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Behavioral differentiation between itch and pain in mouse

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Abstract

The standard rodent model of itch uses scratching with the hind limb as a behavioral response to pruritic stimuli applied to the nape of the neck. The assumption is that scratching is an indicator of the sensation of itch. But because only one type of site-directed behavior is available, one cannot be certain that scratching is not a response to nociceptive or other qualities of sensations in addition to, or instead of, itch. To extend the model, we administered chemical stimuli to the cheek of the mouse and counted scratching with the hind limb as an indicator of itch and wiping with the forelimb as an indicator of pain. An intradermal injection of histamine and capsaicin, known to evoke predominantly itch and pain, respectively, in humans, each elicited hind limb scratching behavior when injected into the nape of the neck of the mouse. In contrast, when the same chemicals were injected into the cheek of the mouse, there were two site-directed behaviors: histamine again elicited scratching with the hind limb, but capsaicin evoked wiping with the forelimb. We conclude that the “cheek model of itch” in the mouse provides a behavioral differentiation of chemicals that elicit predominantly itch in humans from those that evoke nociceptive sensations. That is, the model provides a behavioral differentiation between itch and pain in the mouse.

Keywords

Histamine; Capsaicin; Pruritis; Nociception; Cheek

1. Introduction

The standard model of itch in the mouse has measured scratching in response to pruritic stimuli delivered to the nape of the neck [2,4,5,12]. For such a method to model itch in humans, it would be desirable to meet three criteria. First, the mouse should scratch to stimuli that evoke itch in humans. Second, it should not scratch to stimuli that elicit only pain or other kinds of non-pruritic sensations. Third, it would be desirable if the mouse emitted another type of site-directed behavior, besides scratching, to the non-pruritic stimuli as evidence for sensory detection. In this study we tested the second criterion in the standard model of itch and found that an intradermal injection of the algescic agent, capsaicin, into the nape of the neck does indeed elicit scratching. The third criterion cannot be met if the nape is used as the stimulus site simply because only one type of site-directed behavior is possible, namely scratching with the hind limb. Thus, it was uncertain as to whether scratching to capsaicin delivered to the nape indicated pain or itch, both or neither.

This study tested whether the Kuraishi model [5] could be modified in such a way as to allow discrimination between stimuli that humans describe as primarily painful or itchy. Facial wiping with the forelimb has been used in some studies as an indicator of pain in the mouse

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[e.g., 16,17]. It was therefore decided to use the cheek as the site of application of substances of interest. The substances were chosen based on the dominant qualities of sensation they evoke in humans, i.e., capsaicin (pain) and histamine (itch). It was discovered that in response to histamine, the mouse scratched the cheek with its hind limb; in response to capsaicin, the mouse wiped the cheek with its forelimb.

2. Methods

All protocols were approved by the Yale University Institutional Animal Care and Use Committee. These experiments conformed to the IASP ethical guidelines for animal research published in 1983 [18]. CD-1 mice (Charles River, Wilmington, MA), 69 males, were used, each weighing between 25 and 50 g. Mice were allowed one week after their arrival to the institution before experiments began. During brief anesthesia with isoflurane (2% in 100% oxygen), both cheeks (approx. 5×8 mm area), and the nape of the neck (approx. 1 cm circle) were shaved at least two days before experiments began.

During each experiment, mice were placed in separate, clear, plastic containers, each $9 \times 9 \times 13$ cm. Several small openings in each container allowed air to circulate. A camcorder (Sony Model DCR-DVD 300) was positioned above the mice to video record their behavior. For experiments on the cheek, two mice were tested at the same time. We placed four angled mirrors, one on each side of the mouse, affording the camera a four-sided view in addition to the view from the top. During experiments where substances were applied to the nape of the neck, three mice were tested simultaneously. As the nape of the neck is almost always facing upwards, only a top view was necessary to observe this area of the body.

