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RESEARCH ARTICLE

The nociceptive withdrawal response of the tail in the spinalized rat employs a hybrid categorical–continuous spatial mapping strategy

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Abstract

Complexity in movement planning, arising from diverse temporal and spatial sources, places a computational burden on the central nervous system. However, the efficacy with which humans can perform natural, highly trained movements suggests that they have evolved effective behavioral strategies that simplify the computational burden. The specific aim of our research was to use three-dimensional high-speed video to determine whether the tail nociceptive withdrawal response (NWR) to noxious heat stimuli delivered at locations that varied both circumferentially and rostral-caudally on the tail depended on the location of the stimulus in spinalized rats. In particular, we sought to determine whether the movement strategy was categorical (limited number of directions) or continuous (any variation in stimulus location results in a variation in response direction). In spinalized rats, localized, noxious heat stimuli were delivered at eight locations circumferentially around the tail and at five rostral–caudal levels. Our results demonstrate that at all rostral–caudal levels, response movement direction was bimodal regardless of circumferential stimulus location—either ~ 64° left or right of ventral. However, in spite of tight clustering, movement direction varied significantly but weakly according to circumferential location, in that responses to stimuli were more lateral for lateral stimulus locations. In contrast, changes in stimulus level strongly affected movement direction, in that a localized bend response closely matched the level of the stimulus. Together, our results demonstrate, based on movement analysis in spinalized rats, that the NWR employs a hybrid categorical–continuous strategy that may minimize the harmful consequences of noxious stimuli.

Keywords Spinal · Synergy · Reflex · Flexor · Flexion · Noxious

Introduction

Computational complexity in movement planning arises from diverse temporal and spatial sources, such as nonlinear (Hooper et al. 2016) and multi-segmental (Hollerbach and Flash 1982) inverse dynamics, neural noise (Faisal et al. 2008), large numbers of kinematic and muscular degrees of freedom (Turvey 1990), and redundant degrees of freedom (Bernstein 1967). However, the efficacy with which humans can perform natural, highly trained movements (Brody 2006), suggests that they have evolved effective movement strategies that simplify the computational burden. While all movements present computational challenges in need of solutions, escape and withdrawal responses to potentially injurious stimuli are critically dependent on rapid and accurate responses to insure survival. Thus, the elucidation of underlying spatial movement strategies, finely tuned by evolution, may provide valuable insight into how animals simplify neural and biomechanical computations for movements that are critical for survival.

Previous studies in a myriad of non-mammalian animals (Eaton 1984) have revealed a variety of spatial movement strategies, often closely matched to the behavioral needs of the animal. For example, leeches (Lewis and Kristan 1998) and cockroaches (Kanou et al. 1999) rely on a *continuous* strategy, in which the response direction varies continuously with stimulus location. In contrast, *C. elegans* (Mohammadi et al. 2013) and rapid responses in crayfish (Cattaert and Le Ray 2001) rely on a *categorical* strategy, in which only limited response directions are evoked by widely distributed stimuli.

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In mammals, escape can be manifest as the nociceptive withdrawal response (NWR), in which a body part is rapidly moved in response to noxious cutaneous (Kirkwood et al. 1987; Steffens and Schomburg 1993), muscle (Cleland and Rymer 1990), or joint (Eccles and Lundberg 1959) stimuli. In spinally intact animals that are either anesthetized (Schouenborg and Kalliomaki 1990) or unanesthetized (Grimby 1963; Andersen et al. 1999), the NWR is continuous in that the pattern of muscle activity or movement direction varies with stimulus location, which was designated by Sherrington as "local sign" (Sherrington 1910) and later by Schouenborg and Kalliomaki (1990) as a "modular organization". Although a similar pattern of dependence on stimulus location has been reported in spinally injured or fully transected animals (Sherrington 1910; Creed and Sherrington 1926; Cleland and Bauer 2002; Cleland et al. 2017), the direction of response is often more stereotyped, resulting in an absence (Schmit et al. 2003) or muting (Cleland and Bauer 2002; Andersen et al. 2004; Cleland et al. 2017) of local sign. The diminution of dependence on stimulus location in the spinal state could arise from the mechanisms underlying spatial mapping residing in the brain, or in the spinal cord but disrupted by the loss of descending modulation.

Taken together, existing literature on movement strategies that underlie the dependence of the mammalian NWR in spinalized mammals on variations in stimulus location is both limited and conflicting. Consequently, the goal of our study was to determine the relationships between stimulus location and movement response direction for noxious heat stimuli applied to the tail of spinalized rats that varied (1) circumferentially around the tail and (2) along the rostral–caudal axis of the tail. The rat tail, like the octopus limb (Sumbre et al. 2006), is an ideal model to study neural solutions to computational spatial complexity because it is hyper-redundant (Chirikjian and Burdick 1994), in that there are far more joint and muscular degrees of freedom than necessary to move the skin away from the noxious stimulus during the NWR. Our results demonstrate, based on movement analysis in spinalized rats, that the NWR employs a hybrid categorical–continuous strategy to adapt the NWR to variation in stimulus location.

Methods

Animals and spinalization

Adult, male Sprague–Dawley rats ($n = 18$, 8–14 weeks), bred in-house from rats obtained from Envigo (Indianapolis, IN, USA), were surgically spinalized 16–24 h before experimentation to avoid spinal shock (Schouenborg et al. 1992). Rats were lightly anesthetized with a mixture of 2–5% halothane in 100% oxygen and then injected with sodium pentobarbital (50 mg/kg i.p.). The thoracic spinal cord was exposed at the spinal T8–T9 region via laminectomy and the dura was sectioned. Local anesthetic (40 µl 4% lidocaine hydrochloride) was applied to the spinal cord to block injury-induced activation of descending pathways. The spinal cord was cut using micro-scissors and then fully severed using a right angle surgical micro-knife (von Graefe, Fine Science Tools, Vancouver, BC, Canada); completeness of the transection was confirmed visually. The incision was closed and the rat was allowed to recover with unrestricted food and water. Rats were euthanized after completion of the experiment using sodium pentobarbital (200 mg/kg i.p.). Experiments were approved by the James Madison University Institutional Animal Care and Use Committee.

