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Selective impairment of slowly adapting type 1 mechanoreceptors in mice following vincristine treatment

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Abstract

Loss of the sense of touch in fingertips and toes is one of the earliest sensory dysfunctions in patients receiving chemotherapy with anti-cancer drugs such as vincristine. However, mechanisms underlying this chemotherapy-induced sensory dysfunction is incompletely understood. Whisker hair follicles are tactile organs in non-primate mammals which are functionally equivalent to human fingertips. Here we used mouse whisker hair follicles as a model system and applied the pressure-clamped single-fiber recording technique to explore how vincristine treatment affect mechanoreceptors in whisker hair follicles. We showed that *in vivo* treatment of mice with vincristine impaired whisker tactile behavioral responses. The pressure-clamped single-fiber recordings made from whisker hair follicle afferent nerves showed that mechanical stimulations evoked three types of mechanical responses, rapidly adapting response (RA), slowly adapting type 1 response (SA1) and slowly adapting type 2 response (SA2). Vincristine treatment significantly reduced SA1 responses but did not significantly affect RA and SA2 responses. Our findings suggest that SA1 mechanoreceptors were selectively impaired by vincristine leading to the impairment of *in vivo* whisker tactile behavioral responses.

Keywords

Touch; numbness; chemotherapy; vincristine; mechanoreceptors; Merkel discs

Introduction

Mechanoreceptors in the skin and hair follicles of mammals enable tactile responses, which are important for environmental explorations, social interactions, and tactile discrimination

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Credit Author Statement

Author Contributions: MS designed and performed the experiments and then analyzed the data. HY and SK participated data interpretation and discussion. JGG conceived the study and wrote the manuscript. All authors read and approved the final manuscript.

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(1). Mechanoreceptors can be classified into at least three main functional types, rapidly adapting (RA), slowly adapting type 1 (SA1), and slowly adapting type 2 (SA2) mechanoreceptors (1, 2). Anatomically, mechanoreceptors are specialized structures in the skin and hair follicles (3). For examples, in whisker hair follicle, SA1 mechanoreceptors are Merkel discs in structure, RA type of mechanoreceptors are mainly lanceolate endings, and SA2 mechanoreceptors are mainly reticular endings (4). Mechanoreceptors are highly abundant in human fingertips to allow performing sophisticated tactile tasks (1). In other mammals, whisker hair follicles are functionally equivalent to human fingertips. The three functional types of mechanoreceptors have been detected in mouse whisker hair follicles using electrophysiological recordings including our recent studies with the pressure-clamped single-fiber recordings (2). Recent studies by us and others have demonstrated that SA1 mechanoreceptors or Merkel discs use Piezo2 channels to transduce tactile stimuli and are essential for tactile behaviors in mammals (5–7). More recently, we have shown that Merkel discs in whisker hair follicles are serotonergic synapses that use serotonin to transmit tactile signals (8).

Tactile responses mediated by mechanoreceptors in the skin and hair follicles may become impaired in patients receiving chemotherapy with drugs such as vincristine (9, 10). Vincristine and its analogs are important chemotherapy drugs but their uses in cancer patients are commonly associated with side effects including numbness, tingling sensation, and pain (9, 10). These sensory abnormalities could be experienced soon after receiving the chemotherapies and persist for months to years after the completion of the chemotherapies (9). It adversely affects the quality of life of patients and is an important clinical problem. The sensory abnormalities are dose-limiting factors to prevent patients from continuing the effective treatment regimens with these chemotherapy drugs. Chemotherapy-induced sensory abnormalities are typically present as a “stocking and glove” distribution in toes and fingertips of patients (11). How tactile sensitivity becomes impaired by chemotherapy drugs such as vincristine has not been fully understood. To address this question, we have recently used whisker hair follicles as a model system and shown that vincristine treatment significantly reduced mechanically evoked impulses in whisker afferent nerves (12). However, it remains unknown as which functional type(s) of the mechanoreceptors were impaired in whisker hair follicles following vincristine treatments.

Materials and Methods

Animals:

C57BL/6 mice were obtained from Harlan Laboratories. Mice were used at the age of 8–10 weeks for *in vivo* tactile behavioral assessments and *in vitro* recordings from whisker hair follicle afferent nerves. Animal care and use conformed to NIH guidelines for the care and use of experimental animals. Experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Alabama at Birmingham.