Scratching behavior is susceptible to distractions and easily inhibited. Therefore, efforts were made to reduce distractions to a minimum. Vacuum lines pulled ambient air through the containers at a rate of about 300–500 ml/min so that mouse odors did not circulate between containers. Mice could not see each other during an experiment. A small amount of bedding was placed in each container to absorb any urine voided by the mouse. Experiments were conducted inside a sound proof room. Pseudo-white noise was delivered from a radio, tuned in between stations, to mask extraneous laboratory noises. Ambient temperature was maintained between 23 and 27 °C. Experiments were conducted between 900 and 1400 h.

To adapt mice to the experimental conditions, they were handled, restrained, and placed in containers several times on different days before the experiments began.

A mouse was restrained for cheek injection by first placing it in the palm of the experimenter's gloved right hand, the mouse's head between the thumb and forefinger. Access to the left cheek was gained by placing the thumb on the nape of the neck and pulling the skin of the nape towards the mouse's right shoulder and grasping it between the thumb and the base of the forefinger, with the last three fingers lightly restraining the mouse's body. The right cheek was accessed in the same manner except the skin was pushed towards the left shoulder and the skin held between the thumb and the base of the second or third finger. With the mouse restrained in such a manner, the cheek skin was taut and easily injected superficially. Restraint and injection could be performed in less than 10 s if both the mouse and experimenter were well trained.

To inject the nape of the neck, the experimenter placed the mouse on a grating and held the base of the tail between the thumb and middle finger. With the forefinger of the restraining hand, the skin of the back was pulled rearward to tighten the skin of the nape, thereby aiding the injection. Held by only the tail, the mice were generally motionless.

As mentioned previously, scratching is easily inhibited by distractions and stress. The presence of scratching shortly after injection suggested that the mice were not highly stressed by these restraint and injection procedures.

On the day of an experiment, mice were placed in containers and allowed to habituate for one hour. Then, the behavior of the mouse was video recorded 15 min before (baseline behavior), and 40 min after, a substance was applied to the cheek or the nape of the neck. The experimenter was present briefly – once to start the video recording, and later, to administer the chemical. The video recording was subsequently played back and the behavior scored by laboratory assistants who were blinded to the experimental conditions and unfamiliar with the experimental aims.

The number of wipes of the cheek and the number of bouts of scratching were scored in bins of one minute. Only those unilateral wipes with the forelimb that were not part of grooming behavior were counted. These wipes with the forelimb usually occurred as a gentle, single stroke that began at the back of the cheek, and moved forward in a caudal to rostral direction. There was never a back and forth stroking suggestive of scratching. These strokes were brief, taking perhaps less than 0.5 s to complete, in comparison with the more forceful scratching strokes with the hind limb. Contact during wiping appeared to be with the inner aspect of the forearm. The paw appeared to be closed-fisted, not making contact with the cheek, and facing forward rather than towards the cheek (Fig. 1a). In contrast, grooming behavior consisted of simultaneous wipes with both forelimbs (Fig. 1b), alternating wipes, and wipes that started behind the ears and followed through over the cheek.

Scratching behavior consisted of individual bouts of scratching within which individual scratches sometimes occurred at too rapid a rate to be distinguished. Thus, only the number of bouts was counted. The number of scratches during a bout could be one or many. A bout of scratching could last several seconds and was initiated by lifting of the hind paw to the region of the body to be scratched (Fig. 1c). The bout ended when the hind paw was returned to the floor or to the mouth – the latter behavior presumably a grooming response to rid the hind limb claws of accumulated skin debris and not uncommon after scratching. This was quite different from wiping where the forelimb rarely, if ever, went to the mouth after a wipe.

