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Methods to quantify the velocity dependence of common gait measurements from automated rodent gait analysis devices

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HIGHLIGHTS

- Gait measures are velocity dependent, a confounding factor when using automated gait analysis.
- Instead of limiting this effect, measurement techniques that embrace the velocity dependence of gait are presented.
- Rats move their paws more medially, stretch farther, and are more consistent as they move faster, not so after SCI.

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ABSTRACT

Background: Walking slowly is a different biomechanical task than walking quickly, thus measures of gait will be different at different velocities, such as pre/post injury. It is necessary to determine if the difference in gait measures are from the experimental changes, or simply from traveling at different speeds.
New method: Instead of limiting this effect, we have developed techniques to embrace the velocity dependence of gait measures. By translating the pawprints into a body coordinate frame we are able to measure location of paw placement in addition to the standard gait measures.
Results: At higher velocities rats have greater consistency of steps, place their forelimb initial contact more medially and anteriorly, and place their hindlimb toe off more medially and posteriorly. Interlimb phasing also becomes more consistent at higher velocities. Following a cervical spinal cord injury consistency is reduced and the velocity dependent behaviors are significantly different.
Comparison with existing method: Translating the coordinate frame improves the ability to measure changes in base of support following spinal cord injury. Employing a treadmill, or limiting analysis to a narrow velocity window does address the effects of velocity. We feel that measuring across all velocities is more appropriate than dictating that the animals match speeds.
Conclusions: Quantifying locomotion with automated gait analysis devices is a great way to evaluate the changes that experimental treatments provide. These new methods allow for a more appropriate way to address the confound of many gait measures being velocity dependent.
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1. Introduction

Countless researchers use gait analysis to quantify behavioral changes in groups of animals, and a cottage industry exists to enable researchers to collect and process the locomotor data more quickly and accurately. The more popular automated gait analysis devices are the CatWalk (Noldus Inc, NE), DigiGait (Mouse Specifics Inc, MA), and TreadScan (Cleversys Inc, VA). We have used the CatWalk for many years to help us assess the effectiveness of therapies to restore locomotion following spinal cord injury in rats. But the more we compare our treatment groups to the pre-injury controls the more we realize we do not have a firm grasp on normal rodent locomotion. Our major concern comes from the confound of walking velocity. When walking slowly, one takes short strides infrequently, and long strides rapidly when walking fast. This simple fact that gait parameters have different values at different velocities has been known for quite some time (Heglund et al., 1974; Taylor, 1978; Hruska et al., 1979) but this knowledge rarely makes its way to the users of automated gait analysis devices. A fair amount of researchers will look to limit the effects of velocity by employing a treadmill (Krizsan-Agbas et al., 2014; Redondo-Castro et al., 2013; Tom et al., 2013) or by only analyzing data that falls in similar

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velocity windows (Bozkurt et al., 2011; Deumens et al., 2007). We struggled when we tried to apply that technique to our own data. Prior to injury our animals cross the walkway quickly, and one week after injury the animals are much slower. To limit the confounding effects of velocity we would need to match speeds. We could never determine if it was better to make our pre-injury animals walk slower, or excessively train our post injury animals to walk faster. So instead of limiting the effects of velocity, we have developed techniques that embrace the fact that locomotor measures change as velocity changes.

Our previous work examined how 3 common gait parameters, stride length, cycle time, and duty factor, change with increasing velocity, and how this behavior changes after spinal cord injury in rats (Neckel et al., 2013). Others have found similar velocity dependent measures of locomotion in healthy rats (Koopmans et al., 2007), mice (Batka et al., 2014) and velocity influences on coordination in cats (Frigon et al., 2014). Presented here is an expansion upon these themes, where we show the benefits of not just knowing the stride length or cycle time of a limb, but where that limb is in the context of the animals' body. Yes, animals take longer strides when walking faster. What we show here is that rats move their limbs more medially and stretch farther when walking faster. We also offer a novel technique that quantifies the changes in coordination with increasing velocity. Taken together these findings have great implications on the current standard of rodent gait analysis and should be adopted by fellow researchers who use automated gait analysis devices in their own studies.

2. Material and methods

2.1. Animals and study design

Throughout the experiment animals were housed in the Georgetown University Division of Comparative Medicine and had unlimited access to food and water. The Georgetown University Animal Care and Use Committee approved all protocols. 74 adult female Sprague-Dawley rats were used (appx 5 weeks old, 160–220 g range, 185 ± 12 g mean, Taconic Farms, Germantown, NY). These animals are part of our ongoing robotic gait training studies, and 46 of these animals were part of our previously reported work. Presented here for the first time is novel analysis of existing data.