In vivo vincristine treatment and tactile behavioral assessment:

Vehicle (sterile phosphate-buffered saline) or vincristine (Cayman Chemical, Ann Arbor, Michigan) dissolved in vehicle was *i.p.* administered daily for 5 consecutive days, then 2 day

breaks, and daily for 5 consecutive days again. The dose of vincristine was at 0.3 mg/kg each injection. Unless otherwise indicated, whisker tactile behavioral tests were performed once every 2 or 3 days for up to 21 days. The whisker tactile test was performed in a blinded manner in that one examiner conducted vincristine injections and animal grouping, and another examiner who did not know the grouping performed *in vivo* whisker tactile behavioral tests. To begin the tests, mice were placed in a cage and habituated for 10 min. During habituation and subsequent experiments, the testing room only had a red light on so that animals could not see the examiner and the tactile stimulation filament. After the habituation, right whisker hairs of animals were displaced by the tactile stimulation filament in a caudal to rostral direction. The whisker tactile test was repeated 20 times with an interval of 1 min between trials. A positive whisker tactile behavioral response was considered when the testing animal exhibited an avoidance reaction to the tactile stimulation.

Whisker hair follicle afferent fiber recordings.

Whisker hair follicles were harvested from animals 17 to 31 days following the injections of vehicle or vincristine. Whisker hair follicle preparations were then made and whisker afferent recordings were performed using our previously described method (2). In brief, mice were anesthetized with isoflurane and sacrificed by decapitation. Whisker hair follicles with attached afferent fiber bundles were dissected out and anchored in a recording chamber. The whisker hair follicles were submerged and perfused in the oxygenated Krebs solution that contained (in mM): 117 NaCl, 3.5 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 1.2 NaH₂PO₄, 25 NaHCO₃, and 11 glucose, bubbled with 95% O₂ and 5% CO₂, adjusted pH to 7.3 and osmolarity to 325 mOsm, and was maintained at 24°C. The end of each follicle capsule was cut open to facilitate bath diffusion into the whisker hair follicle. In one set of experiments, compound action potentials evoked by mechanical stimulation and conducted on whisker afferent fibers were recorded using a suction electrode. In most of experiments, action potentials evoked by mechanical stimulation and conducted on a single whisker afferent fiber was recorded using the pressure-clamped single-fiber recording technique that we developed recently (2, 13, 14). In brief, under a 40x objective, individual fibers in the cutting end of the whisker afferent nerve bundle were separated by a positive pressure of approximately +10 mmHg delivered from the recording electrode. The end part of a single nerve fiber was then aspirated into the recording electrode by a negative pressure at approximately -10 mmHg. Once the nerve end reached approximately 10 µm in length within the recording electrode, the pressure in the recording electrode was readjusted to -5 to -1 mmHg and maintained throughout the experiment. In a different set of experiments, individual whisker hair follicles were obtained from normal animals not treated *in vivo* with vincristine. The hair follicles were then *in vitro* incubated with 2 µM vincristine (vincristine-treated group) or vehicle (control) for 2 hours, and the pressure-clamped single-fiber recordings were performed from whisker afferent fibers. In all electrophysiological recordings, nerve impulses were recorded using a Multiclamp 700A amplifier and signals sampled at 20 KHz with low pass filter set at 1 KHz. All recording experiments were performed at 24°C.

Mechanical Stimulation.

Mechanical stimulation was applied to the body of each whisker hair follicle using a blunted 20-gauge needle as a probe. The needle was mounted on a holder and attached to a piezo device. The tip of the needle was positioned at an angle of 45 degrees to the surface of the whisker hair follicle. The piezo device with the mechanical probe was mounted on a Sutter MPC-200 micromanipulator. The piezo device was computer-programmable with the pCLAMP10 software to deliver forward stepwise mechanical stimulation. In each experiment, a receptive field was first probed manually with the mechanical probe controlled by the micromanipulator. Once identified, the vertical position of the probe tip was adjusted such that no nerve impulses were evoked at this position but a 1- μ m forward movement of the probe would evoke nerve impulses. Unless otherwise indicated, the stepwise forward movement of the probe consisted of a 100-ms ramp to 38- μ m step (dynamic phase) followed by a 2500-ms holding position at the 38- μ m step (static phase) and then a 100-ms ramp back to baseline.