Experimental setup

Spinalized rats were restrained inside a fenestrated acrylic tube. Ten equally spaced 2 mm bands were marked with permanent black marker along the length of the tail and extending around the entire circumference at each location, so that the movement of each band could be tracked (Fig. 1a). Red dots corresponding to dorsal, ventral, left, and right locations were marked 2.5 mm above bands two, four, six, eight and ten to guide targeting of the laser stimulus. The rat was then suspended vertically with its tail hanging down through a large hole relative to tail width (2 cm in diameter) in the base of the tube that did not restrict movement of the tail in any direction. The rat was allowed to acclimatize for 30 min.

Movement recording and heat stimulation

Heat stimuli were applied to the tail with a fixed, 980 nm fiber-coupled laser diode (BWF-5, 0.5–15 W, B&W Tek, Wilmington, DE, USA; Tzabazis et al. 2005) focused through a condenser lens to a 1.5 mm spot. Location was determined by a low-power, collinear, targeting laser. Eight circumferential stimulus locations (dorsal, ventral, left, right, left-dorsal, left-ventral, right-dorsal, and right-ventral) at five rostral–caudal levels (band numbers 2, 4, 6, 8 and 10) were stimulated (Fig. 1a). Latencies were typically less than 1 s and always less than 3 s, consistent with primarily Aδ nociceptor activation (Yeomans and Proudfit 1996). To stimulate a given location, the tube restraining the rat was rotated on its long axis until the desired stimulus location was positioned in front of the laser beam (Fig. 1c). Correct rotation was visually confirmed by alignment of the targeting laser to the red marks denoting dorsal, ventral, left and right. The peak temperature at radially adjacent stimulation sites never exceeded 32° (Cleland and Bauer 2002), verifying that the stimulus primarily excited nearby thermoreceptors.

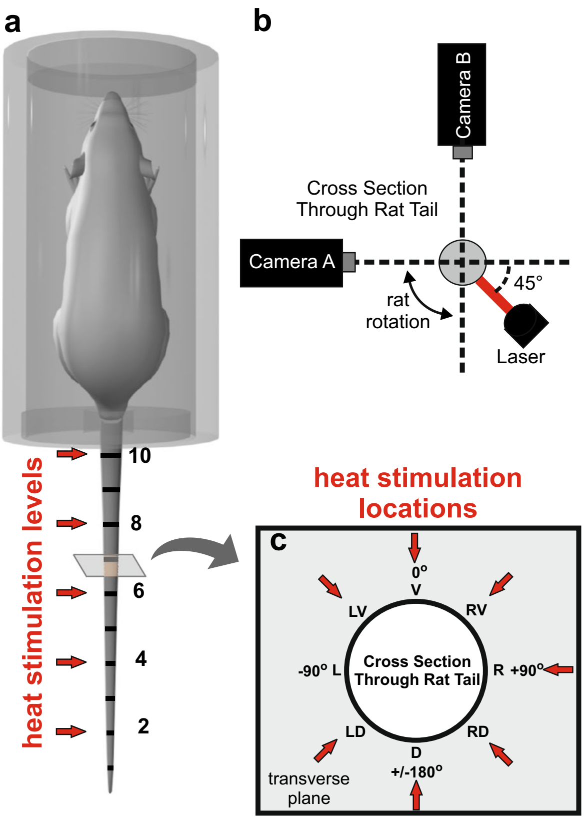

Fig. 1 Methods. **a** The spinalized rat's tail was marked with ten equally spaced black bands along its length for tracking and laser targeting (only band numbers 2, 4, 6, 8, 10 are labeled). The rat was then suspended vertically in an acrylic tube with its tail hanging down freely. The five stimulus levels are shown by arrows. **b** Two high-speed video cameras (A and B) were positioned 90° with respect to each other 50 cm from the tail. The laser diode with condenser lens was positioned 45° with respect to each camera axis. The laser was focused just above the black bands marked on the tail. Changes in stimulus location were accomplished by rotating the tube containing the rat and changes in stimulus level were made by sliding the tube up and down within a tube clamp. **c** The rat was rotated so that stimuli fell on each of eight circumferential locations [dorsal (D), ventral (V), left (L), right (R), left-dorsal (LD), left-ventral (LV), right-dorsal (RD), and right-ventral (RV)] within the transverse plane, indicated by arrows. The polar coordinate system was aligned with 0° corresponding to ventral and clockwise increasing angle to + 180° and counterclockwise decreasing angle to − 180°

Movement was recorded by two high-speed video cameras (Fig. 1b; 250 fps, 1/1000 s shutter speed, 6 mm f/1.2 lens, 480 × 420 pixels, Motionscope, IDT, CA, USA). To achieve maximal visual contrast between the black marked bands and the background, white foam boards were placed behind the tail and low heat compact fluorescent lights (730 W) illuminated the tail and background. Video data were saved as series of .jpg files over 400 ms (100 frames) before and 4 s (1000 frames) after the initiation of a response. Three-dimensional calibration utilized a custom-built bi-planar fixture constructed from Legos™.

