensory input, if learned previously, can influence future responses of the same input through these long loop connections (Evarts, 1973).

Reflexes can also undergo supraspinal modulation in both animals (Schomburg, 1990) and humans (Andersen, 2007). Supraspinal modulation differs from long-loop reflexes in that phasic signals do not travel to the brain and then back to the spinal cord, but rather the brain acts to modulate spinal reflexes. Stretch reflexes in particular are modulated by supraspinal descending pathways (Shemmell et al. 2010). The process of walking provides a nice example of this such that modulation acts to decrease the gain, or strength, of the stretch reflex in order to preserve balance (Rietdyk and Patla, 1998). As an individual walks, the stretch reflex is decreased in order to allow desirable changes in muscle length. In contrast, when an individual is standing still, the gain, or strength, of the stretch reflex is increased in order to maintain proper balance and posture.

B. Nociceptive Withdrawal Reflex

The focus of this research is the nociceptive withdrawal reflex (NWR), sometimes referred to as the “flexor” or “flexion” (Hultborn, 2006) reflex, which is a rapid limb (Sandrini et al., 2005) or tail (Cleland and Bauer, 2002) withdrawal movement that acts to protect the animal from noxious (actual or potential tissue-damaging stimuli) stimuli. Typically, the response is stereotyped (Sherrington, 1910) and occurs at short latency (Jolly, 1910). Common examples of noxious stimuli are heat, such as touching a hot stove, pressure such as the pinch of your skin with tweezers, or chemical, such as eating hot chili peppers (Cleland and Gebhart, 1997).

The NWR was described extensively by Sherrington (1906, 1910) based on his studies in spinalized cats, where he found that stimulating the leg in different places resulted in a stereotyped flexion of the limb. Sherrington also stimulated different nerves in the leg and visually observed muscles to determine which muscles were activated during the response. Sherrington divided the participating muscles into two functional groups – flexors and extensors – with flexor muscle activate and extensor muscles inactive, perhaps even inhibited, during the NWR. Thus, Sherrington proposed that the NWR of the hind limb arose from the widespread excitation of flexor muscles and inhibition of extensor muscles. It was also noted that the individual reflexes that occurred from stimulating different nerves differed slightly, which he described as “local sign”, but all largely conformed to the stereotypical “flexion reflex” pattern of withdrawal. The NWR is a spinal reflex, as it was found that humans (Andersen 2007) with spinal cord injuries as well as spinalized animals still exhibit a NWR reflex (Cleland and Bauer, 2002).

Descending pathways can either inhibit or excite spinal nociceptive processing. Inhibitory and excitatory pathways are physically separated within the brainstem and descending tracts, and often utilize different neurotransmitter and corresponding receptor systems (Gebhart, 2004).

Noxious stimuli that evoke the NWR also simultaneously activate other reflex pathways. Prior to the NWR, posture is adjusted in response to a noxious stimulus apparently to help maintain balance during the NWR (McIlroy et al., 1999). One well studied example is the crossed-extension reflex, in which the limb that is opposite to the withdrawn limb undergoes the opposite reaction as the withdrawn limb - widespread excitation of extensors (Solomon et al.,

1990). For example, if the stimulated limb flexes to withdraw from the stimulus, the other limb will extend in order to support the weight of the body

While the presence of modulation has been shown to occur in the NWR, there is only scant evidence of long loop processes influencing the withdrawal response (Sandrini et al., 2005).

C. Dependence of the NWR on Stimulus Location

The NWR only serves its intended purpose if the movement keeps the animal from injury, and thus there are distinct spatial strategies of the NWR. The potential spatial strategies regarding organization of the NWR are the *continuous* and *categorical* strategies. The continuous strategy predicts that each stimulation point will result in a separate direction of movement, and that a small change of stimulus location will result in a small change in direction or magnitude. An example of the continuous strategy was found in the leech by Lewis and Kristan (1998), in which the local bend of the body was 180° directly away from the stimulus location. The continuous strategy may be the most accurate at separating the affected area from the stimulus. In contrast, the categorical strategy predicts that regardless of the site of stimulation, withdrawal movement occurs only in a finite number of directions. An example of this strategy was found in the crayfish, whereby abrupt stimuli caused stereotyped responses in only the forward or backward direction (Edwards et al., 1999). The brain can only process a certain maximum amount of information at one particular time (Fitts, 1954), and so limiting the number of responses with the categorical strategy may beneficially decrease the latency of response (Hick, 1952).

In non-human mammals, Hagbarth (1952), and Schouenborg and colleagues (1990,1992) demonstrated a continuous pattern of withdrawal. Schouenborg found through electromyography (EMG) which muscles were excited during the response, and used these results to infer the direction of movement. Andersen (2007) also supported a continuous pattern of withdrawal in his study of humans where he found that the dorsi-flexion/plantar-flexion as well as the inversion/eversion of the human foot NWR gradually changed angle as electrical stimulus location changed on the plantar surface of the was foot. Support for the continuous model has also been found in lightly anesthetized rabbits by Clarke and Harris (2002) where they observed that changing the mechanical stimulus location along the rostral-caudal axis of the plantar aspect of the foot resulted in a continual change in movement. In rats, support for the continuous strategy has also been found by Wyatt (2010) in which heat stimulation of the plantar aspect of the hind paw of intact rats resulted in a continuous withdrawal pattern, though weak in magnitude. Similarly, Seamon (2012), who also studied intact rats, found that stimulation in different locations of the foot were continuous with respect to changes in magnitude but not direction.

The categorical model is also supported by several studies. Sherrington's (1910) study of cats showed different stimulus locations resulted in similar responses. Another study in humans by Spaich et al. (2003) found a categorical pattern of withdrawal magnitude when stimulating the sole of the foot. In spinalized rats, withdrawal of the rat paw was found to follow a categorical pattern by which it was found that the foot moves in the rostral and medial directions without any dorsal or ventral components (Esquivel, 2010). Similarly, Davis (2009), who also studied spinalized rats, discovered that the response of the hind limb was largely in the rostral/medial direction.

There is also support for the idea that the continuous and categorical strategies are not necessarily exclusive. For example, some experiments support a hybrid of the continuous and categorical strategies. Cleland and Bauer (2002) found that the response directions of the rat tail after heat stimulation was in only two directions (± 15 degrees from ventral); and also depended slightly on stimulus location. Weiss (2007) studied spinalized rats in which the tail was stimulated at two separate locations at the same time; and if there were a continuous pattern one would expect the tail to withdraw in a direction somewhere between the two stimuli. However the tail moved in only one of two directions, supporting a categorical strategy.

D. Dependence of the NWR on Posture

The effect of initial posture on the NWR has been studied largely, though not exclusively, in humans. The effects of initial posture can be broken down into two categories, its effect on *magnitude* of response and its effect on *direction* of response. The magnitude of response has been found to be influenced by initial posture in cats by Baxendale and Ferrell (1980), in which the strength of the NWR varied according to initial limb position. In particular, the excitability of the flexion reflex decreased as the knee was initially in a more flexed position, which they attributed to modulation from knee joint afferents. In another study, Spaich et al., (2003) determined that the magnitude of withdrawal response in the human foot depended on the phase of the walking cycle.

The effects of initial posture on the direction of the response have also been studied, and are of particular interest for our research because the influence of initial posture on the direction of response is the main focus of this study. In humans, there have been conflicting results

regarding the effects of initial posture. For example, Peterson et al. (2013) found that as the human arm was moved from a flexed position to an extended position, the NWR changed from favoring the posterior-lateral direction to favoring the posterior direction. In contrast, Eckert and Riley (2013), who also studied humans, found that the configuration of the upper limb did not influence the response direction.

E. Specific Aims

Although there have been numerous studies investigating the effects of *stimulus location* on the NWR, there have been few, and conflicting, accounts of the dependence of the NWR on *initial foot position*. Thus, the primary aim of this study was to determine whether there is an influence of the initial limb position on the pattern of response to heat stimulus delivered to different locations on the plantar surface of the paw in intact, unanesthetized rats.

Methods

Male Sprague Dawley rats (n=7), aged 10 weeks to 2 years, with a mean weight of 494g (+/- 12.0g S.D.), were bred from rats acquired from Harlan (Indianapolis, IN). The rats were housed in polypropylene cages with females kept in different cages than the males (except during breeding). The room where the rats were stored was inspected daily to ensure proper living conditions were met, including maintaining the humidity between 30-70%, the temperature between 68-79° F, and checking availability of food and water. The animal care facility and protocol were approved by the James Madison University Institutional Animal Care and Use Committee (Protocol A08-10) and conformed to the Ethical Guidelines for Investigation of Experimental Pain in Conscious Animals (Zimmerman, 1983).

A. Rat Preparation

In order to be anesthetized, rats were placed in an acrylic box and exposed to Isoflurane at 5% with an oxygen flow rate of 1 liter/minute. Anesthesia was necessary to mark the foot (see later) and shave the legs and torso. Once anesthetized, the hair on the animal was removed with a hair clipper from all areas posterior to the front feet. The removal of hair was helpful to accurately visualize movements.

Five locations on the plantar surface of a rat foot were marked using an ultra-fine black permanent marker (Sharpie). The mark sizes were approximately 1.5 mm and organized into medial-lateral (locations: 5,2,4) and rostral-caudal (locations: 1,2,3) progressions (Figure 1). The first location was rostral, between the superior three pads. Posterior to that, near the middle of

the foot, was the second location between the last two pads of the foot. The third location was placed equidistant below the second location. The fourth location was marked lateral to the second location (if viewed from the bottom of the foot) and the fifth location was medial to the second location

The paws were marked for three reasons. The first was to improve absorption of the laser stimulus. Second, the marks limited the stimulus to the marked area to avoid extraneous heating of unintended areas because unblackened areas absorb radiant energy less efficiently. Lastly, the marks allowed for tracking of movement using ProAnalyst software on a PC computer. Location 2 (middle location) corresponded to the approximate center of mass (COM) of the paw and was used to measure translational response movements of the entire paw. Locations 1 and 3 were used in order to define an axis along the length of the foot that was used to calculate changes in the angle of the foot.

Prior to the start of the experiment the rat was provided forty-five minutes to recover in order to diminish any influence of the Isoflurane on the animal's response.

B. Experimental Apparatus

The rat was placed on a platform of glass (50 cm x 25 cm). The platform was supported by two horizontal steel bars each of length 30 cm, perpendicular to the length of the glass. Lack of further attachment allowed the glass to be manually shifted in order to move the animal into position for each trial. The animal was constrained on the platform in an acrylic box (22.5 cm x 8 cm x 8.5 cm) which contained fenestrations in the walls in order to ensure that both the tail could fully extend and that the rat could breathe.