Animals were pre-trained on the CatWalk XT9.1 gait analysis system on 3 non-consecutive days before pre-operative overground locomotion was recorded. Neither food deprivation nor food rewards were used as motivators, but a goal box (not the homecage) was located at one end of the walkway. Rats were allowed to transverse the walkway at their own self-selected walking speed and no time, velocity, or directional constraints were placed on the trials. Once several walking steps were recorded from each limb the trial was deemed complete (this could be accomplished from as few as 1 complete pass, or from several partial passes). Trotting or galloping steps were omitted.

All rats then received a right overhemisection injury at the C4–5 level (previously described in Bregman et al., 1993; Lynskey et al., 2006). Briefly, rats were anesthetized with 4% chloral hydrate (0.01 cc/g intraperitoneally), a partial C4/C5 laminectomy was done, and iridectomy scissors were used to create a lesion at C4–5. The lesion bilaterally ablates the dorsal corticospinal pathway, and unilaterally ablates the contralateral rubrospinal pathway. At the end of the study all lesion sites were reconstructed from serial cresyl violet sections and only the 61 animals with appropriate injuries were included in post-injury analysis.

The overground locomotion of all rats was re-assessed with the CatWalk 1 week after injury, with no re-training. A subset of 17 animals were then tested weekly for an additional 6 weeks starting on post-injury day 11 and ending on post-injury day 46 (hereafter referred to as weeks 2 through 7).

2.2. The need for a new coordinate frame

As a rodent crosses the glass walkway of the CatWalk the pawprints reflect the light down toward the digital camera where they are recorded as a pixel array with values of time, position, and color. The time value is simply a measure of seconds from frame to frame in 9.983 ms intervals (in version XT9.1). The position is a grid with the x coordinate along the length of the glass walkway, and the y coordinate along the width of the glass. The resolution of this grid is user defined, user calibrated, and based on the distance of the camera from the glass plate. When the user labels a group of pixels as a pawprint, such as "right forelimb" the software recognizes the time when those pixels first surpass the color threshold (initial contact), when that group of pixels covers its widest area (max contact) and when those pixels are below the color threshold (toe off). The mean position of the group of pixels at max contact is used as the location of the footprint for the duration of stance phase. Once the user has labeled all the successive prints from all four limbs the software can calculate a myriad of gait measures from just that one crossing of the walkway. Fig. 1A depicts one of our rats crossing the glass walkway of the CatWalk, and the user defined pawprints.

Two of the more commonly reported gait measures are stride length and base of support. Stride length is the distance from one initial contact to the next. For animals traveling in straight lines this distance is along the direction of travel. For animals walking in circles the measure of stride length is more complex as it needs to be adjusted to accommodate the path of the animal. Base of support is the distance between the forelimb or hindlimb pairs. In a stationary animal, this distance is along the minor body axis. For animals walking in straight lines this distance is perpendicular to the direction of travel. Again, for animals walking in circles the measure of base of support is more complex as it needs to be adjusted to accommodate the path of the animal as well as the timing of the paw placement. The CatWalk software measures stride length as the distance between two successive pawprints and base of support as the difference between the average y position of the left and right limbs.

There is a difference between the space between labeled pixels on a computer grid and the paw placement of a walking rat. If all animals walked in straight lines that were perfectly parallel to the grid established by the CatWalk (world coordinate frame) the difference would be zero. Unfortunately, rats rarely walk in straight lines and to our knowledge are unaware of the world coordinate frame of the CatWalk (most CatWalk users are unaware of the world coordinate frame!) To remedy this we do not look at measures in the world coordinate frame, but in the body coordinate frame of the rat. This body coordinate frame has one axis along the major axis of the animal, the second axis along the minor axis of the animal, and the origin at the center of mass of the animal. Fig. 1B depicts the same animal crossing as in 1A, but measured in the body coordinate frame.

In the body coordinate frame base of support is now the medial/distal placement of the paw. This measure is consistent even if the rat is walking in a non-straight line or a straight line that is not parallel to the edge of the glass plate (Fig. 1B1). The measure of stride length is the anterior/posterior distance between the toe-off of one step to the initial contact of the next step. Again, these measures in the body coordinate frame of the animal are consistent with the changing position of the rat (Fig. 1B2). With our body coordinate frame measures it is even possible to track the position of a paw print as it progresses through stance phase. The 4th left hindlimb step from Fig. 1A is seen in body coordinate frame in Fig. 1B2 as

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