Although non-nociceptive mechanoreceptors can evoke flexion reflexes (Le Bars et al. 2001), withdrawal responses were highly likely to have arisen from nociceptive heat rather than non-nociceptor "warm" receptor activation because the tail withdrawal response in spinalized rats (Cleland et al. 1994), 46°, exceeds threshold for cutaneous Aδ heat nociceptors, 43.8° (Yeomans and Proudfit 1996). Further, warm receptors in the rat reach maximum firing frequency at 43° and decrease with higher temperatures, dropping to zero at > 47° (Hellon et al. 1975).

One of the two stimulation protocols was employed for each experiment. The first stimulation protocol ($n = 9$ rats) consisted of 0.5 s pulse stimuli applied at 2 × threshold to each of the eight circumferential stimulus locations just above band 8 on the tail (stimuli were not applied directly to the black ink). Threshold intensities were determined individually for each stimulus location by progressively increasing intensity from 2.0 W (which never evoked a response) in increments of 0.4 W; threshold was taken as the minimum intensity that evoked a response. The experimental protocol consisted of 40 trials in which stimuli were applied to each of the 8 circumferential locations every 2 min in the same random order for 5 repetitions. At least 16 min were thus provided between stimulation of a single point to minimize habituation (Carstens and Wilson 1993). The mean response latency was 0.69 s (range 0.41–1.24 s).

The second stimulation protocol ($n = 9$ rats) consisted of continuous, lower intensity stimuli that were applied until the rat withdrew its tail. Stimuli were applied randomly to 40 locations that varied both circumferentially (8 locations as above) around the tail and rostral-caudally along the length of the tail (5 levels). The mean latency, 1.07 s (range 0.56–2.98 s), did not depend (two way ANOVA, insignificant interaction $F(16, 360) = 0.99$, $p = 0.47$) on stimulus location ($F(8, 359) = 1.38$, $p = 0.24$) or level ($F(4, 359) = 0.89$, $p = 0.47$).

Analysis

Digitized video were aligned in time to the onset of response at the stimulated level and all 10 bands from both cameras were tracked automatically in software (ProAnalyst, Xcitex, Cambridge, MA, USA) to yield the three-dimensional trajectory of each band in rat coordinates. Data were then exported to Matlab (Mathworks, Natick, MA, USA) where the units were scaled and the origin, based on the frame immediately preceding movement, was set to the site of stimulation (protocol 1, level 8) or level 10 (protocol 2).

Movement vectors in polar coordinates in the transverse plane (Fig. 2c) for each trial were measured from the origin to a second point of the movement that was manually identified based on a slowing or stopping and subsequent abrupt change in direction (see Fig. 2c). To account for differences