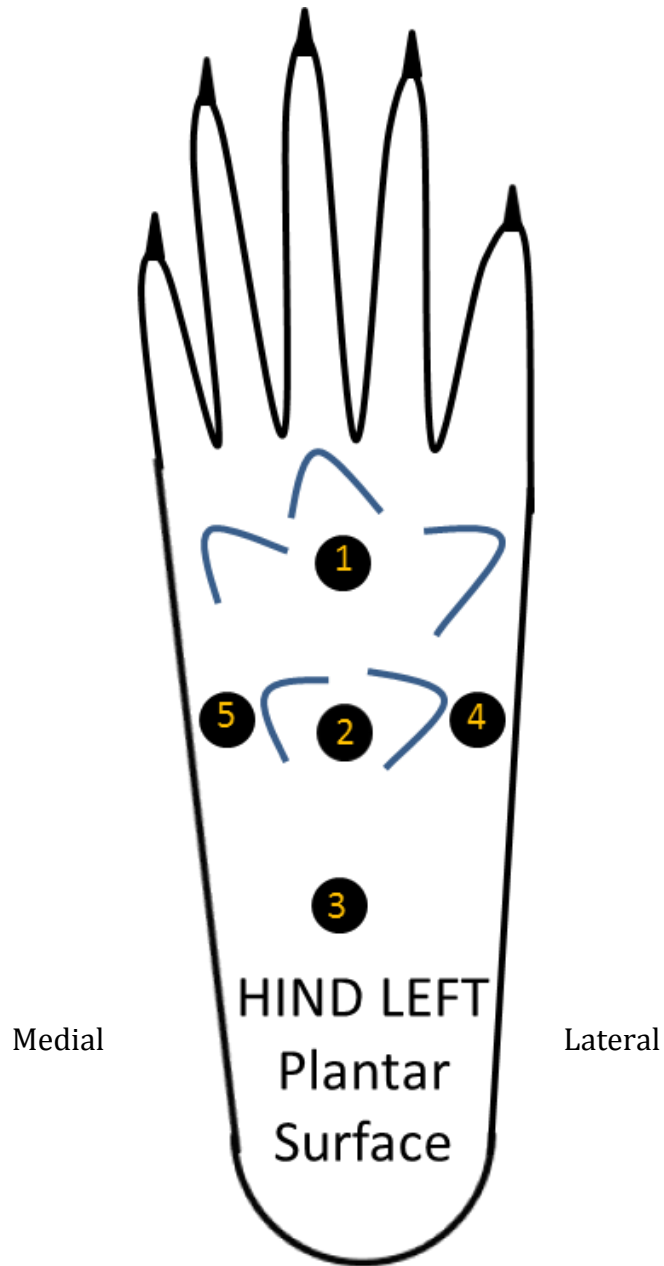


Figure 1. The five stimulus locations on the bottom of the left hind left foot. The view is shown from the plantar surface as if the viewer were looking up at the animal. The 2nd location was chosen to represent the center of mass of the paw, and locations 1 and 3 define the axis of the paw used to measure the angle of the foot. The pads of the paw (represented by the curved lines) were used as a reference when creating markings on each rat.

The platform was raised 50 cm from the surface of the table in order to allow adequate clearance for the video camera, light emitting diode (LED) lights, and stimulus apparatus (Figure 2).

Illumination was provided by two high power LED fiber-coupled light sources (SugarCube Quad LED Illuminators; Vergennes, VT). LED's were used to minimize heat that might have influenced the health of the rat or the withdrawal response. Two LED light sources were positioned below the platform offset at angles of roughly 135° and -45° from the hind left leg of the rat. The LED lights were triggered to illuminate at the same time the stimulus was delivered, thus allowing identification of the frame in which the stimulus was delivered.

The heat stimulus used to evoke the withdrawal response was delivered at 8 Watts using a 980nm infrared laser diode (BWTEK; Newark, DE). A visually red targeting laser allowed the desired location on the rat's foot to be aligned with the locus of stimulus delivery. The laser was focused with a large condenser lens at a diameter equivalent to that of the black marked location marker on the foot (approximately 1.5 mm). The latency of response ranged from 267 milliseconds to 3467 ms with a mean latency of 870 ms (+/- 772 ms S.D.). The rapid response suggests that A δ nociceptors rather than C fiber nociceptors were responsible for sensory perception (Tzabazis, 2005). In no instance was burning of the tissue or damage of any sort observed, and the rat never vocalized in response to the heat stimulus.

A video camera (Sony Handycam HDR CX160) was used to record the animal's foot movement. The resolution and format was 1080p (1920x1080 progressive scan) and set at a speed of 60 frames per second in order to provide high spatial and temporal resolution. The camera was positioned and secured to the table using Manfrotto clamps (Upper Saddle River,

NJ) and Newport (Irvine, CA) magnetic clamps. The focus, exposure, and zoom on the camera were adjusted prior to each experiment. The camera recorded movement in pixels, and thus it was necessary to convert pixels to millimeters in order to determine proper distances of movement. In order to perform this calibration, in each experiment one frame was recorded with a ruler in the field of view that served as a known distance marker.

C. Experimental Protocol

Each trial began with moving the glass platform so that the hind left foot was in view of the video camera beneath. The video camera was set to “record”, the stimulus was positioned in the correct location, and the stimulus was delivered using a foot-activated switch. Following the animal’s withdrawal response the video recording was stopped. The rat was provided at least four minutes to recover before initiating the next trial to ensure that stimulation and withdrawal from the prior withdrawal had minimal influence on the next trial, as increased sensitization from repeated stimuli can lead to shorter latencies of withdrawal (Sunkin, 2009).

The order in which the locations on the foot received the stimulus was ordered at random by Stat Trek (stattrek.com). After each of the five locations was stimulated in order, the entire sequence was repeated twice. The randomization of stimulus location decreased any interference that the NWR from one trial may have had on subsequent trials by balancing the effect across locations. A total of 15 trials, with each of the locations being stimulated thrice, were conducted for each rat. Following the completion of an experiment the rat was exposed to CO₂ gas until breathing stopped and cervical dislocation was performed to ensure death.

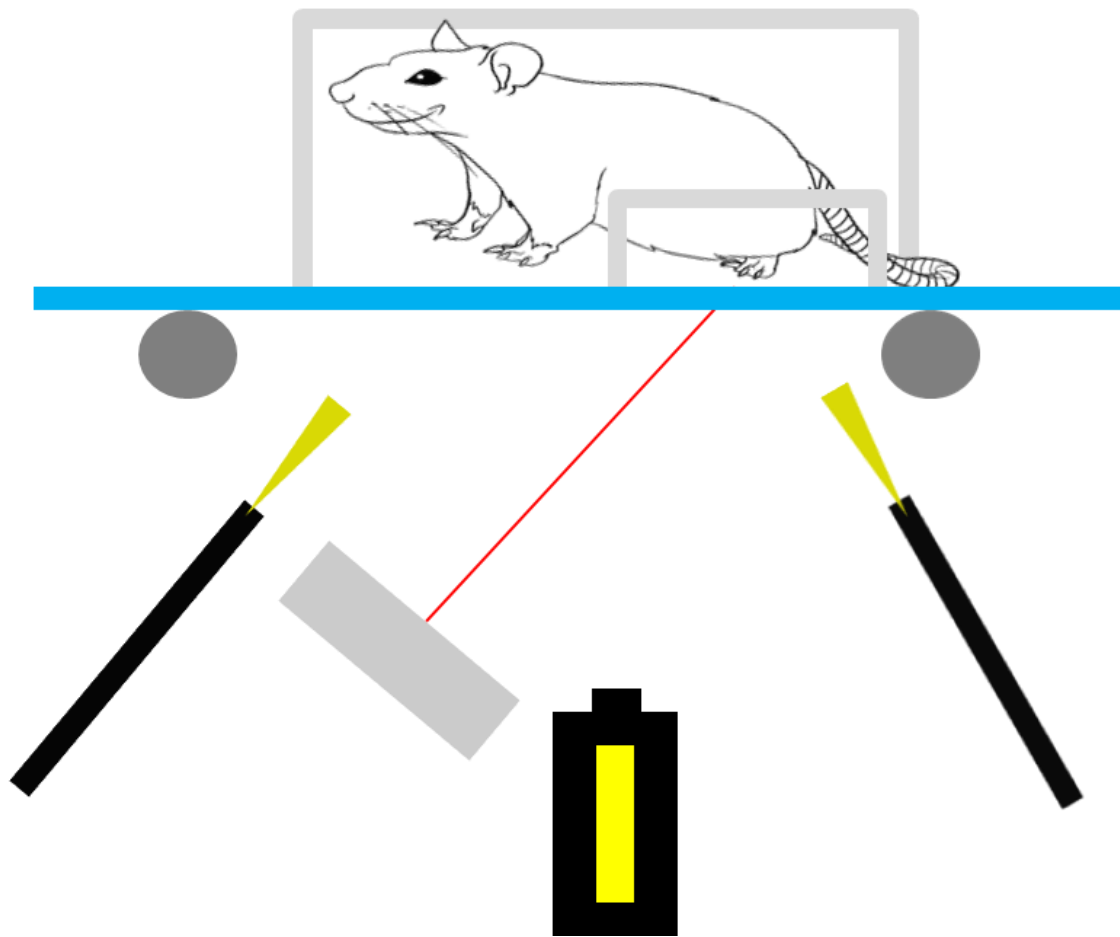


Figure 2. Diagram of experimental apparatus. The glass platform was supported by two horizontal steel bars (grey circles). The two LED light sources below the animal were positioned for proper illumination of the foot. The camera was positioned directly below the animal and was positioned as to not obstruct the laser diode.

At the end of the experiment the video files were uploaded to a PC computer. The video was then converted to a sequence of “jpg” image files using TMPGEnc Video Mastering Works 5 (Pegasys). Extraneous video more than five frames before and after the response were deleted. Video was then analyzed using ProAnalyst (Cambridge, MA) to determine the position of the location 2 (translational movement) and locations 1 and 3 (to determine foot angle rotation) in the frames immediately preceding the movement and immediately after the foot was replaced on the glass, typically separated by a movement that lasted 2-3 frames (50 ms).

The Cartesian coordinate system was arranged by setting the origin of a coordinate system at the urinary orifice; the positive Y direction was set directly parallel to the long axis of the animal, and the positive X direction was set in the medial direction if viewed from above (Figure 3).