Capsaicin (Fluka) was dissolved in a vehicle of 7% Tween-80 in saline. Histamine dihydrochloride (Sigma-Aldrich Chem. Co.) was dissolved in a vehicle of sterile saline. A volume of 10 μ l of a given chemical, or its vehicle alone, was injected intradermally into the nape or into the cheek using a 0.3 ml insulin syringe with a 29 ga needle (Terumo). The doses of capsaicin delivered to the cheek were 0 (vehicle only), 1, 10, and 40 μ g ($n = 6$ for vehicle and $n = 8$ for all other groups) and, to the nape of the neck, 0, 1, and 10 μ g (12 mice for each dose). The doses of histamine were 0, 10, 20, and 50 μ g for both the cheek and neck. For the cheek, the number of mice tested was 8 for the vehicle of saline and 10, 8, and 8, respectively, for the doses of 10, 20, and 50 μ g of histamine. For the nape of the neck, there were 8 mice in each group each receiving either the saline vehicle or one of the doses of histamine. Each cheek, or the nape of the neck, received only one application of chemical.

Behavioral responses subsided within 20 min after the injection of a chemical. The mean number of bouts of scratching or the mean number of wipes (and standard errors of the mean) during this period were calculated for each group. One-way analyses of variance (ANOVAs) were used to determine the significance of differences in means obtained for each group tested with a given chemical, followed by Bonferroni post-hoc tests to examine differences among groups. The criterion for statistical significance was a probability value of 0.05.

3. Results

An injection of histamine or capsaicin each evoked scratching when applied to the nape of the neck. The temporal profile of scratching in bouts/min rapidly reached a peak and gradually subsided to baseline within 20 min (Fig. 2a). During this period, each dose of histamine evoked a significantly greater mean number of bouts of scratching than the vehicle (Fig. 2b). Although all doses of histamine evoked more bouts of scratching than the vehicle ($F_{3,28} = 6.470$, $p = .0018$), there were no significant differences in the mean number of bouts obtained in response to the different doses. The vehicle for capsaicin evoked significantly more scratching than the vehicle for histamine ($p < 0.001$, Welch's unpaired t -test) (Fig. 2b and c). The higher dose of capsaicin (10 μg) evoked significantly more scratching than the vehicle (Fig. 2c, $F_{2,33} = 3.194$, $p = 0.0298$), but there was no significant difference between the two doses of capsaicin.

When the same chemical substances were applied to the cheek, both wiping with the forelimb and scratching with the hind limb were observed (Fig. 3). Histamine again evoked site-directed scratching with the hind limb just as it had when applied to the back. The mean number of bouts of scratching in response to an injection of 50 μg of histamine into the cheek reached a peak within several minutes, and then returned to near baseline levels by 20 min (Fig. 3a). The small amount of wiping of the cheek that also occurred (Fig. 3b) usually took place right after scratching, and, thus, may have been a reaction to the nociceptive effects of one or more bouts of particularly vigorous scratching. Scratching and wiping, in response to histamine, were always directed with the ipsilateral limb to the site of injection and never directed (with any limb) toward the un-injected cheek.

When 40 μg of capsaicin was injected into the cheek, a behavior was observed that was completely different from that elicited when applied to the nape of the neck. Instead of eliciting scratching with the hind limb, capsaicin evoked site-directed wiping with the ipsilateral forelimb (Fig. 3d). There was very little scratching directed toward the cheek (Fig. 3c). Although the number of wipes induced by capsaicin exceeded the number of scratching bouts evoked by histamine, it is not clear that the capsaicin response is "greater" than the histamine response. A scratching bout can contain many scratching strokes and can last several seconds in comparison with a brief single wipe. Thus, although not directly comparable, a bout of scratching may be equivalent to multiple wipes, and the histamine-induced scratching response in Fig. 3a may in fact be greater than the capsaicin evoked wiping response in Fig. 3d.

The mean total number of bouts of scratching and wipes, simultaneously recorded and occurring over the 20 min after an injection into the cheek were obtained for all mice tested with each vehicle and dose of each chemical (Fig. 4). For scratching of the cheek after histamine, ANOVA revealed a significant effect of dose ($F_{3,33} = 4.791$, $p = 0.0070$). In comparison with responses to the vehicle, each dose of histamine evoked significantly more bouts of scratching (Fig. 4a). There was no significant dose effect for wipes (Fig. 4b, $F_{3,30} = 1.76$, $p = 0.176$) nor were there any significant differences among the doses for either type of behavior.