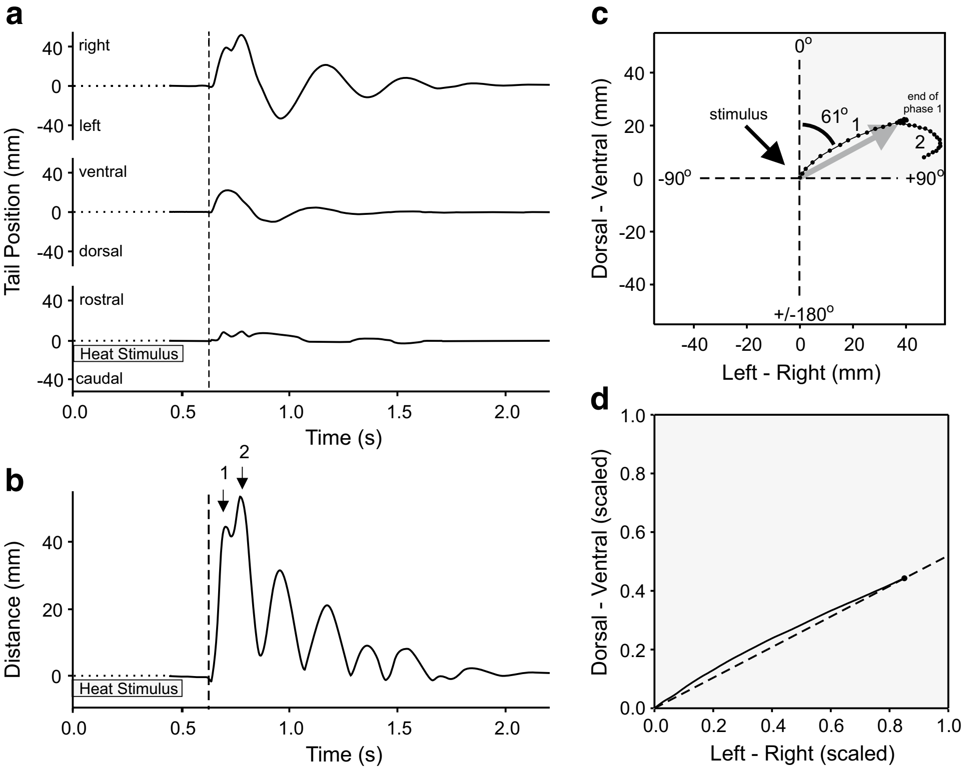

Fig. 2 Single trial and average movement responses to heat stimuli. **a** Representative example of movement for a typical response along the left–right, dorsal–ventral, and rostral–caudal axes in response to a 0.5 s heat stimulus applied to the left-ventral aspect of the rat's tail at level 8. Latency of movement response for this trial was 0.62 s after stimulus onset (dashed line). This typical response displayed initial tail movement primarily in the right-ventral direction (top, middle panels), though there was a small movement along the rostral–caudal axis (bottom panel). **b** Total distance traveled in the transverse plane for the same representative trial shown in **a**. The first phase of the response peaked ~0.07 s after movement initiation with a magnitude of 44.5 mm (indicated by the arrow numbered "1"). The second phase of the response peaked ~0.06 s later, with a magnitude of 53.3 mm (indicated by the arrow numbered "2"). The tail then continued to oscillate around the origin for another ~0.8 s. **c** Path of movement in the transverse plane for the same representative trial shown in **a** and **b**. Points correspond to video frames separated by 4 ms. The solid black arrow represents the location of stimulation (left-ventral/−45°). The response consisted of two phases. The first component ("1") followed a relatively straight and temporally symmetric (speed increased and decreased at similar rates) path that ended at the first peak (in distance, **b**) and slowed in speed (closely spaced points). The second component underwent a change in direction and an increase in speed ("2"). The end of the first component was manually identified based on speed and directional changes. Response direction was quantified by a vector from the origin to the point that the first component ended and the second began (indicated by the gray arrow). For this trial, the response direction was 61°. **d** Average response path in the transverse plane for all trials from 18 experiments (only the top-right quadrant from **c** is shown). To calculate average path, the absolute distance from response origin to the end of phase 1 was first scaled to 100% for each individual trial ($n=144$), and then the individual paths were resampled ($n=200$) so that the re-sampled points were distributed evenly along the path. The resulting average path of all individual responses was roughly linear (a perfect linear relationship is displayed by the dashed line), which justified the use of single response vectors for each trial to be used in subsequent analysis

between rats, distance was normalized by dividing by the average distance for each rat.

Central tendency and variability are expressed as mean ± SEM unless otherwise indicated. Statistical significance was taken at $p=0.05$. Small p values were capped at 0.0001. Parametric statistics were selected based on distribution conformity. Subject effects were accounted for using linear mixed effect models with random slopes. Circular statistics for direction were avoided by choosing a polar coordinate system that avoided discontinuities in direction (Zar 1984), which also permitted the use of more powerful mixed effect models. Additional analyses and graphs were conducted using Sigmaplot (Systat, San Jose, CA, USA) and custom programs in Matlab.

Results

Localized heat stimuli delivered to the skin of the rat's tail evoked a short latency (~ 1 s), brief (~ 150 ms), two-phase movement of the tail, which was then followed by diminishing oscillations (Fig. 2a, b). Movement trajectory in the transverse plane (Fig. 2c) was composed of two distinct components. The first component was relatively straight (Fig. 2c, d) and temporally symmetric, while the second component typically increased in speed and curved in either a clockwise or counterclockwise direction. The response was quantified by the vector ending at the end of the first phase of movement (gray arrow). Only the first, more consistent, response component was considered in this study.

Distribution of movement vectors

Individual (gray) and average (black) movement vectors in the transverse plane for each of eight stimulus locations at level 8 over all 18 experiments are displayed in Fig. 3a. Vectors directed toward the inside of the circle represent responses that moved away from the stimulus, while vectors directed outward represent responses that moved toward the stimulus. For dorsal and ventral stimuli, average response vectors were determined separately for responses that were directed left of the midline and those that were directed right of the midline. Responses appear to be grouped into two