The measures obtained from ProAnalyst were exported to an Excel spreadsheet. The Excel spreadsheet was used to calculate the coordinates of the center of mass (COM) of the foot before and after completion of the movement which were used to calculate the magnitude and direction of movement of the foot, as well as the foot angle before and after movement, for each trial (Figure 3).

Analysis and graphing were performed using Oriana circular statistics (Kovach Computing Services), SPSS (IBM Corp), and SigmaPlot (Systat Software). Data were analyzed using correlation (normal and circular), regression, and ANOVA (normal and circular). Significance (α) was set at a p-value of 0.05 or less, and error bars are standard error of the mean (SEM) unless otherwise indicated.

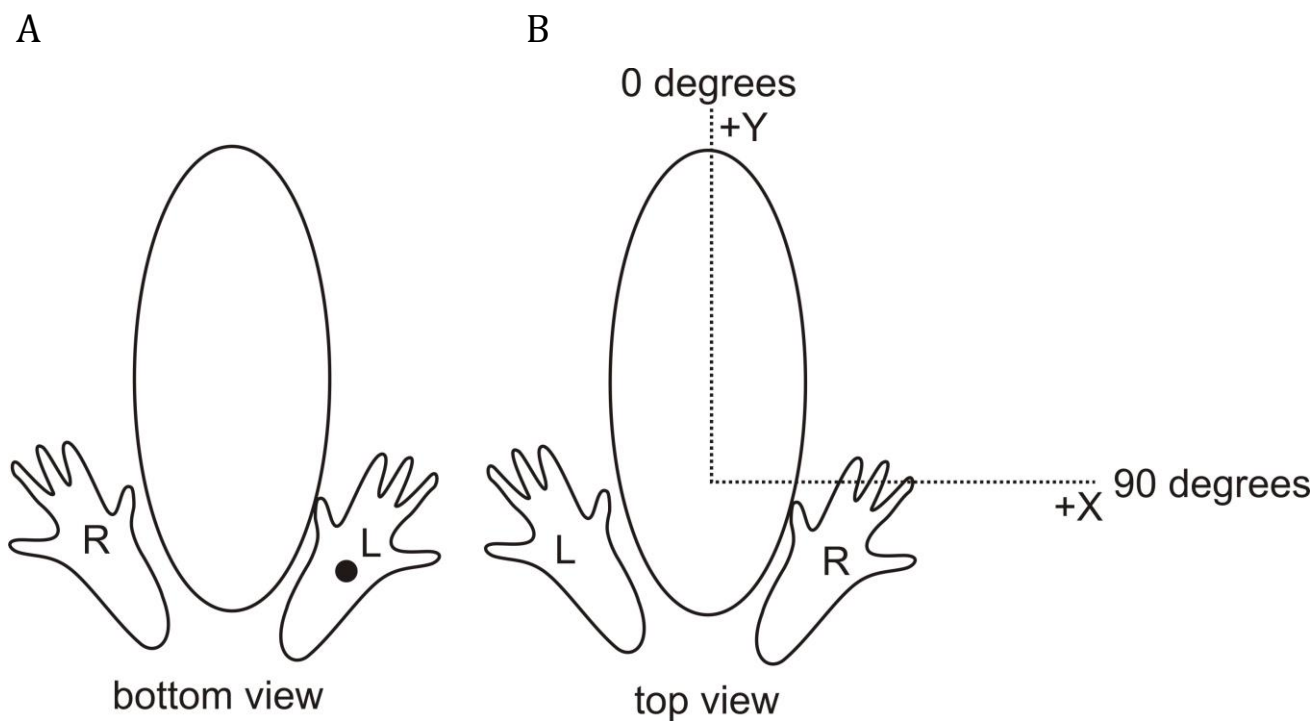


Figure 3. Axis of measurement. The hind left paw of the rat was used in the experiment. A) The view as if looking upward from the bottom of the rat; the stimulated foot is present on the right side of the rat diagram. B) A view as if looking downward from the top of the rat, with the left foot of the animal placed to the left of the diagram. The axes were part of a Cartesian coordinate system interpreted as if placed above the rat with the positive X-axis to the right and the positive Y-axis toward the rostral end of the rat.

Results

The withdrawal response of the foot to heat stimuli delivered to the plantar surface of the foot consisted of three components: direction of foot movement, magnitude of foot movement, and change in angle of the foot. Figures 4A and B are frames from the video that illustrate movement of the paw (visualized from below the animal). Figure 4A shows the initial location of the paw, while Figure 4B shows the final position of the paw, with the initial position located with a shaded gray region. Figures 4C and D show how the measurements were made for each component of the response. Figure 4C, which is now viewed from above the animal in the same plane as the coordinate system, shows the angle of movement, θ_1 , and the magnitude of movement, d . In Figure 4D, the drawing shows how the change in foot angle was measured (via the axis determined by locations 1 and 3) and is represented by θ_2 .

When all response directions are represented ($N=101$), and when taking direction and magnitude into consideration, there is an apparent preference for caudal-lateral movement (Figure 5A). Figure 5B demonstrates the change in foot angle for each of the 101 trials, showing a seemingly minimal change in foot angle.

A histogram of response directions shows a preference for foot movement along an axis that was $15^\circ/195^\circ$ from the vertical, denoted by the dashed line (Figure 6A). Figure 6B is a histogram of frequency of response magnitudes. The magnitudes of movement ranged from 0.6 - 29.1 mm with a mean response magnitude of 9.2 mm represented by the dotted line. The distribution is slightly skewed, with a large majority of responses occurring in the 0-15 mm movement range, and fewer movements occurring at magnitudes greater than 15 mm. Figure 7 shows the frequency histogram for change in foot angle between the initial and final foot

positions for each trial. The mean change in foot angle was only 3 degrees (or 357°), represented by a solid line extending outward from the circle, indicating that the angle of the foot changed little during the withdrawal movement.

A. Dependence of Stimulus Location

The first question this study was focused on was whether or not the stimulus location on the plantar surface of the foot influenced the withdrawal movement. A variety of response directions occurred at each stimulus location, though the medial position showed a preference for lateral movement and the lateral position showed a preference for medial movement (Figure 8A). Figure 8B displays change in foot angle during the response, again divided into the five stimulus locations. The response angle appears to remain relatively constant throughout the response, irrespective of stimulus location.

In order to determine if stimulus location significantly influenced response direction, the five stimulus locations are shown in regards to their mean direction (Figure 9A), magnitude (Figure 9B) and foot angle (Figure 10). The circular mean direction of movement for each of the five stimulus locations was not significantly affected by stimulus location ($p=0.45$, Watson Williams F Test) (Figure 9A). Similarly, the effect of stimulus location on distance of movement was not significant ($p=0.21$, one-way ANOVA) (Figure 9B). In addition, stimulus location also did not significantly influence the change in foot angle ($P=0.10$) (Figure 10).

Taken together, stimulus location had no significant effect on the withdrawal direction, magnitude, or foot angle, which raises the question if other variables may have influenced the withdrawal response and accounted for the remaining variance

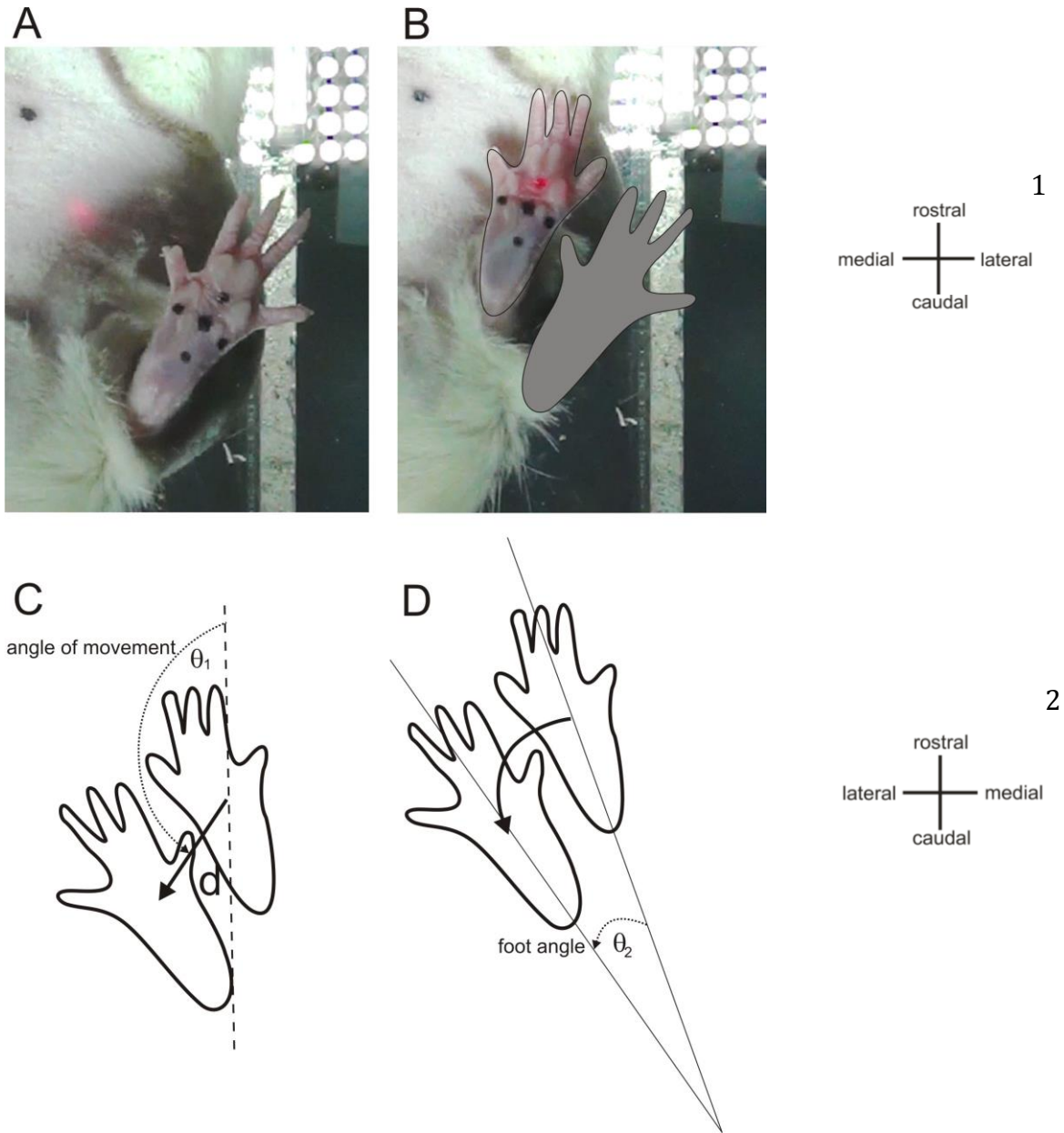
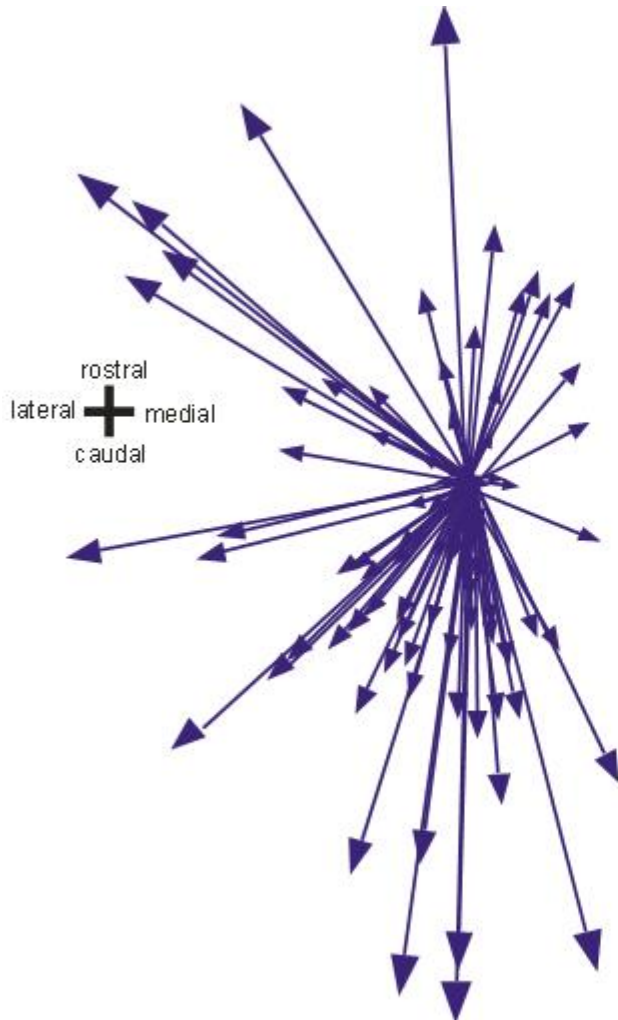