Data Analysis:

Electrophysiological data were analyzed using Clampfit software. Data are presented as mean $\pm$ SEM. Statistical significance was evaluated using two-way ANOVA with Bonferroni post hoc test or Student's t-test, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Results

The body weights were not significantly different between the saline-injected group and the *in vivo* vincristine-treated group (Figure 1A). Tactile behavioral assessments were performed once every two or three days in both groups (Figure 1B&C). As shown in figure 1C, the vehicle-injected group showed high response rates to tactile stimuli ($n = 6$) during the course of ~3 weeks of whisker tactile behavioral tests. The vincristine-treated group ($n = 6$) also displayed high response rates during the initial 12 days of the tests, which was not significantly different from those of the vehicle-injected group (Figure 1C). However, a significant reduction in the response rates were observed 15 days following the start of the vincristine treatment regimen. The significant reduction of whisker tactile behavioral responses lasted for approximately 9 days after the end of the vincristine treatment regimen, and animals did not undergo further whisker tactile behavioral tests after day 21 (Figure 1C).

We determined if mechanically evoked whisker afferent impulses were impaired in vincristine-treated group. In this set of experiments, whisker hair follicles were harvested from the vehicle-injected and the vincristine-treated animals. Impulses on whisker afferent bundles were evoked by a mechanical stimulation probe with a 38- μ m displacement and recorded using a suction electrode (Figure 1D, nerve-bundle recording). As shown in Figure 1D&E, robust whisker afferent impulses were elicited by the mechanical stimulation in recordings made from whisker hair follicles of vehicle-injected group ($n = 6$ mice). In contrast, there were much fewer impulses elicited in recordings made from whisker hair follicles of the vincristine-treated group (Figure 1D&E, $n = 6$ mice). In the dynamic phase, impulses induced by the mechanical stimulation were 39.4 ± 0.8 ($n = 6$) in the saline-injected group and the impulse numbers were reduced to 35.0 ± 1.2 ($n = 6$, $p < 0.05$) in the

vincristine-treated group. In the static phase, impulses induced by mechanical stimulation were 755.7 ± 15.0 ($n = 6$) in the saline-injected group and reduced to 448.5 ± 25.4 ($n = 6$, $p < 0.05$) in the vincristine-treated group.

Three functional types of mechanoreceptors, including the rapidly adapting (RA), the slow adapting type 1 (SA1), and the slowly type 2 (SA2) mechanoreceptors, are present in whisker hair follicles (13, 14). We determined which type(s) of mechanoreceptors was/were affected following the *in vivo* vincristine treatment. In this set of experiments, whisker hair follicles were harvested from the saline-injected animals and the vincristine-treated animals, and mechanically evoked impulses were recorded using the pressure-clamped single-fiber recording technique (Figure 2A) (2, 13, 14). As shown in figure 2B–D, robust SA1 impulses were recorded from the afferent nerves of whisker hair follicles harvested from the saline-injected group (control, $n = 9$). In contrast, fewer SA1 impulses were elicited by the mechanical stimulation in the recordings made from afferent nerves of whisker hair follicles that were harvested from vincristine-treated group (Figure 2B–D, $n = 8$). The SA1 impulse numbers during the static phase were shown to be significantly reduced by the *in vivo* vincristine treatment. For example, at the displacement of $38 \mu\text{m}$, the impulse numbers in the course of 2.5 sec static phase were 71 ± 11.6 ($n = 9$) in the saline-injected group and significantly reduced to 35.3 ± 4.4 ($n = 8$, $p < 0.05$, Figure 2C) in the vincristine-treated group. We compared the areas under the curves (Figure 2D), which consistently showed that the *in vivo* vincristine treatment ($n = 9$, $p < 0.01$) resulted in a significant decrease of SA1 impulse numbers in the static phase in comparison with the saline-injected group ($n = 8$).

We next determined if RA responses were affected following the *in vivo* vincristine treatment. As shown in Figure 3A–C, mechanically evoked RA impulses were recorded from whisker hair follicles of both the saline-injected group ($n = 12$) and the vincristine-treated group ($n = 12$). RA impulses did not show a statistically significant decrease in impulse numbers following vincristine treatment ($p = 0.15$, Figure 3B&C, $n = 12$). We determined if SA2 responses were affected following the *in vivo* vincristine treatment. As shown in Figure 3D–F, robust SA2 impulses were elicited by the mechanical stimulation in recordings made from whisker hair follicles of both the saline-injected group ($n = 5$) and the vincristine-treated group ($n = 6$). SA2 impulse numbers showed no significant difference between the saline-injected group ($n = 5$) and the vincristine-treated group ($n = 6$, Figure 3E&F).