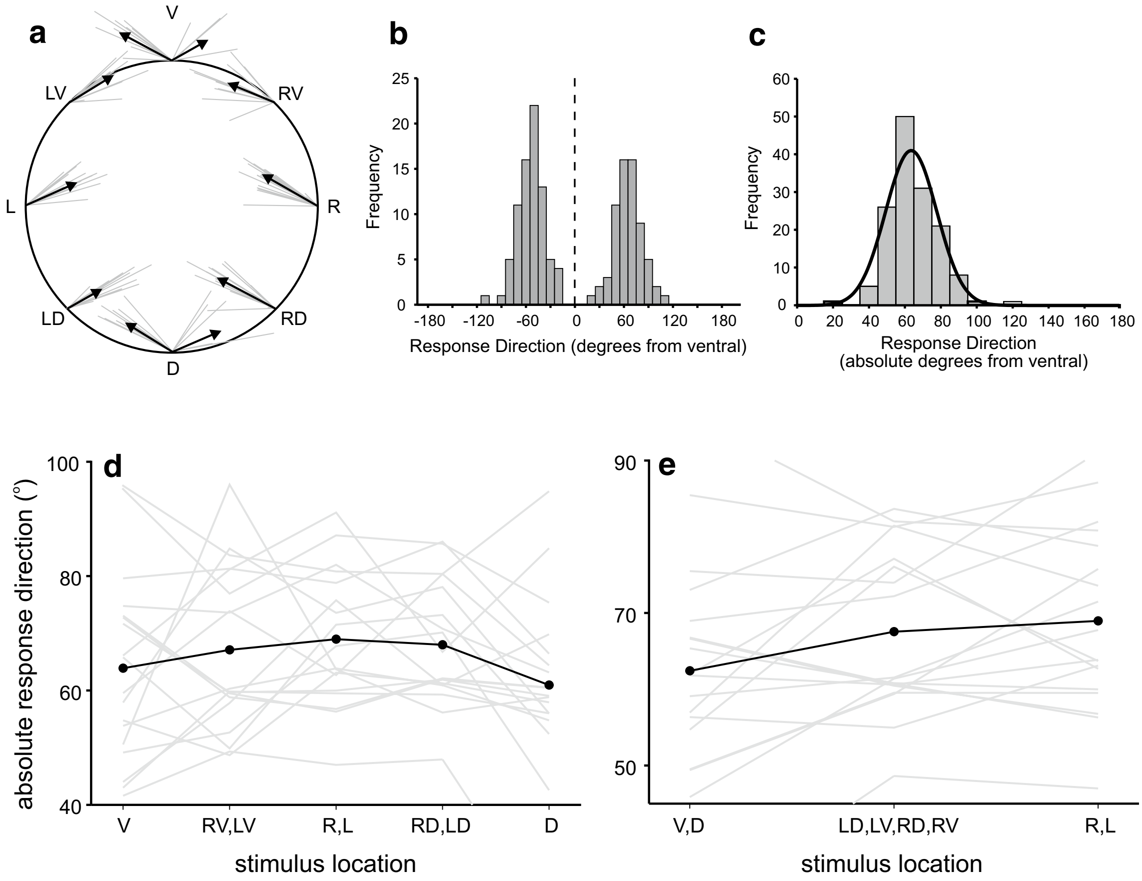

Fig. 3 Response directions. **a** The circle represents a cross-section through the rat tail, with stimuli applied into the circle along its edge from eight circumferential locations (D, V, L, R, RD, LD, RV, LV). Individual (gray lines) and average (black arrows) movement vectors (indicating response direction and magnitude) in the transverse plane for each of eight stimulus locations at level 8 over all experiments ($n = 18$ rats; five replicates from protocol 1 were averaged to yield one vector per rat). Separate averages were computed for left- and right-directed responses to pure ventral and dorsal stimuli. **b** Frequency of movement direction for all eight stimulus locations combined revealed that all responses were grouped in only two directions, $-64.4° \pm 14.3$ SD and $64.1° \pm 14.2$ SD from ventral [95% CI $\pm 7.5°$ (left) and 8.6° (right)]. Cluster analysis showed that the two clusters of movement directions differed significantly (k-means, $p < 0.0005$). **c** Reflecting the responses over the dorsal–ventral axis by calculating their absolute value yielded a single, normal (Shapiro–Wilk, $n = 144$, $p = 0.008$) distribution with a mean of $64.8° \pm 13.7$ SD, as indicated by the overlaid best-fit normal curve. **d**, **e** Dependence of movement direction on stimulus location. **d** Movement direction relative to ventral varied with reflected stimulus location (gray lines correspond to each of 18 rats, black line is the mean), with more lateral response directions suggested for right and left stimuli. **e** After response directions were expressed as their degree from purely lateral (by further reflecting directions around the left–right axis), response direction significantly (linear mixed model with random slopes, $n = 432$) increased with degree of laterality ($p = 0.0005$, slope 20.8 response degrees/360 stimulus degrees)

general directions, ventral-left and ventral-right, and the magnitudes are broadly similar throughout.

To further examine the apparent bimodal distribution of movement directions, movement directions for all eight stimulus locations combined are represented as frequency histograms (Fig. 3b). Because the mean directions of the two clusters (−64.4° ± 14.3 SD and 64.1° ± 14.2 SD) significantly differed (*k*-means, $p < 0.0005$), the negative vector directions and associated stimulus locations were reflected across the rostral–caudal axis to yield a single, normal (Shapiro–Wilk, $p = 0.008$) distribution (Fig. 2c; 64.2° ± 13.7° SD) of absolute movement directions.

Dependence on circumferential stimulus location

Although responses overall were shown to be tightly grouped around 64°, it remained possible that movement directions still depended, though perhaps slightly, on circumferential stimulus location. To test this possibility, we analyzed absolute movement direction as a function of reflected stimulus location (Fig. 3d). Although correlation (Pearson, $p > 0.05$) showed no significant dependence between movement direction and stimulus location, the responses shown in Fig. 3d seem to display a shallow curve in which movement directions due to left and right lateral stimuli are greater (more lateral) than movement directions due to stimuli at any other location. To further explore this potential relationship, we reflected response directions a second time around the left–right axis by combining stimulus locations based on their deviations from lateral (Fig. 3e). Deviation of stimulus location from lateral significantly decreased response direction (linear mixed effects, $p = 0.0005$), with more lateral stimuli (R, L) evoking greater (more lateral) responses. However, this dependence was slight, in that a 90° change in stimulus location (e.g., from V to R) corresponds to only a 5.2° change in movement direction. Movement distance did not show a significant dependence on stimulus location (linear mixed effects, $p > 0.05$).

Dependence on rostral–caudal stimulus level

Eight circumferential stimuli were also delivered at five different rostral–caudal levels (Fig. 1a). Figure 4a shows examples of three lateral stimuli delivered to levels 2, 6 and 10 in which the stimulus results in a local bend of the tail close to the point of stimulation. To determine the dependence of the level of local bend on rostral–caudal stimulus location, for each trial the black mark that first moved over 1 mm in the transverse plane was identified as the level of the local bend (Fig. 4b). The relation between stimulus level and local bend level (Fig. 4c) revealed a significant linear relation ($p < 0.00005$, Pearson correlation) and a close match between stimulus and local bend levels for distal stimuli (dashed line represents a perfect match). However, at more proximal stimulus locations the bend levels off near level 8, possibly due to the increased stiffness of the proximal tail.

Movement direction in the transverse plane varied with both stimulus level and circumferential location (Fig. 5). Examples of trajectories (Fig. 5a) reveal consistent ventral-lateral responses that progress laterally with more caudal stimuli. Frequency histograms of movement directions (Fig. 5b) and superimposed best-fit normal curves (Fig. 5b, c) similarly suggest an effect of stimulus level. Combining the effects of stimulus level and location (Fig. 5d) revealed significant (linear mixed effect) effects of both stimulus level ($p < 0.0001$) and, as shown previously for only level 8 stimulation, location ($p = 0.01$; 9.2° change in response direction per 90° change in stimulus location).