Figure 4. Translational and rotational movement of the foot. A) Video frames from below the animal showing the initial position of the left hind foot before stimulus; the foot also displays location markings. B) Video frames showing the position of the foot following the stimulus-induced movement. The withdrawal movement duration was 50 ms, and the initial foot location prior to the stimulus is represented by shaded region. C) An illustration of two of the measurements recorded for translation of the paw. The illustration shows the initial and ending locations of the COM. θ_1 represents the angle of movement, as measured from the dotted vertical line, and “d” indicates the magnitude of the movement. D) The change in foot angle from initial position to the final foot position after movement is represented by θ_2 .

A



B

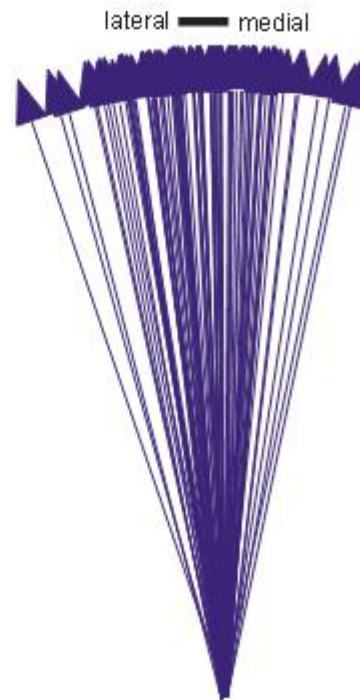


Figure 5. Changes in response direction and foot angle. A) Each trial in the study (n=7 rats) is represented by a vector, showing the relative direction and magnitude of movement. All directions are represented, with an apparent preference for the caudal-lateral direction suggested by the direction and magnitude of the responses. B) Each trial is represented as a unit vector showing the change in foot angle; the mean change in foot angle was 3° (357°).

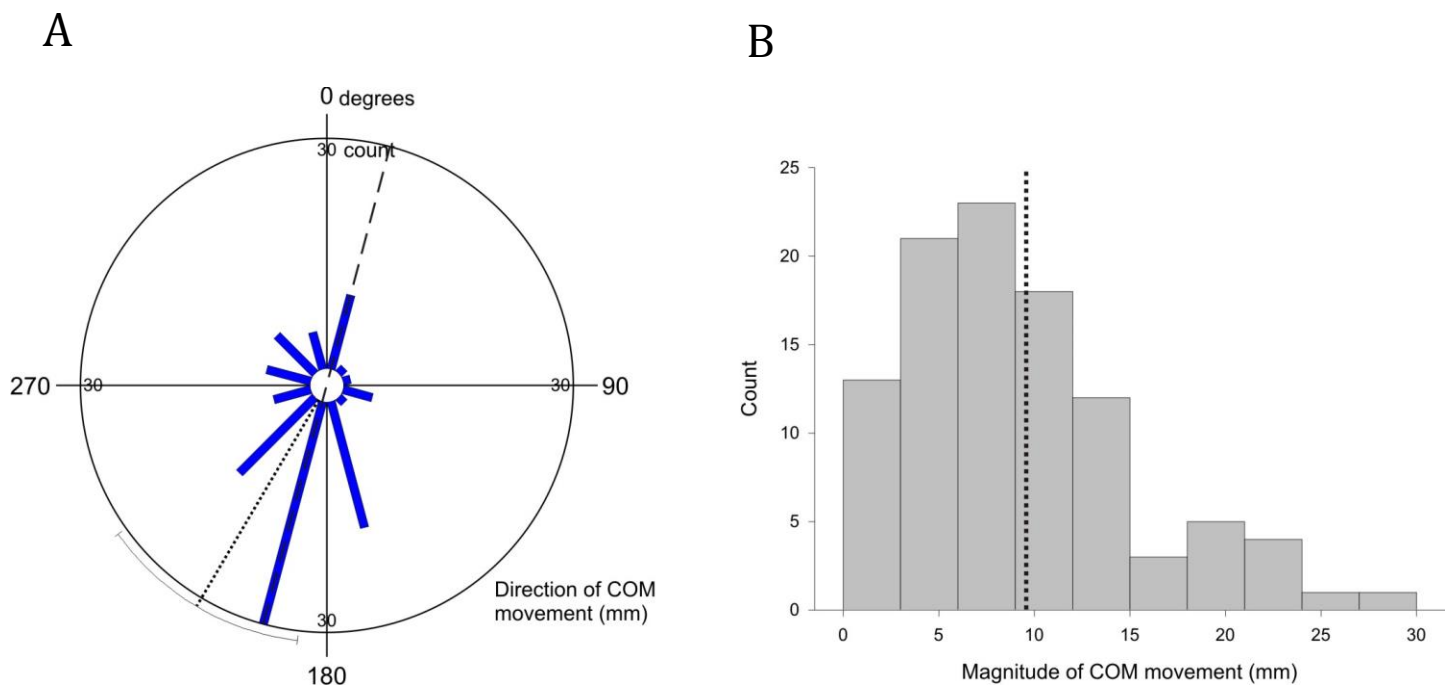


Figure 6. Frequency of direction and magnitude of movement. A) The circular histogram displays the mean frequency of response of the center of mass of the foot in specific directions. The histogram consists of 12 bins, each spanning 30° . The circular mean direction was 210° (dotted line) $\pm 76^\circ$ S.D., and the arc at the bottom left denotes a 99% confidence interval of $\pm 26^\circ$. $N=101$. A dashed line was placed through the movement axis that was most commonly seen for rostral and caudal movements. B) The histogram displays the frequency of specific magnitudes of response. The mean magnitude was 9.2 mm, represented by the dotted vertical line. All trials ($N=101$) are included.

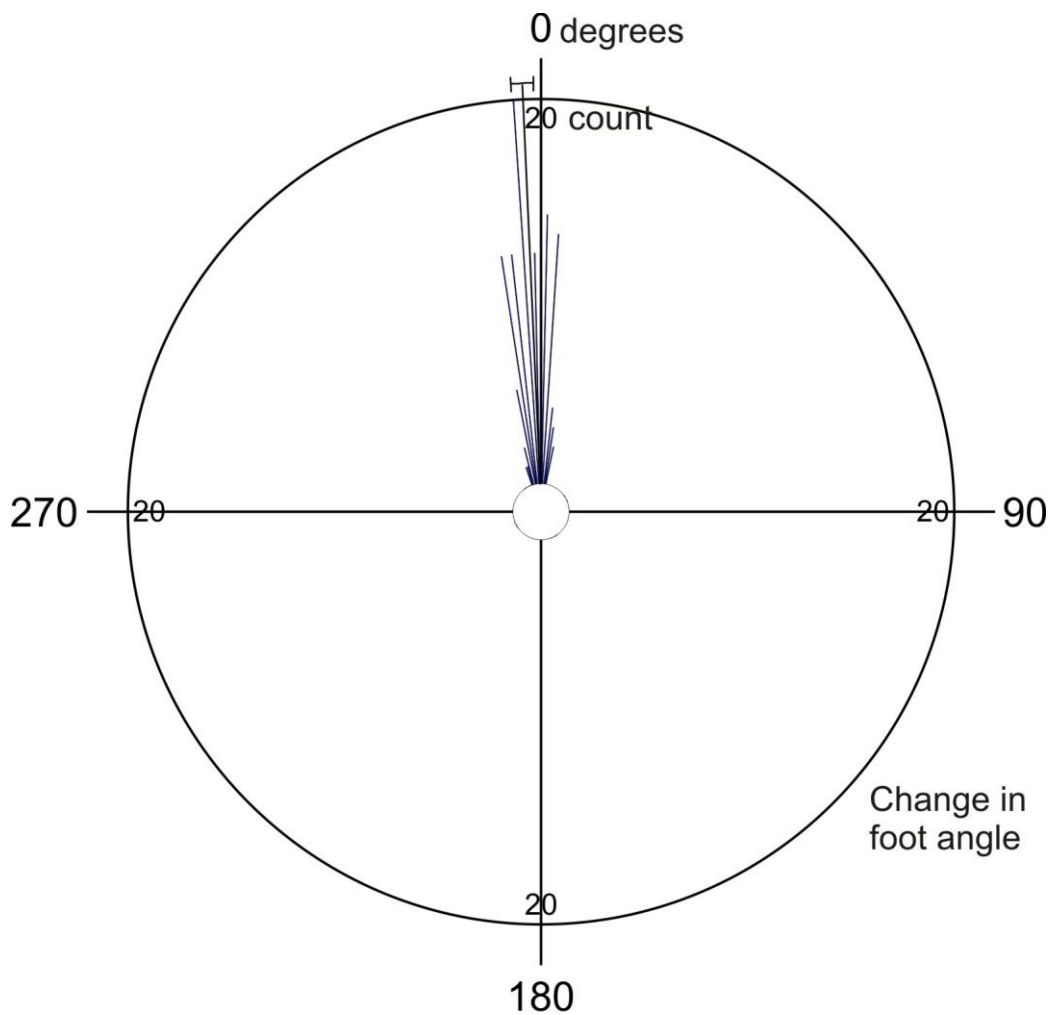


Figure 7. The frequency of change in foot angle. The circular frequency histogram displays the change in foot angle that results from the movement response. The distance from the center of the histogram to the edge of the circle represents 20 counts. The angles were divided into 144 bins, with 2.5° width of each bin. The mean change in foot angle was 357°, +/- 4.9° S.D., all trials (N=101) are included.