We determined whether vincristine had direct effects on mechanoreceptors in isolated whisker hair follicles. In this set of experiments, whisker hair follicles isolated from normal animals were used, mechanically evoked impulses were examined following the incubation of the whisker hair follicles with saline (control) or with $2 \mu\text{M}$ vincristine for 2 hours. As shown in Figure 4A–C, robust SA1 impulses were elicited by the mechanical stimulation in the control group ($n = 10$), but SA1 impulse numbers were substantially lower in both dynamic phase and static phase in the vincristine-treated group ($n = 8$, Figure 4C). For example, at the displacement of $38 \mu\text{m}$, impulse numbers in the static phase were 65.5 ± 12.4 ($n = 10$) in the control group and significantly reduced to 28.1 ± 8.1 ($n = 8$, $p < 0.05$) in the vincristine-treated group (Figure 4B). We compared the areas under the curves of the impulse numbers at different displacements (Figure 4C), which showed that the *in vitro*

vincristine treatment for 2 hours resulted in a significant decrease of SA1 impulses in both dynamic ($n = 8$, $p < 0.05$) and static phases ($n = 8$, $p < 0.05$) in comparison with the control group ($n = 10$).

We determined if RA responses were affected following the *in vitro* vincristine treatment. As shown in Figure 5A&B, RA impulses were elicited by the mechanical stimulation in both the control group incubated in saline ($n = 9$) and the vincristine-treated group incubated with 2 μ m vincristine for 2 hours ($n = 12$). RA impulses did not show significant difference in impulse numbers between the control and the *in vitro* vincristine-treated groups (Figure 5B&C). We also determined if SA2 responses were affected following the *in vitro* vincristine treatment. As shown in Figure 5D–F, robust SA2 impulses were elicited by the mechanical stimulation in both control ($n = 5$) and vincristine-treated groups ($n = 11$). SA2 impulse numbers, in both the dynamic and the static phases, showed no significant difference between the control group and the *in vitro* vincristine-treated group (Figure 5D–F).

Discussion

In the present study we have shown that vincristine selectively impairs SA1 mechanoreceptors in whisker hair follicles of mice. This appears to be a cause of the reduction of tactile sensitivity in the whisker tactile behavioral responses in our animals following the *in vivo* vincristine treatment regimen. The selective impairment by vincristine of SA1 mechanoreceptors in whisker hair follicles may have clinical implications since the loss of tactile sensitivity is one of the earliest symptoms experienced by patients receiving vincristine treatment as a cancer chemotherapy.

The treatment of cancers with vincristine and its analogs in human patients often causes sensory abnormalities including numbness, tingling sensation, and pain in the body, with the fingertips and toes are more severely affected (11, 15). In the present study, we have used whisker hair follicles, which functionally resemble human fingertips (16–18), to show that both tactile behavioral responses and SA1 responses are impaired following the *in vivo* vincristine treatment regimen. Interestingly, the *in vivo* vincristine treatment regimen did not significantly affect RA and SA2 mechanoreceptor responses in whisker hair follicles of mice. Different from RA and SA2 mechanoreceptors, SA1 mechanoreceptors consist of Merkel cells and their associated afferent endings (19). Recent studies have shown that both Merkel cells and their associated afferent endings transduce tactile stimuli via Piezo2 channels (6, 7). We have previously shown that Piezo2-mediated currents in Merkel cells were reduced following the *in vivo* vincristine treatment regimen (12). This supports our present study that vincristine would impair SA1 mechanoreceptors because SA1 mechanoreceptors are primarily Merkel discs in whisker hair follicles. The lack of effects by vincristine on RA and SA2 mechanoreceptors may suggest that the molecular identities of RA and SA2 mechanoreceptors may be different from SA1 mechanoreceptors in whisker hair follicles. However, a previous study has provided anatomic evidence showing that Piezo2 channels are expressed at lanceolate endings, a major type of mechanoreceptors mediating RA responses (20). If this is the case, it may suggest that effects of vincristine on

mechanotransduction via Piezo2 channels may be cell type specific, and Merkel cells may be more vulnerable to the impairment by vincristine.