Discussion

The specific aim of our research was to use three-dimensional high-speed video to determine whether movement of the tail in response to noxious heat stimuli delivered at locations that varied both circumferentially and rostral-caudally on the tail depended on the location of the stimulus in spinalized rats. In particular, we sought to determine whether the movement strategy was *categorical* or *continuous* (Lewis and Kristan 1998; Cleland and Bauer 2002). Our results demonstrate that at all rostral–caudal levels, response direction was bimodal regardless of circumferential stimulus location—either ~ 64° left or right of ventral. However, in spite of tight clustering, movement direction varied significantly but weakly according to circumferential location, in that responses to stimuli were more lateral for lateral stimulus locations. In contrast, changes in stimulus level resulted in a localized bend that closely matched the level of the stimulus. Together, our results demonstrate, based on movement analysis in spinalized rats, that the NWR employs a hybrid *categorical–continuous strategy* that may minimize the harmful consequences of noxious stimuli.

Evidence for a continuous movement strategy

Previous studies of escape and withdrawal responses in diverse animals have revealed two directional mapping strategies—*categorical* and *continuous* (Lewis and Kristan 1998; Cleland and Bauer 2002; Cleland et al. 2017). Continuous mapping arises when variation in stimulus location results in a variation in response direction. Categorical mapping corresponds to response directions that cluster in a limited number of directions. For example, leeches employ a continuous strategy in their local bend (Lewis and Kristan 1998), while crayfish use a categorical tail-flip strategy for strong stimuli (Cattaert and Le Ray 2001).

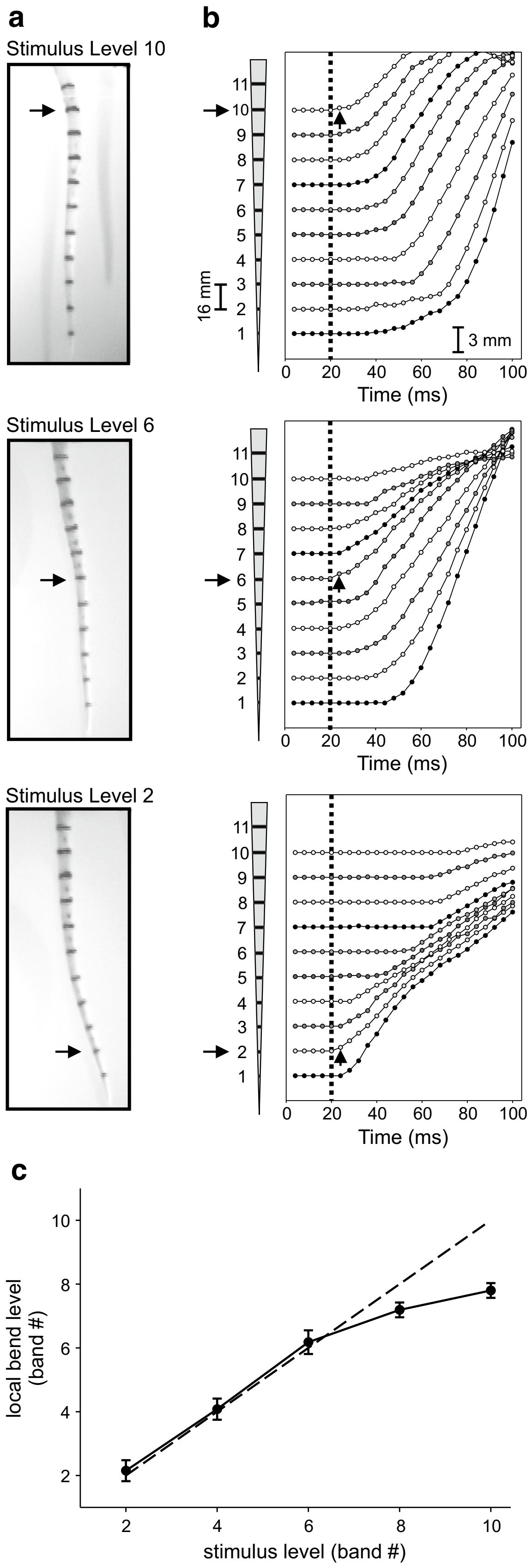

Fig. 4 Level of movement initiation depended on rostral–caudal ▸ stimulus level. **a** Video frames of representative responses shortly after movement onset to stimuli applied at varying rostral–caudal tail levels. For each of the three stimulus levels shown (2, 6 and 10), the level of movement initiation is represented by a local bend in the tail at that location (indicated by the black arrows). **b** Representative initial response trajectories of each marked band on the tail for the same three representative trials shown in **a**. Rostral–caudal stimulus level is indicated on the tail diagram by a black horizontal arrow, and each line of data points within the associated panel represents the movement trajectory of the corresponding tail band in response to that stimulus. Response trajectories of bands 1–10 are shown for each trial, and movement is measured as total distance traveled away from resting tail position within the transverse plane. The vertical arrow within a panel represents the initiation (first point) of movement away from resting tail position (indicated by the dashed vertical line). Level of response initiation was determined by setting a threshold distance in the transverse plane from the origin at 1.0 mm; the first tail band to cross this threshold during a response was considered the response initiation level. **c** Response initiation level increased significantly (Pearson correlation, $n = 9$ rats, $n = 360$ trials, $p < 0.0001$) with, and closely matched, stimulus level, except at the most rostral levels. The dashed line represents a perfect linear relationship between response initiation level and stimulus level

In intact, unanesthetized animals, the direction of the lower limb (Grimby 1963; Hagbarth and Finer 1963; Andersen et al. 1999; Levinsson et al. 1999), upper limb (Kofler 2003; Peterson et al. 2014) or tail (Cleland and Bauer 2002) NWR depends on noxious stimulus location. In unanesthetized spinalized animals, however, stimulus dependence can be present (Hagbarth 1952; Schouenborg et al. 1992; Thelin and Schouenborg 2008), but is often weak (Sherrington 1910), abnormal (Levinsson et al. 1999), less pronounced than in intact animals (Grimby 1965) or absent (Schmit et al. 2003), suggesting that the neural basis for stimulus location dependence requires intact descending supraspinal pathways (Andersen et al. 2003; Schmit et al. 2003). Our results, in which response direction significantly depended—strongly for rostral–caudal variation in stimulation level and weakly for circumferential stimulus locations—support the existence of a continuous stimulus–response mapping strategy at the spinal level, although it remains possible that supraspinal modulation or long loop reflexes further refine mapping. It is also possible that the variation in response direction in both circumferential and rostral–caudal axes arose at least in part from variations in stiffness and inertia rather than neural organization.