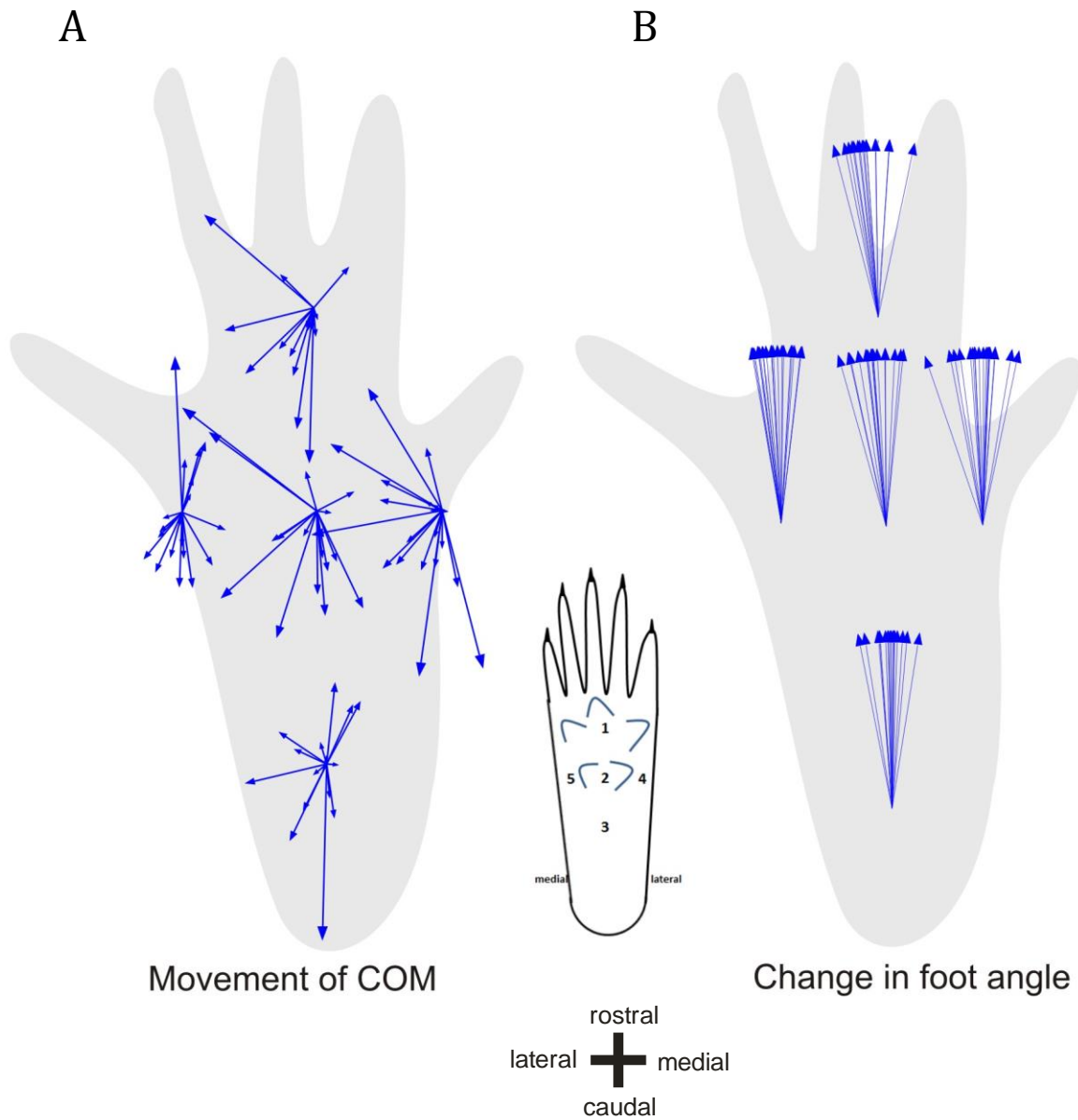


Figure 8. The movement of the foot for each stimulus location. A) The withdrawal response of the five stimulus locations are represented on the foot (see small figure for reference) by vectors. A longer vector represents a larger magnitude of movement, while the direction of the arrow represents response direction. Each arrow represents a separate trial (N=101). B) Unit vectors indicating the change in foot angle, divided into the five stimulus locations. Each arrow represents a separate trial (N=101).

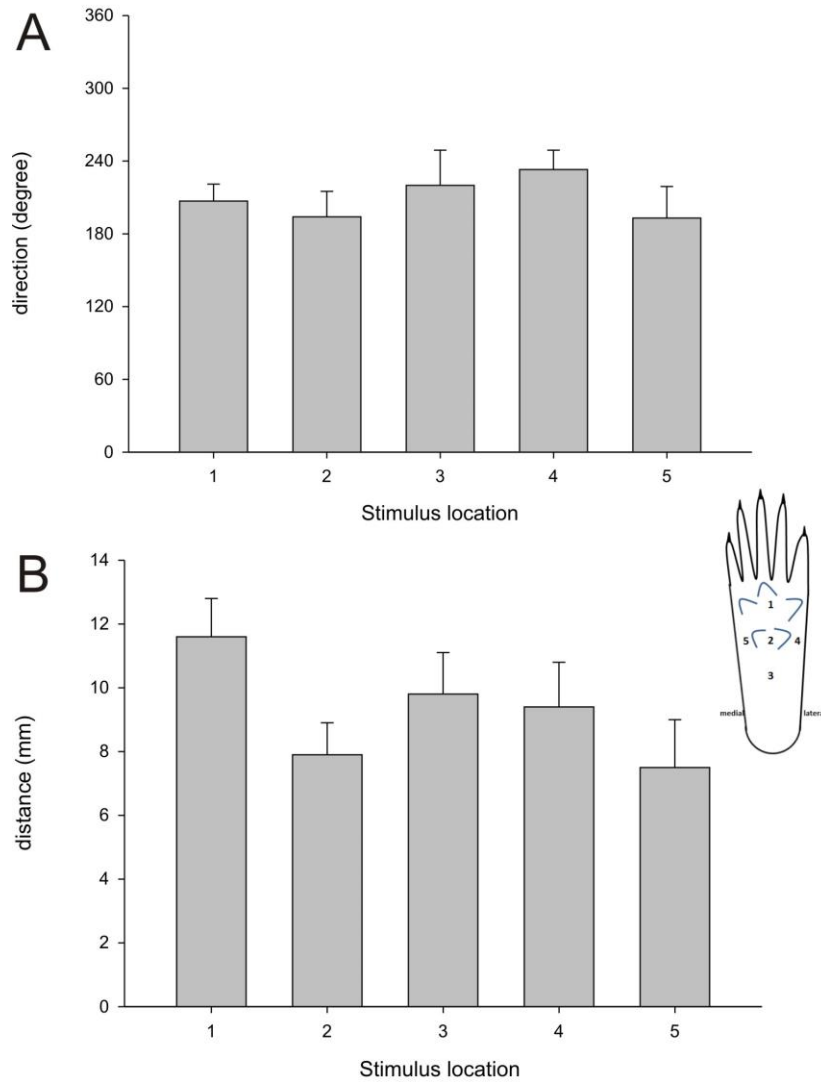


Figure 9. Dependence of mean direction and magnitude of response on stimulus location. A) The mean direction of movement for each stimulus location did not significantly depend on stimulus location (Watson Williams F Test $p=0.45$, $N=101$.) B) Similarly, the mean distance of movement for each stimulus location did not depend on stimulus location (one-way ANOVA $P=0.21$, $N=101$).

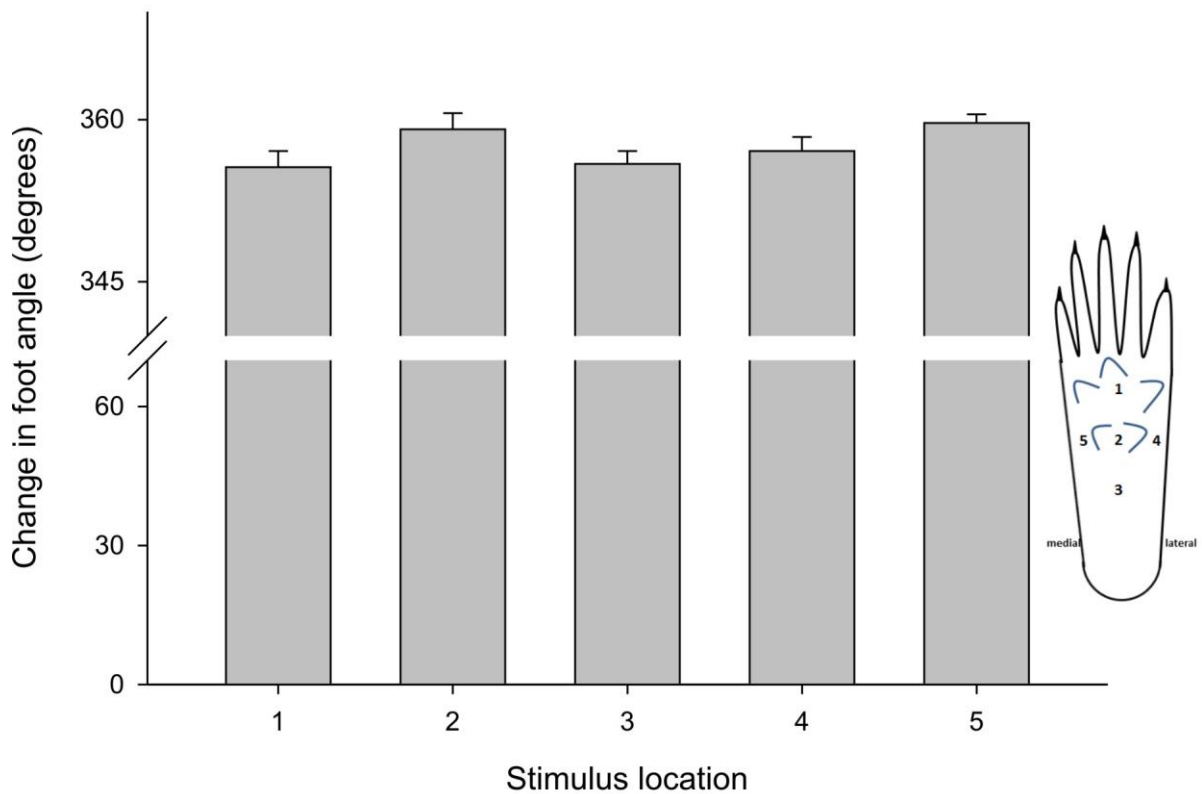


Figure 10. The change in foot angle between the initial and final positions for each stimulus location. The mean change in foot angle for each stimulus location did not significantly depend on stimulus location (one-way ANOVA $p=0.10$, $F=1.99$, $N=101$).

