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X-ray kinematics analysis of vaginal scent marking in female Syrian hamsters (*Mesocricetus auratus*)

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ABSTRACT

Vaginal marking is a stereotyped scent marking behavior in female Syrian hamsters used to attract male hamsters for mating. Although the modulation of vaginal marking by hormones and odors is well understood, the motor control of this proceptive reproductive behavior remains unknown. Therefore, we used X-ray videography to visualize individual bone movements during vaginal marking. Kinematic analyses revealed several consistent motor patterns of vaginal marking. Despite exhibiting a diversity of trial-to-trial non-marking behaviors (e.g. locomotor stepping), we found that lowering and raising the pelvis consistently corresponded with coordinated flexion and extension cycles of the hip, knee, and tail, suggesting that these movements are fundamental to vaginal marking behavior. Surprisingly, we observed only small changes in the angles of the pelvic and sacral regions, suggesting previous reports of pelvic rotation during vaginal marking may need to be reconsidered. From these kinematic data, we inferred that vaginal marking is primarily due to the actions of hip and knee extensor muscles of the trailing leg working against gravity to support the weight of the animal as it controls the descent of the pelvis to the ground. The cutaneous trunci muscle likely mediates the characteristic flexion of the tail. Interestingly, this tail movement occurred on the same time scale as the joint kinematics suggesting possible synergistic recruitment of these muscle groups. These data therefore provide new targets for future studies examining the peripheral control of female reproductive behaviors.

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1. Introduction

Many species use scent marking as a means of conveying information between individuals, including the sex, reproductive status, individual identity, ownership, competitive ability, and health status of the marker [1]. Scent marking is particularly important for reproductive synchrony in solitary species such as Syrian hamsters [2]. Female hamsters scent mark by depositing vaginal secretion onto a substrate, a behavior termed vaginal marking. Converging lines of evidence suggest that vaginal marking functions as an advertisement of a female hamster's impending sexual receptivity. First, vaginal marking increases across the estrous cycle, peaking on the day prior to behavioral receptivity [3], but is not observed when females are sexually receptive [4,5]. Second, female hamsters will preferentially direct their vaginal marks to male odors over female odors [4]. Lastly, males are highly attracted to the secretion deposited by vaginal marking [6–8].

Although studies have examined how hormones and odors stimulate vaginal marking both systemically and at the level of the forebrain [9–14], the descending motor control of vaginal marking is unknown. Unlike more complex social behaviors, vaginal marking is relatively simple and can be divided into several discrete and stereotyped behavioral events, including the lowering of the pelvis, deflection of the tail from the substrate, pelvic rotation and/or forward motion, and expulsion of vaginal secretion [4]. The stereotyped nature of these events provides tractable means for identifying the peripheral targets controlling this behavior. Indeed, previous attempts at taking a "bottom-up" approach to the study of sexually motivated behaviors, including the use of X-ray cinematography and kinematics analyses [15], have yielded substantial progress in understanding the neuromuscular control of the lordotic receptive posture in female rats [2,16–19] and hamsters [2,20–22].

To delineate the motor circuits involved in vaginal marking, it is first necessary to identify the relevant musculature involved in the behavior. This is difficult, however, as overlying soft tissues (fat, skin, fur) obscure skeletomuscular movements during marking. Therefore, we used digital X-ray video analysis [23] to quantify the skeletal movement patterns of the hindquarters of female Syrian hamsters during vaginal marking, focusing on the motor pattern of the marking behavior, rather than the expulsion of secretion itself.

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We used the resulting kinematic analysis to infer which muscle groups were active during vaginal marking behavior [15].

2. Materials and methods

2.1. Animals

Adult, female Syrian hamsters (*Mesocricetus auratus*; $n=21$) were purchased from Charles River Laboratories (Wilmington, MA, USA) at 8 weeks of age. Upon arrival at Georgia State University, subjects were singly housed in solid-bottom Plexiglas cages ($43\times22\times20$ cm) with corncob bedding (The Andersons, Maumee, OH, USA) and cotton nesting material (Ancare, Bellmore, NY, USA). To determine estrous cyclicity, the consistency of females' vaginal secretion was examined daily for eight consecutive days as described previously [13]. Only females with a consistent, four-day cycle were included in the study.

A separate group of gonadally intact, adult male hamsters (Charles River Laboratories; $n=16$) were used to provide stimulus cages for behavioral data collection. These males were unrelated to, and had no previous contact with, female subjects. In order to provide an odor environment that would stimulate high levels of vaginal marking, stimulus cages were not changed for 1 week prior to pre-screening or data collection, at which time the male was removed and the soiled cage was sealed with Parafilm (SPI Supplies, West Chester, PA, USA) until use later that day.