Evidence for a categorical movement strategy

In intact, unanesthetized animals, response directions can tightly cluster in spite of large variations in stimulus location. In the rat tail, a 360° circumferential change in stimulus location resulted in a change in movement direction of only about 80° (Cleland and Bauer 2002). In the human arm,

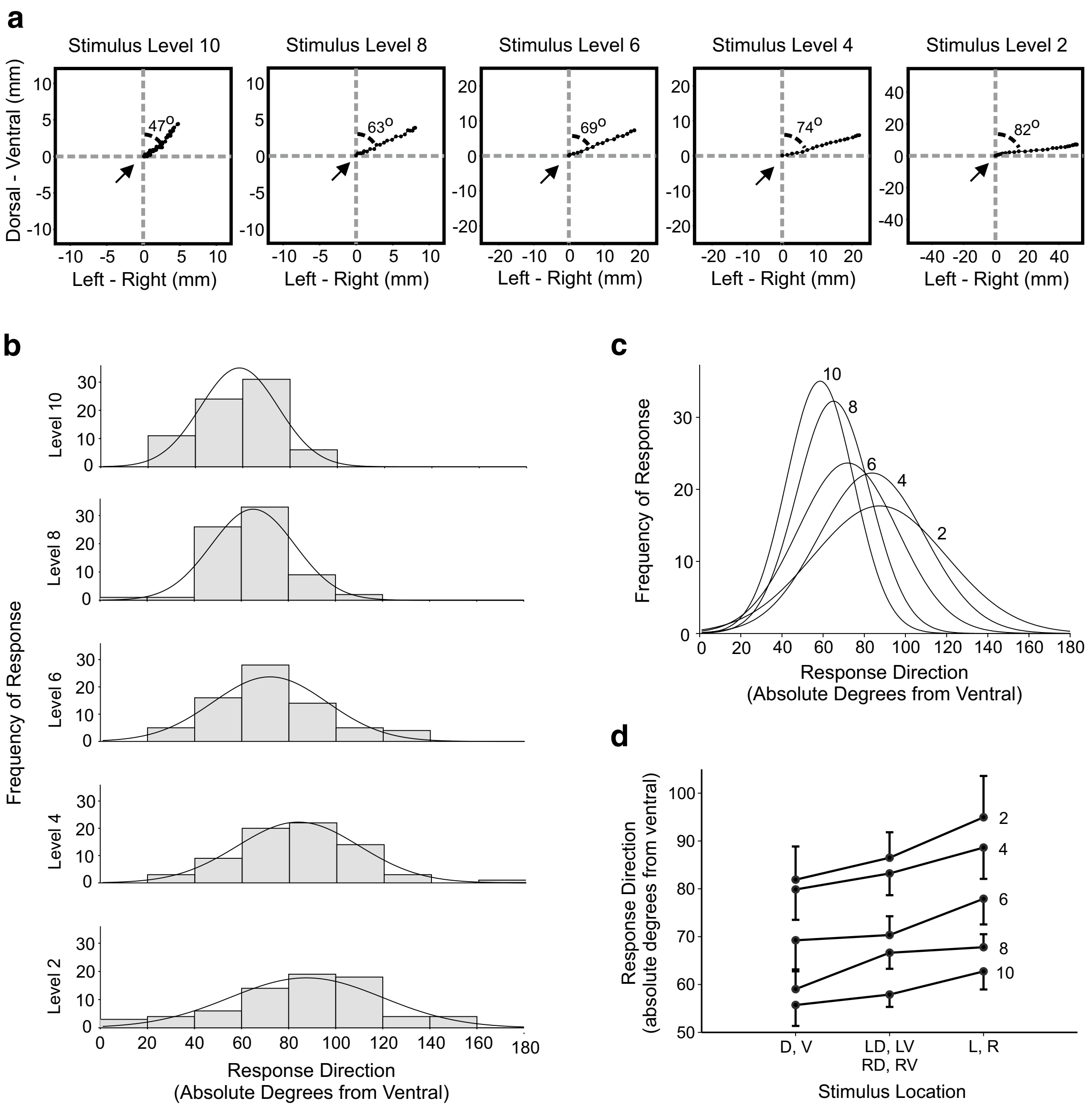

Fig. 5 Dependence of response direction on stimulus level and location. **a** Movement in the transverse plane for five sample trials in which stimuli were applied to different rostral–caudal levels on the tail (level 10 was most rostral, 2 was most caudal). Stimuli were applied to the left-dorsal aspect of the tail for each trial, and all responses were directed into the ventral-right quadrant. As stimuli moved to caudal levels, the movement became more lateral. **b** Frequency of response direction histograms for each of five rostral–caudal tail levels (10, 8, 6, 4 and 2) across all nine rats. Normal curves have been fitted to illustrate the mean and range of movement directions at each level. As stimuli moved caudally, the mean direction of movement shifted right (lateral) and the variability increased. **c** Normal curves fitted to the frequency of movement direction versus stimulus level data (from **b**) have been superimposed to illustrate the increasing laterality and range of movement directions as stimuli shift caudally along the tail. Stimulus levels are indicated by the numbers corresponding to each curve. **d** Response direction depended significantly (linear mixed model with random slopes, $n = 360$) on both stimulus level ($n = 9$ rats, $n = 360$ trials, $p < 0.0001$, -15.2 response degrees/tail length from levels 2 to 10) and location ($p = 0.01$, slope 36.8 response degrees/360 stimulus degrees), with response direction becoming more lateral with both more caudal and more lateral stimuli

varying the stimulus across all five digits resulted in only a 23° change in force direction.