B. Dependence of Initial Position

The initial foot location prior to the NWR varied spontaneously between trials, suggesting that foot location might influence the response. The examination of initial foot location was performed in an attempt to explain the observed results in variation of the withdrawal. In order to study the effect of initial position of the left stimulated foot on the withdrawal response, it was first necessary to verify that different initial positions occurred spontaneously throughout the experiment. Figure 11 illustrates the range of different initial positions of the rat paw prior to stimulation; a drawn-to-scale image of the rat paw is also included. The dots within Figure 11 demonstrate a wide variety of initial positions.

In order to determine if initial foot position influenced the response, movement in the rostral-caudal axis was analyzed separately from movement in the medial-lateral axis. There was a significant correlation between initial rostral-caudal position and movement along the rostral-caudal axis (correlation test; $p < 0.001$, $R^2 = 0.272$) (Figure 12A). Analysis showed that 27.2% of the variance in the change in rostral-caudal position is accounted for by the initial rostral-caudal position of the paw. There was also a significant correlation between initial lateral-medial position and movement along the lateral-medial axis (correlation test; $p < 0.001$, $R^2 = 0.197$), revealing that 19.7% of the variance of the change in medial-lateral movement is accounted for by the initial medial-lateral position of the paw (Figure 12B). The initial foot angle compared to the final foot angle is shown in Figure 13. The figure shows a significant effect of initial foot angle influencing the final foot angle ($p < 0.001$, $R^2 = 0.613$), indicating 61.3% of the final foot angles are accounted for by the initial foot angles.

Together, these results demonstrate that all three measurements of response movement – direction, magnitude and change in foot angle depended on the initial foot location and angle.

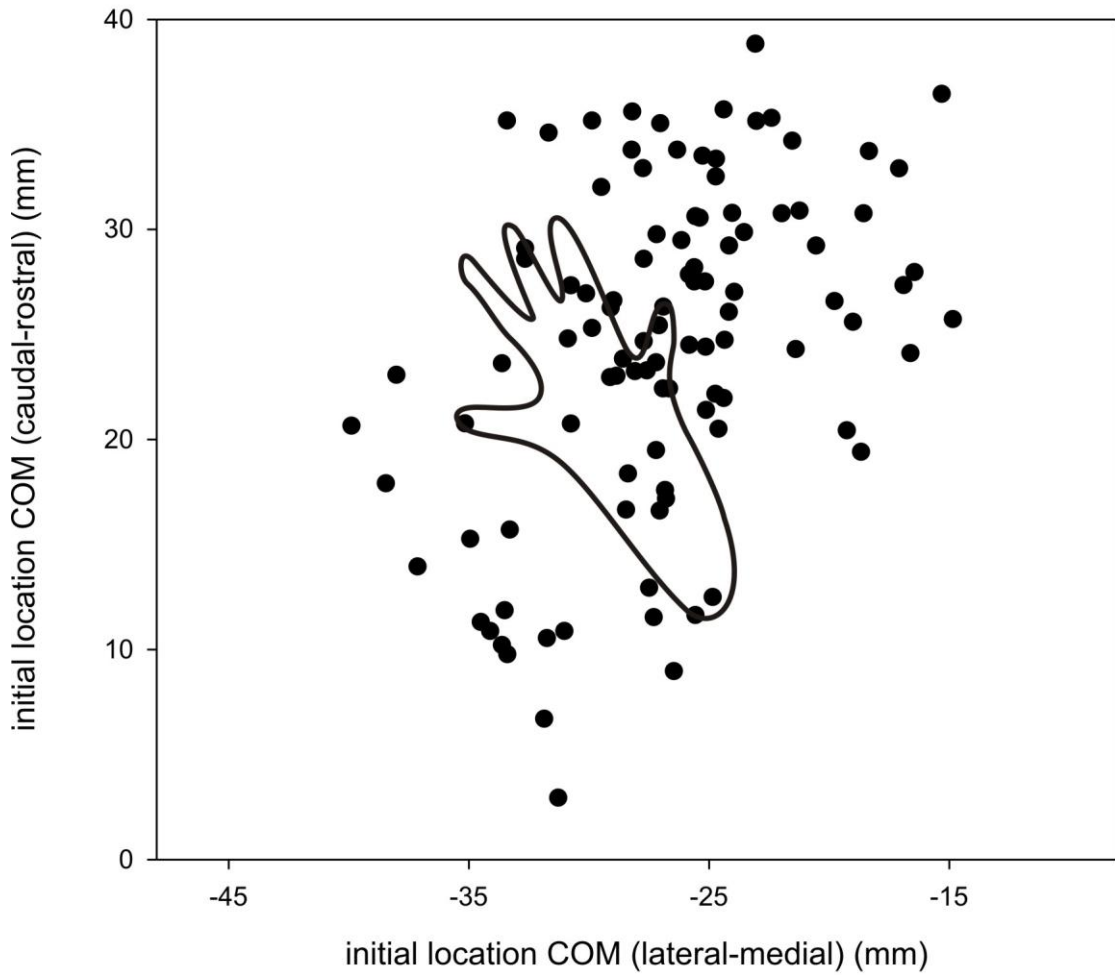


Figure 11. Initial locations of the foot prior to stimulation. The initial locations of the COM of the foot are displayed in the lateral-medial and caudal-rostral plane. A figure of the rat paw drawn to scale gives perspective of location variation. All trials at all stimulus locations are included (N=101).

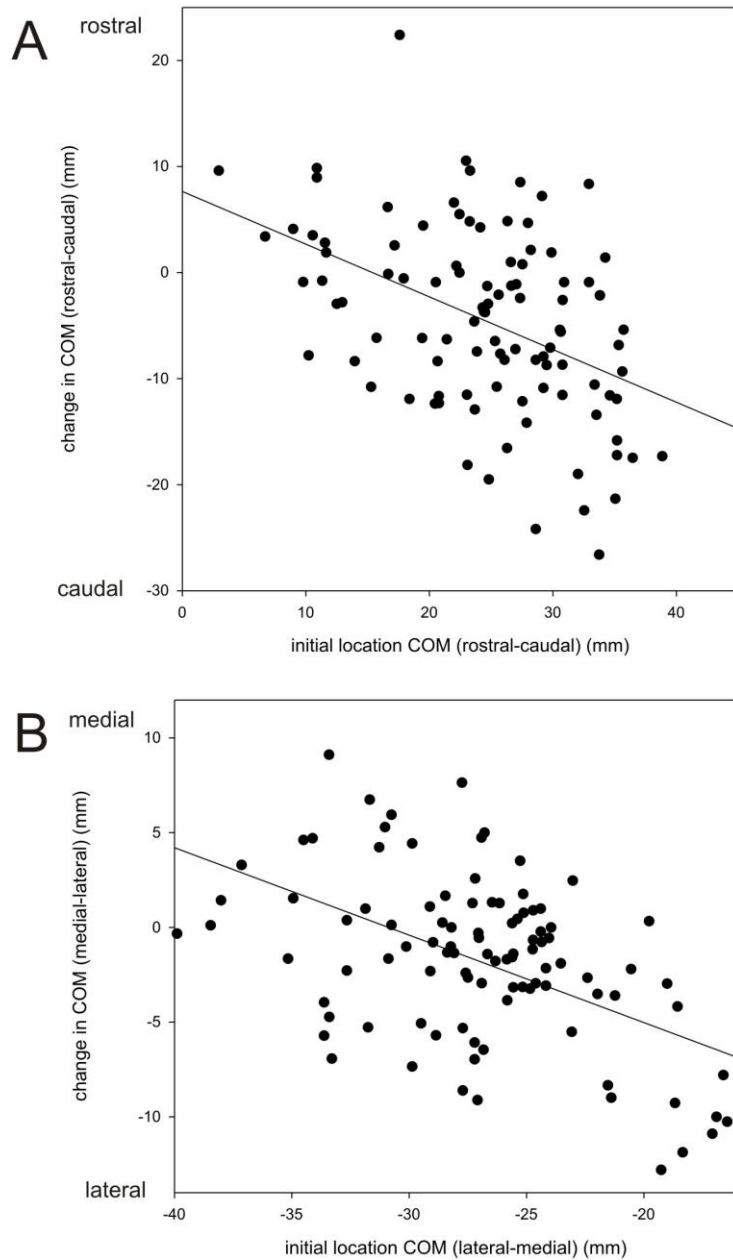


Figure 12. The effect of initial foot location on movement. A) The initial location of the foot in the rostral-caudal axis is graphed against the corresponding movement of the foot in the rostral-caudal direction. A correlation test was performed that indicated a significant dependence of movement along the rostral-caudal axis on initial rostral-caudal location (Correlation test; $F=36.9$, $P<0.001$, $R=-0.52$, $R^2=0.272$, $N=101$). B) The initial location of the foot in the medial-lateral axis is graphed against the corresponding movement of the foot in the medial-lateral direction. A correlation test was performed that indicated a significant dependence of movement along the medial-lateral axis on initial medial-lateral position (correlation test; $F=28.8$, $P<0.001$, $R=-0.44$, $R^2=0.197$, $N=101$).