The *in vivo* vincristine treatment regimen may result in a reduction of Piezo2 channel expression to impair SA1 mechanoreceptors. However, we found that *in vitro* acute treatment of isolated whisker hair follicles with vincristine for 2 hours also impaired SA1 but had no effects on RA and SA2 mechanoreceptors. This acute effect of vincristine does not favor the idea that a change in the expression of Piezo2 channels is a main cause of the impairment by vincristine of SA1 mechanoreceptors. Alternatively, vincristine, a microtubule destabilizer, may disrupt cytoskeletons to change Merkel cell mechanics, which may subsequently affect mechanotransduction via Piezo2 channels in Merkel cells. Piezo2-mediated mechanotransduction has been shown previously to rely on actin filaments, another major type of cytoskeletons in cells that is essential for membrane mechanics (21). A further study will be needed to determine whether, similar to actin filaments, microtubules also are essential cytoskeletons for mechanotransduction in Merkel cells.

In our study, SA1 mechanoreceptors were impaired by acutely exposing dissociated whisker hair follicles to vincristine *in vitro*. On the other hand, whisker tactile behavioral responses were impaired days after the *in vivo* vincristine treatment regimen. The delayed impairment of the sensory behavioral responses could be due to slow accumulation of vincristine in whisker hair follicles during the *in vivo* vincristine treatment. In addition, SA1 mechanoreceptors or Merkel discs are located deep in hair follicles and shielded by a layer of glassy membranes, which may not favor the diffusion of vincristine into Merkel disc regions during the *in vivo* vincristine treatment. Alternatively, whisker tactile responses may be compensated by RA and SA2 when the impairment of SA1 mechanoreceptors by vincristine was not very severe in the early stage of the *in vivo* vincristine treatment. Although the *in vivo* vincristine treatment regimen used in the present study did not impair RA and SA2 mechanoreceptors, it will be interesting to examine whether a prolonged *in vivo* vincristine treatment may eventually impair these two mechanoreceptors as well. It will also be interesting to examine whether and how long the vincristine-induced impairment of mechanoreceptors can be recovered following the termination of a vincristine treatment regimen.

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Highlights:

- Vincristine treatment impaired tactile behavioral responses *in vivo*.
- It selectively impaired functions of SA1, but not RA and SA2 mechanoreceptors.
- The effect may underlie tactile dysfunctions in patients treated with vincristine.

Figure 1. Impairment of tactile behavioral responses following *in vivo* vincristine treatment.

(A) Body weights during and following a vincristine treatment regimen ($n = 6$) or saline injections (control, $n = 6$). Vincristine was *i.p.* administered at daily dose of 0.3 mg/kg. Arrows indicate vincristine administrations. (B) Diagram illustrates tactile behavioral assessments. (C) Tactile behavioral responses over time in animals of saline-injected group (control, $n = 6$) and vincristine-injected group ($n = 6$). The tactile behavioral responses were measured as the percent of avoidance reactions in responses to 20 times of tactile stimuli. (D) Left panel, schematic diagram shows experimental setting of the nerve-bundle recording of whisker afferent impulses. Right panel, sample traces show whisker afferent impulses evoked by a 38- μ m displacement stimulation in the saline-treated (control, top) and the vincristine-treated group (bottom). (E) Comparison of the impulse numbers in the dynamic (left panel, $n = 6$) and static phases ($n = 6$) during the whisker hair displacement in the saline-injected group and the vincristine-injected group. Three follicles from each animal were recorded and a total of 6 animals ($n = 6$) were used in each group. Data represent Mean $\pm$ SEM, * $p < 0.05$, *** $p < 0.001$, two-way ANOVA with Bonferroni post hoc test or Student's *t*-test.

Figure 2. Reduction of SA1 responses in whisker afferent nerves following *in vivo* treatment of mice with vincristine.