Both subject females and stimulus males were maintained on a reversed 14-hour light/10-hour dark photoperiod (lights off at 9 AM) and food and water were available *ad libitum*. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23; revised 1996) and approved by the Institutional Animal Care and Use Committees at both Georgia State University and the Georgia Institute of Technology.

2.2. Vaginal marking pre-screening

Prior to X-ray video data collection, female subjects were pre-screened for high levels of vaginal marking. All pre-screening tests took place during the first 6 h of the dark phase on the cycle day immediately prior to sexual receptivity (proestrus). Females were placed into a soiled, vacated male stimulus cage and allowed to freely explore the cage for 5 min. The total number of vaginal marks was scored live using a hand counter. Five of the females displayed less than five vaginal marks during the pre-test and were therefore eliminated from the experiment. The remaining 16 females displayed an average of 13.44 ± 5.06 (mean $\pm$ standard deviation) vaginal marks during pre-testing and were used for further data collection.

2.3. Data collection

Data collection took place during the first 6 h of the dark phase on proestrus. Female subjects and soiled stimulus cages were transported to the Georgia Institute of Technology for behavioral testing. After transport, females were left undisturbed in their cages for at least 1 h prior to the beginning of data collection. During behavioral experiments, each female was placed inside of a vacated, soiled male stimulus cage and allowed to explore freely. To maximize the amount of time females spent in the line of sight of the X-ray beam during behavioral data collection, stimulus males were housed in smaller, solid-bottom Plexiglas cages ($28\text{ cm}\times22\times12$ cm). The stimulus cage containing the female was positioned in line of sight of the X-ray beam and the subject was observed via a live optical video feed. To collect X-ray video data, we exposed the female to a beam of X-ray photons (80 kV, 2 mA, Monoblock 160, VJ Technologies, Bohemia, NY, USA), which passed through the cage and the female into an image intensifier (225 mm diameter, TH-9438-HX, VJ Technologies). The image intensifier converted the X-rays into visible light (548 nm wavelength) that was recorded by a digital video camera (30 Hz, A504k, Basler Vision Technologies, Exton, PA, USA) and saved to computer using commercial software (Streampix, Norpix, Inc., Montreal, Quebec, Canada). As it was not possible to anticipate when the female would vaginal mark, we activated the X-ray beam only when there was a clear sagittal view of the female and when she was not engaged in behaviors incompatible with marking (i.e. grooming or rearing), thereby maximizing optimal conditions for analysis and minimizing radiation exposure. We collected up to 15 X-ray video trials per animal lasting up to 10 s each. Upon completion, each animal was returned to her home cage and transported back to Georgia State University.

2.4. Data processing and analysis

After data collection, we visually assessed each video for vaginal marking behavior and cropped the videos into shorter clips that each contained a single marking event (see Supplementary video online). Video clips were excluded from analysis ($n=35$) if they did not exhibit a clear sagittal plane view of the animal during the marking behavior. In total, 16 video clips were analyzed across 8 animals (1–5 clips per animal). Given the limited amount of usable data, each vaginal mark was treated as an independent event in the analysis.

We transformed the video clips from each marking event into individual sequences of single images and then batch processed the images using commercial software (NI Vision software, National Instruments, Austin, TX, USA). Image processing consisted of two steps: distortion correction and contrast enhancement. To correct for nonlinear visual distortion, before each data collection session

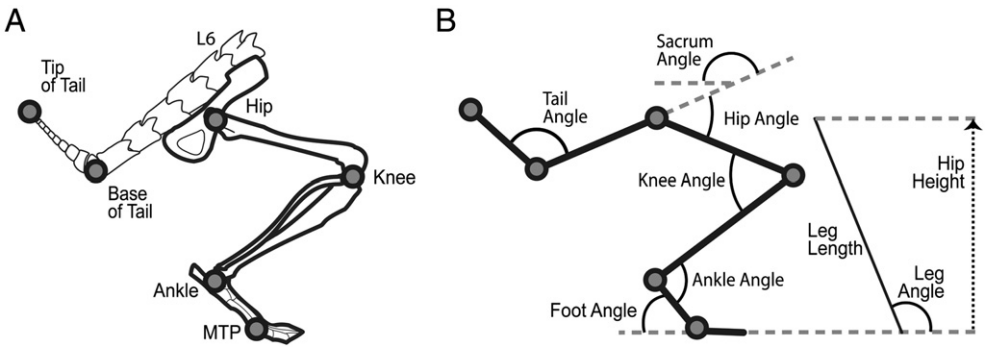

Fig. 1. Model of female hamster skeletal anatomy and kinematic measures. A) Six digitized bony landmarks (filled circles) of a female hamster were used to generate a linked segment geometric model of the hindlimb and pelvic anatomy. B) Foot, ankle, knee, leg, hip, sacrum, and tail joint angles, as well as leg length, leg angle, and hip height were quantified. L6, 6th lumbar vertebra; MTP, metatarsal phalangeal joint.