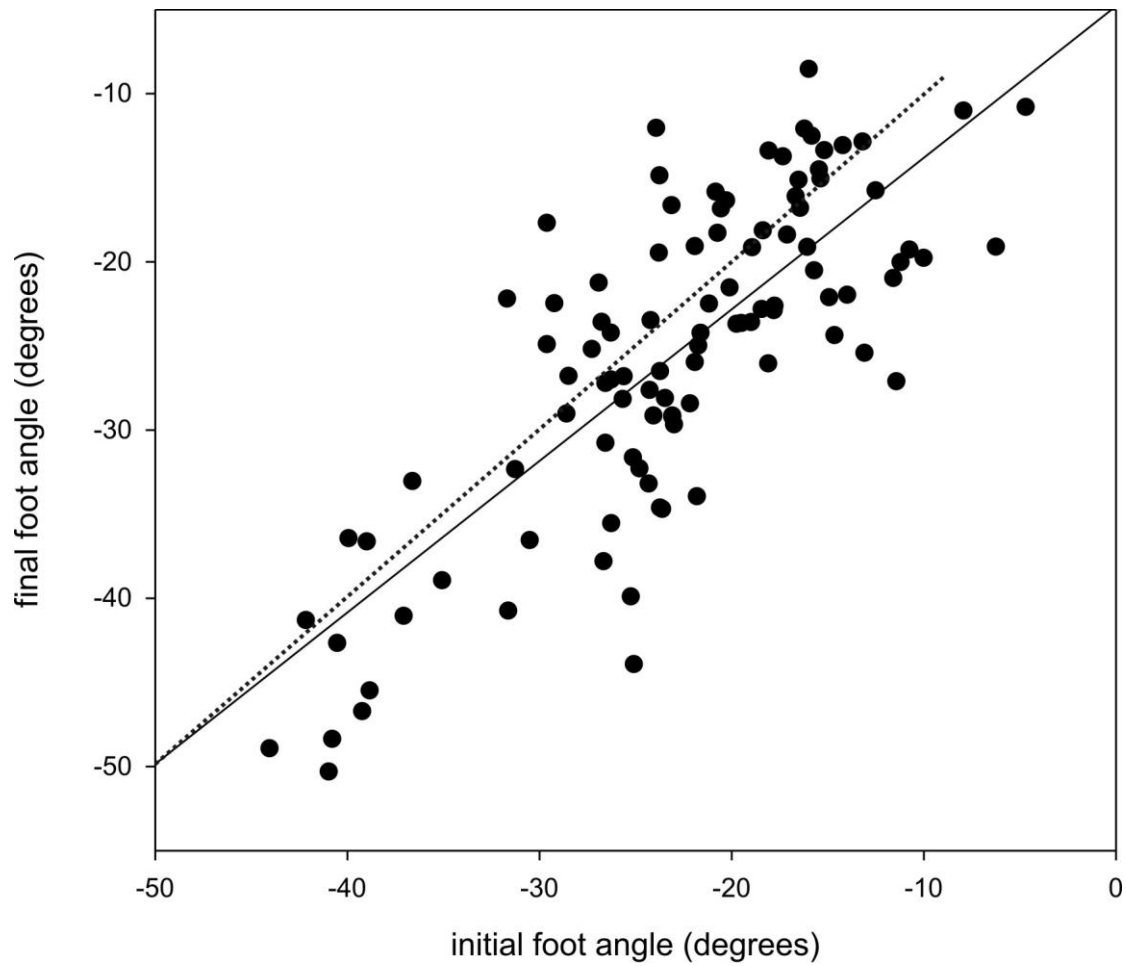


Figure 13. Final foot angle after movement as a function of original foot angle. Each dot symbolizes a separate trial whereby the initial foot angle is graphed against the final foot angle. A correlation test was performed which found a significant and strong influence of initial foot angle on final foot angle ($F=156.9$, $P < 0.001$, $R=0.78$, $R^2=0.613$, $N=101$).

Discussion

A. Summary

The NWR has previously been shown to be dependent on stimulus location and initial limb position in humans; however, there are no studies in intact, unanesthetized non-human mammals.

The influence of stimulus location on the NWR in intact, unanesthetized rats was determined by stimulating the plantar surface of the rat paw with heat localized at five different locations in order to evoke a NWR. The NWR consisted of a withdrawal of the paw which was rapidly replaced on the platform. In contrast to previous studies, the differing stimulus locations did not significantly affect the direction of movement, magnitude of movement, or the change in foot angle between the initial and final positions of the response in the horizontal plane.

However, the NWR movement in the horizontal plane was significantly influenced by the initial position of the hind left limb. In order to determine the influence of spontaneous change in initial position of the hind left paw, the paw was stimulated on the plantar surface while it was located at different initial positions relative to the body and the withdrawal movement was observed. As the paw was positioned laterally in relation to the body the NWR caused the paw to move medially (a more medial initial position caused lateral movement). As the paw was positioned caudally in relation to the body the NWR caused the paw to move rostrally (a more rostral initial positioned caused caudal movement). The initial angle of the foot was also a significant factor in influencing the final angle of the foot after the response. There was a small change in foot angle between the initial and final foot positions, and thus the final foot angle was very similar to the initial foot angle.

B. Comparison to Previous Results

Stimulus Location

One purpose of this study was to determine whether stimulus location affected the NWR in intact, unanesthetized rats. The magnitude of withdrawal, direction of withdrawal, and change in foot angle from the initial to final position were all found to be independent of stimulus location, which contradicts patterns of withdrawal based on stimulus location found in previous studies. A particular pattern of withdrawal, often observed in previous studies, is that of the categorical pattern. This pattern was supported by findings from Sherrington (1910) who studied cats. Sherrington observed the muscle contraction visually rather than using EMG like Schouenborg had in some of his studies. Sherrington noted that changing the stimulus location on the leg resulted in only a few movement directions. Similarly, Spaich et al. (2003) who studied the withdrawal response after stimulating the sole of the foot during the walking cycle found a categorical response. The decreased gain of the stretch reflex during the walking cycle may have accounted for a greater sensitization to stimuli and thus may have led to an effect that would not normally have been seen. Esquivel (2010) and Davis (2009) who studied spinalized rats noticed that changing stimulus location on the sole of the rat foot resulted in only a set number of responses. Esquivel observed two possible movements for a response, the more common movement being rostral-medial and the less common being in the dorsal direction, though with greater magnitude. However, neither movement significantly depended on stimulus

location. Davis, in contrast, noted a weak relationship between response direction and stimulus location, yet no significance of the stimulus location on the magnitude of the movement.

Another pattern of withdrawal that has been observed in previous studies is that of the continuous pattern whereby changing stimulus location results in an equal change in response. Hagbarth (1952) found in his studies of rats that altering stimulus location always resulted in electroneurogram recordings that showed activity of muscles that would support movement away from the stimulus, consistent with a continuous pattern of withdrawal. Similarly, Schouenborg (1990) who also studied rats, found that stimulating distinct areas resulted in differences in the muscles that were activated. However, Hagbarth and Schouenborg inferred movement based on analysis of electric recordings of nerves of muscle, and thus withdrawal was not actually observed. Recent studies from our laboratory (Wyatt 2010, Seamon 2012) also support continuous patterns of withdrawal of the foot. Wyatt (2010) studied the varying stimulus locations on the hind paw of intact, unanesthetized rats similar to this study. The difference in results may have occurred because Wyatt used different stimulation points on the sole of the paw that included a toe, whereas this study used stimulation points that were grouped closer together in the center of the foot. Seamon (2012) also demonstrated a continuous pattern of withdrawal when stimulating the sole of the rat hind paw. The stimulus locations of Seamon's study were grouped in similar proximity to the markings of this study; however, Seamon had four more stimulus locations, which provided a continuous axis of stimulation. Andersen (2007) found that changing the stimulus location on the sole of the foot in humans resulted in a change in foot angle (inversion/eversion) that was continuous. However, the change in inversion/eversion was not studied in this experiment as this experiment only recorded the initial and final positions of the foot. It may have been the case that inversion/eversion were occurring during the responses

observed in this study and were absent at the points where the paw contacted the platform. Clarke and Harris (2002), who studied rabbits, also determined a continuous pattern of withdrawal upon stimulating the foot in different locations although their study was using lightly anesthetized rabbits.

Initial Posture

The second purpose of this study was to determine whether the initial posture of the left stimulated hind limb prior to stimulation influenced the NWR. Taking advantage of spontaneous change in initial foot placement, we determined that the direction of response significantly depended on the initial position of the COM of the paw in the horizontal plane. Correlation revealed an R^2 of 0.272, implying that 27.2% of the in change in rostral-caudal position was accounted for by the initial rostral-caudal position of the paw. If the paw was initially positioned rostral, it would move caudally during the response. If the paw was initially positioned caudally, it would move rostral during the response. Similarly, correlation revealed an R^2 of 0.197, implying that 19.7% of the change in medial-lateral movement is accounted for by the initial medial-lateral position of the paw. If the paw was initially positioned laterally, it would move medially during the response. If the paw was initially positioned medially, it would move laterally during the response. The correlation statistics demonstrate that although there was a significant effect of initial position of the limb on the NWR, the effect is inadequate in both the rostral-caudal and medial-lateral movements to account for a majority of the variance.

The findings of this study support studies by Peterson et al. (2013) in their observations on the influence of the position of the human arm on the NWR. Peterson et al. (2013) found that

the position of the arm influenced the direction of the withdrawal response. Similarly, our findings are consistent with the findings of Baxendale and Ferrell (1980), in their study of decerebrate cats, who found that the knee joint angle influenced the NWR. Finally, Kim et al. (2006) added support to this finding in that during their studies on humans with spinal cord injuries, the NWR was modulated presumably by hip proprioceptors, particularly the muscle spindles.

Our results, which show that initial position of the foot influenced the response direction, differ from that of Eckert and Riley (2013) who found that the upper limb of humans did not influence the response direction, while this study found a dependence of the NWR on the initial limb position. Though the study was done on humans and not rats, one would expect a possible similarity in the neural mechanisms as both animals are mammals. Eckert and Riley noted in their study that specific feedback mechanisms which alter the NWR may have been occurring, yet the modulation was to such a small extent that it was unnoticeable.

C. Mechanisms

It was found that withdrawal movement was modulated by the spontaneous initial posture of the limb, which resulted in changes in ankle, knee, and hip joint angle. This raises the question regarding possible mechanisms of modulation. First, the influence of posture on the reflex direction may result from supraspinal descending modulation, whereby changes in commands for foot placement would be accompanied by changes in modulation of the NWR. Secondly, afferent proprioceptors may modulate the NWR.

Afferent proprioceptors are known to converge onto interneurons in the spinal cord (Kim et al., 2006), and may modulate the NWR. There are four classes of proprioceptors located in the limb. Muscle spindles, located within the muscle, measure the length of muscle. Golgi tendon organs, located within muscle tendons, measure muscle force. Joint afferents may measure the angle of joints, and finally the cutaneous receptors (though not proprioceptors), are located within the skin and may provide joint angle information through skin stretch (Ruskell, 1999).