(A) Schematic diagram shows experimental setting of single-fiber recordings of mechanical responses from whisker afferent fibers. (B) Sample traces show SA1 responses recorded from a single whisker afferent nerve fiber in a whisker hair follicle of a saline-injected mouse (top) and a single whisker afferent nerve fiber in a whisker hair follicle of a vincristine-treated mouse (bottom). SA1 impulses were evoked by a 38- μm whisker hair follicle displacement. (C) Summary data show impulse numbers in the dynamic phase (left) and the static phase (right) in responses to the displacements at 2, 14, 26 and 38 μm . Solid circles, data of saline-injected group ($n = 9$), open triangles, data of vincristine-treated group ($n = 8$). (D) Bar graphs show the areas under the curve of the dynamic phase (left) and the static phase (right) for a comparison of SA1 responses between the saline group ($n = 9$) and the vincristine-treated group ($n = 8$). The area under the curves were constructed from data in C. Data represent the mean $\pm$ SEM. ** $p < 0.01$, Student's t -test.

Figure 3. Lack of significant effects on RA and SA2 responses by *in vivo* vincristine treatment. (A) Sample traces show RA impulses in response to a 38-μm displacement in a whisker hair follicle obtained from a saline-injected mouse (top panel) and a whisker hair follicle obtained from a vincristine-treated mouse (bottom panel). In each panel, inset indicated by an arrow shows individual RA impulses at an expanded time scale. (B) Summary data of total numbers of RA impulses in responses to mechanical displacement at 2, 14, 26, and 38 μm. Solid circles, saline-injected group (n = 12), open triangles, vincristine-treated group (n = 12). (C) A comparison of RA responses between the saline-injected group (n = 12) and the vincristine-treated group (n = 12) using the area under the curve shown in B. (D) Sample traces show SA2 impulses in response to a 38-μm displacement in a whisker hair follicle obtained from a saline-injected mouse (top) and a whisker hair follicle obtained from a vincristine-treated mouse (bottom). (E) Summary data of total numbers of SA2 impulses in the dynamic phase (left) and the static phase (right) in responses to mechanical displacements at 2, 14, 26, and 38 μm. Solid circles, saline-injected group (n = 5), open triangles, vincristine-treated group (n = 6). (F) A comparison of SA2 responses between the saline-injected group (n = 5) and the vincristine-treated group (n = 6) using the area under

the curve shown in E. Data represent Mean $\pm$ SEM, ns, not significantly different, Student's t-test.

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Figure 4. Reduction of SA1 responses in whisker afferent nerves following *in vitro* acute treatment of whisker hair follicles with vincristine.

(A) Sample traces show SA1 responses recorded from a single whisker afferent nerve of a whisker hair follicle incubated with saline for 2 hours (control, top) and a different whisker hair follicle incubated with 2 μM vincristine for 2 hours (bottom). SA1 impulses were evoked by a 38- μm whisker hair displacement. (B) Summary data show impulse numbers in the dynamic phase (left) and the static phase (right) of the mechanical stimulation with displacements at 2, 14, 26, and 38 μm . Solid circles, data from the control group ($n = 10$); open triangles, data from the vincristine-treated group ($n = 8$). (C) Bar graphs show the areas under the curve of the dynamic phase (left) and the static phase (right) for comparisons of SA1 responses between the control group ($n = 10$) and the vincristine-treated group ($n = 8$). Data represent the mean $\pm$ SEM. * $p < 0.05$, Student's t-test.

Figure 5. Lack of effects on RA and SA2 responses in whisker afferent nerves following *in vitro* acute treatment of whisker hair follicles with vincristine.

(A) Sample traces show RA impulses in responses to a 38-μm displacement in a whisker hair follicle incubated with saline for 2 hours (control, top panel) and a different hair follicle incubated with 2 μM vincristine for 2 hours (bottom panel). (B) Summary data of total numbers of RA impulses in responses to mechanical displacements at 2, 14, 26, and 38 μm. Solid circles, control group (n = 9); open triangles, vincristine-treated group (n = 12). (C) A comparison of RA responses in whisker hair follicles of the control group (n = 9) and the vincristine-treated group (n = 12) using the area under the curve shown in B. (D) Sample traces show SA2 impulses in response to a 38-μm displacement in a whisker hair follicle incubated with saline (top) and a different whisker hair follicle incubated with vincristine (bottom). (E) Summary data of total numbers of SA2 impulses in the dynamic phase (left) and the static phase (right) in responses to mechanical displacements at 2, 14, 26, and 38 μm. Solid circles, the control group (n = 5); open triangles, the vincristine-treated group (n = 11). (F) A comparison of SA2 responses between the control group (n = 5) and the

vincristine-treated group ($n = 11$) using the area under the curve shown in E. Data represent Mean $\pm$ SEM, ns, not significantly different, Student's t-test.

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