Similarly, in unanesthetized spinalized animals, Sherrington (1910) observed that, in spite of the presence of local sign (Creed and Sherrington 1926), the mammalian flexor reflex was highly stereotyped. More recently, a 360° circumferential change in stimulus location around the tail (Cleland and Bauer 2002) and the lower leg (Cleland et al. 2017) resulted in a change in movement direction of only about 100° and 91°, respectively. Our results for variation in circumferential stimulus location around the tail strongly support a categorical contribution because a 360°

circumferential change in stimulus location around the tail resulted in a change in direction of only 21°–37°.

Evidence for a hybrid categorical–continuous movement strategy

Taken together, previous mammalian studies have provided conflicting support for categorical and continuous strategies. Our previous and current results suggest a hybrid categorical–continuous strategy. For heat stimuli delivered to the foot and lower leg in the rat (Cleland and Bauer 2002), response directions were clumped in only two directions (rostral–medial–dorsal and caudal–medial–dorsal) but also depended, albeit weakly, on stimulus location. Similarly in the tail (Cleland et al. 2017), for both intact and spinalized rats, responses were clustered in only two directions (left-ventral and right-ventral) but still could depend weakly on stimulus location. Our results from the present study—for circumferential stimulus locations—paralleled those from our earlier study in that response directions for stimuli at level 8 were clustered in two directions, ~ 64° lateral to ventral. Further, at the four other levels response direction was similarly clustered at directions between 55° and 85°. Together, these findings reflected a categorical component. Yet, supporting the continuous component, our results for varying rostral–caudal levels strongly differed; there was a tight correlation between stimulus level and the level of the tail at which the movement was initiated.

Biomechanical and neural mechanisms

Heat stimuli delivered to the tail evoked a local bend response whose location closely matched the level of the stimulus. Although there are multiple possible patterns of extrinsic and intrinsic tail muscle activity that could have accomplished the observed local bend, one intriguing possibility is that the bend arose from contraction of only the few unilateral muscle fascicles whose tendons insert at the same level as the local bend. Possibilities include the sacrocaudalis dorsalis lateralis and sacrocaudalis ventralis lateralis muscles, which insert into all 28 coccygeal levels of tail vertebrae (Hori et al. 2011).

For stimuli delivered to circumferentially varying locations, it was striking that response directions were tightly clustered around ~ 64° lateral to ventral and that the distribution never included purely ventral. There are two possible explanations. First, the movement was biomechanically constrained. Second, there was a neural preference, possibly linked to the organization of spinal muscle synergies. Biomechanical constraints are unlikely since movements in all directions have been reported (Cleland and Bauer 2002) and the geometry of the extrinsic muscles should permit movement in all radial directions (Brink and Pfaff 1980; Hori et al. 2011), although variations in stiffness and inertia could alter the magnitude of movement. Since there are no purely ventral muscles (all have a lateral component), the lack of pure ventral movement could arise if muscle synergies were strictly lateralized (that is, never including muscles that act on both the left and right sides of the tail).

Functional organization of the NWR

A hybrid categorical–continuous movement strategy may allow the effective removal and protection of the affected body part across diverse conditions. The continuous component of the response, which is tightly coupled to stimulus location, may be sufficient, even if weak, to move the body part out of harm’s way. For example, even limited dorsiflexion around the ankle might free the foot from an offending thorn. However, for noxious stimuli with a greater “reach”, such as stinging insect, a large movement, which could be in any direction, might be needed. The categorical component of the NWR, especially at the hip where angular changes produce especially large translational movement, would be beneficial.

The categorical component of the NWR could also simplify the neural control of movement by reducing the number of kinematic and potentially muscular degrees of freedom. In the hyper-redundant (Chirikjian and Burdick 1994) rat tail, there are 28 inter-vertebral joints that move in three axes resulting in 84 joint degrees of freedom (Hori et al. 2011; ignoring the tissue coupling between tail segments that would reduce the effective number of joint degrees of freedom). Further, there are 168 inter-vertebral intrinsic muscles and > 100 extrinsic muscles or fascicles that control the tail through individual long tendons (Brink and Pfaff 1980). Consequently, a categorical strategy that reduces the number of degrees of freedom could decrease the computational burden resulting in potentially shorter response latencies (Cattaert and Le Ray 2001), which might be more functionally important than movement accuracy for protection of the body part from noxious stimuli. Alternatively, the observed reduction in degrees of freedom in tail movement could reflect task requirements (e.g., in a sitting rat the tail cannot typically move pure ventrally) or tail biomechanics (tail stiffness and inertia).

Rat tail NWR as a model system for studying the spatial organization of NWR

Although the rat tail contributes to heat exchange (Gordon 1990) and reproduction (Brink and Pfaff 1980), it is also engaged during voluntary and reflex movement (Hori et al. 2011). While the reflex tail “flick” response is widely studied in pain research (Le Bars et al. 2001) and as a model of spasticity (Bennett et al. 1999), few studies have addressed the underlying organization of motor control. Due to the large numbers of redundant degrees of freedom, experimental accessibility, and ubiquity across diverse animals, the tail may be an ideal mammalian model system for studying neural and biomechanical solutions to the computational complexities arising from multi-segment movement planning and execution.

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