Each of these proprioceptors may play a role in aiding the brain to determine the position of the limb in relation to the body. However, the two proprioceptors that seem to be able to provide the most useful information regarding limb position are the joint afferents and the muscle spindles. The information sensed by joint afferents provides a direct measurement of joint angle, whereas the information provided by the muscle spindle can be used to calculate limb position indirectly.

Despite the existence of multiple types of proprioceptors, there is evidence that one proprioceptor in particular is used in determining the position of the limb in order to modulate the NWR. Kim et al. (2006) observed in their studies on humans with spinal cord injuries that reflex excitability and magnitude was enhanced when the hip was placed in an extended position. This modulation was attributed to the activity of hip proprioceptors interacting with neurons in the reflex pathway. In particular, the proprioceptor that Kim and colleagues thought to be most responsible was that of the muscle spindle.

D. Significance

The environment is often threatening to an animal, and the NWR functions to protect an animal from injury. Thus, the ability of an animal to alter the NWR in order to produce the most effective movement is highly advantageous. If the initial foot position of the rat is such that a withdrawal in one direction would result in the animal losing balance, the withdrawal is changed such that the direction becomes more manageable. This is essentially what was found in this study. As the paw was positioned more laterally the stimulus would cause the paw to be withdrawn more medially. This is advantageous because movement in the lateral direction, even further from the rat, would be cumbersome for the process of maintaining balance.

It is also advantageous for a movement from noxious stimuli to be fast in order to decrease tissue damage. The rat paw is small in size, and thus having different directions and magnitudes of movement based off small changes in stimulus location may require modulation or other complex interactions, thus greatly increasing the latency of response. It is much more advantageous for the rat to initiate the best possible withdrawal of the foot as a whole. These tendencies were observed in the rat, as stimulus location had little effect on the withdrawal response.

Literature Cited

- Andersen OK (2007) Studies of the organization of the human nociceptive withdrawal reflex. Focus on sensory convergence and stimulation site dependency. *Acta Physiologica Scandinavica*. 189:1-35.
- Andersen OK, Sonnenborg FA (1999) Modular organization of human leg withdrawal reflexes elicited by electrical stimulation of the foot sole. *Muscle and Nerve*. 11:1520-1530.
- Baxendale RH, Ferrell WR (1980) The effect of knee joint afferent discharge on transmission in flexion reflex pathways in decerebrate cats. *Journal of Physiology*. 315:231-242.
- Camhi JM, Tom W, Volman S (1978) The escape behavior of the cockroach *Periplaneta Americana*. II. Detection of natural predators by air displacement. *Journal of Comparative Physiology*. 128:203-212.
- Cleland CL, Bauer RE (2002) Spatial transformations in the withdrawal response of the tail in intact and spinalized rats. *Journal of Neuroscience*. 22:5265-5270.
- Cleland CL, Gebhart GF (1997) Principles of nociception and pain. in *Expert Pain Management*. Springhouse.
- Cooper WE, Stankowich T (2010) Prey or predator? Body size of an approaching animal affects decisions to attack or escape. *Journal of Behavioral Ecology*. 21:1278-1284.
- Davis H (2009) Dependence of the nociceptive withdrawal response of the hind limb on stimulus location in spinalized rats. Honor's Thesis. Dept. of Biology: James Madison University.
- Delcomyn F (1998) *Foundations of Neurobiology*, New York: W.H. Freeman and Company.

- Domenici P, Blagburn JM, Bacon JP (2011) Animal escapology II: escape trajectory case studies. *Journal of Experimental Biology*. 214:2474-2494.
- Drewes CD (1984) Escape reflexes in earthworms and other annelids. in *Neural Mechanisms of Startle Behavior*. Plenum press. 43-91.
- Eaton RC (1984) *Neural mechanisms of startle behavior*. Plenum Press.
- Eckert N, Riley ZA (2013) Adaptability of the nociceptive withdrawal reflex. *F1000Research*. 2:158-167.
- Edwards DH, Heitler WJ, Krasne FB (1999) Fifty years of a command neuron: The neurobiology of escape behavior in the crayfish. *Trends in Neuroscience*. 22:153-161.
- Ellrich J, Hopf HC (1998) Cerebral potentials are not evoked by activation of golgi tendon organ afferents in human abductor hallucis muscle. *Electromyography and Clinical Neurophysiology*. 38:137-139.
- Esquivel C (2010) *Spatial Analysis of the Nociceptive Withdrawal Response in the Hindlimb of Spinalized Rats*. Honor's Thesis. Dept. of Biology: James Madison University.
- Evarts EV (1973) Motor cortex reflexes associated with learned movement. *Science*. 179:501-503.
- Fitts PM (1954) The information capacity of the human motor system in controlling the amplitude of movement. *Journal of Experimental Psychology*. 47:381-391.
- Gebhart GF (2004) Descending modulation of pain. *Neuroscience and Biobehavioral Reviews*. 27:729-737.

- Hagbarth KE (1952) Excitatory and inhibitory skin areas for flexor and extensor motoneurons. *Acta Physiological Scandinavica*. 26:7-52.
- Hammond PH (1955) Involuntary activity in biceps following the sudden application of velocity to the abducted forearm. *Journal of Physiology-London*. 127:23-25
- Harris J, Clarke RW (2002) Organization of sensitization of hind limb withdrawal reflexes from acute noxious stimuli in the rabbit. *Journal of Physiology*. 546:251-265.
- Hassenstein B, Hustert R (1999) Hiding responses of locusts to approaching objects. *Journal of Experimental Biology*. 202:1701-1710.
- Hick WE (1952) On the rate of gain of information. *Quarterly Journal of Experimental Physiology*. 4:11-26.
- Hultborn H (2006) Spinal reflexes, mechanisms and concepts: from Eccles to Lundberg and beyond. *Progress in Neurobiology*. 78:215-232.
- Jolly WA (1910) On the time relations of the knee-jerk and simple reflexes. *Experimental Physiology*. 4:67-87.
- Kim Y, Youm Y, Wu M, Schmit BD (2006) Modulation of flexor reflexes by static and dynamic hip proprioceptors in chronic human spinal cord injury. *Journal of Clinical Neuroscience*. 14:1078-1088.
- Lewis JE, Kristan WB (1998) Quantitative analysis of a directed behavior in the medicinal leech: Implications for organizing motor output. *Journal of Neuroscience*. 18:1571-1582.

- Matthews PB (1991) The human stretch reflex and the motor cortex. *Trends in Neuroscience*. 14:87-91.
- McIlroy, WE, Bent LR, Potvin JR, Brooke JD, Maki BE (1999) Preparatory balance adjustments precede withdrawal response to noxious stimulation in standing humans. *Neuroscience Letters*. 267:197-200.
- Mohammadi A, Rodgers JB, Kotera I, Ryu WS (2013) Behavioral response of *Caenorhabditis elegans* to localized thermal stimuli. *BMC Neuroscience*. 14:66-78
- Peterson CL, Riley ZA, Krepkovich ET, Murray WM, Perreault EJ (2013) Withdrawal reflexes in the upper limb adapt to arm posture and stimulus location. *Muscle and Nerve*. Electronic Publication.
- Prochazka A, Clarac F, Loeb GE, Rothwell JC, Wolpaw JR (1999) What do reflex and voluntary mean? Modern views on an ancient debate. *Experimental Brain Research*. 130:417-432.
- Rietdyk S, Patla AE (1998) Context dependent reflex control: some insights into the role of balance. *Experimental Brain Research*. 119:251-259.
- Rozanowski K, Murawski K (2013) Optical sensor to monitor pupillary light reflex. *Acta Physica Polonica*. 124:558-562.
- Ruskell, GL (1999) Extraocular muscle proprioceptors and proprioception. *Progress in Retinal and Eye Research*. 18:269-291.
- Sandrini G, Serrao M, Rossi P, Romaniello A, Cruccu G, Willer JC (2005) The lower limb flexion reflex in humans. *Progress in Neurobiology*. 77:353-395.

Schomburg ED (1990) Spinal sensorimotor systems and their supraspinal control. *Neuroscience Research*. 7:265-340.

Schouenborg J, Holmberg H, Weng HR (1992) Functional organization of the nociceptive withdrawal reflexes. II. Changes of excitability and receptive fields after spinalization in the rat. *Experimental Brain Research*. 90:469-478.

Schouenborg J, Kalliomaki J (1990) Functional organization of the nociceptive withdrawal reflexes. I. Activation of hindlimb muscles in the rat. *Experimental Brain Research*. 83:67-78.

Seamon MA (2012) The effect of variations in stimulus location on the direction and magnitude of the nociceptive withdrawal reflex in intact rats. Honor's Thesis. Dept. of Biology: James Madison University.

Shemmell J, Krutky MA, Perreault EJ (2010) Stretch reflexes as an adaptive mechanism for maintaining limb stability. *Clinical Neurophysiology*. 121:1680-1689.

Sherrington CS (1906) *The Integrative Action of the Nervous System*. New Haven Yale University Press.

Sherrington CS (1910) Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. *J. Physiol. London*. 40:28-121.

Solomon EP, Schmidt RR, Adragna PJ (1990) *Human Anatomy and Physiology*. Saunders College Publisher.

Spaich EG, Arendt-Nielsen L, Andersen OK (2003) Modulation of lower limb withdrawal reflexes during gait: a topographical study. *Journal of Neurophysiology*. 91:258-266.

Sunkin J (2009) Habituation of the nociceptive withdrawal reflex of the tail in spinalized rats.

Honor's Thesis. Dept. of Biology: James Madison University.

Tzabazis A, Klyukinov M, Manering N, Nemenov MI, Shafer SL, Yeomans DC (2005)

Differential activation of trigeminal C or Adelta nociceptors by infrared diode laser in rats: behavioral evidence. *Brain Research*. 1037:148-156.

Weiss D (2007) Tail withdrawal patterns after application of multiple stimuli to tails of

spinalized rats. Master's Thesis. Dept. of Biology: James Madison University.

Windhorst U (1996) The peripheral motor apparatus and its nervous system: General problems

to be solved and general problem solvers. *Comparative Human Physiology*. 1:969-985.

Wyatt L (2010) Spatial transformations of the nociceptive withdrawal reflex in the hindlimb of

unanesthetized, intact rats. Honor's Thesis. Dept. of Biology: James Madison University.

Zimmermann M (1983) Ethical guidelines for investigations of experimental pain in conscious

animals. *Pain*. 16:109-110.