The vehicle for capsaicin evoked a small amount of scratching. Addition of capsaicin to the vehicle reduced this scratching ($F_{3,26} = 3.558$, $p = 0.0279$), significantly so for the highest dose (Fig. 4c), in comparison with the effects of vehicle alone. This is exactly opposite to the significant increase in scratching with an increase in the dose of capsaicin applied to the nape of the neck (Fig. 2c). In contrast, and in comparison with the response to vehicle, the mean number of wipes was significantly increased ($F_{3,26} = 9.828$, $p = 0.0002$) by the addition of capsaicin to the vehicle, reaching significance at the two highest doses (Fig. 4d). This was opposite to the effects of capsaicin on hind limb scratching directed toward the cheek (Fig. 4c).

There were no significant differences among the doses of capsaicin for either scratching or wiping.

The two different behaviors elicited in each mouse by each chemical injected into the cheek are summarized in a scatter gram (Fig. 5). For each mouse and each dose of each chemical, the total number of bouts of scratching with the hind limb is plotted against the total number of wipes. The histamine data are grouped along the axis for scratching whereas the capsaicin data are clustered near the axis for wiping thereby suggesting the painful and pruritic characteristics of each substance.

4. Discussion

There is only one behavior available to mice that can be directed toward the nape of the neck: scratching with the hind limb. This confounds the interpretation of responses that use only the nape of the neck to deliver pruritic and algescic chemicals as demonstrated in this study. This is illustrated by the scratching evoked by capsaicin applied to the nape (Fig. 2) which would be interpreted as evidence that capsaicin causes itch. In contrast, two behaviors, hind limb scratching, and forelimb wiping, can be directed toward the cheek. Vos et al. [15] delivered both noxious and non-painful stimuli to the face. They concluded that facial wiping is a nocifensive specific behavior in rodents. Since then, facial wiping has been used as an indicator of pain in mice [16,17]. Forelimb movements that are directed toward the ocular area in rodents in response to irritants applied to the eye may be interpreted as behavioral measures of nociceptive behavior [8] or even itch [7]. However, it has not been determined for such tests whether it is the skin of the forelimb that contacts the ocular tissues or the claws.

The present finding that mice emit different behaviors in response to capsaicin and histamine applied to the cheek is in agreement with human observations that the former is nociceptive and the latter, pruritic. Future studies will be necessary to establish the precise relationship between the dose of capsaicin or histamine delivered to the cheek and the corresponding amount of wiping and scratching elicited. The limited goal of this study was to demonstrate the degree to which suprathreshold doses of each chemical elicit two, qualitatively different kinds of behavior: wiping with the forelimb and scratching with the hind limb.

In humans, an intradermal injection of histamine evoked an itch that was dose-dependent up to the highest dose of 100 μ g [14]. The duration of the itch was approximately 25 min for the highest dose but less than 20 min for lower doses. Although Simone et al. [14] reported that histamine produced a pure itch, we recently observed that when subjects are given a choice of pricking/stinging and burning, in addition to itch, that one or both of these nociceptive qualities can be experienced as well, although typically at lesser magnitudes (unpublished observations). This finding is consistent with anecdotal reports of itch and nociceptive qualities produced by iontophoretically applied histamine [10], and psychophysical measurements of both the itch and nociceptive sensations evoked either by the pruritic spicules of cowhage, a tropical legume [6,11], or by the protease contained within these spicules [9]. The present observations of the behavioral responses of mice to histamine applied to the cheek are consistent with the findings reported for humans. If scratching and wiping are respectively indicative of pruritic and nociceptive sensations, then histamine injected into the cheek of the mouse evoked itch lasting nearly 20 min, and nociceptive sensations lasting only a few minutes. Scratching in mice might be expected to be of lesser duration than pruritic sensations in humans due to its effects in alleviating itch.

In humans, topically applied capsaicin elicited sting, burn and itch [3]. However, intradermal injections of capsaicin always evoked only pain, typically described as burning or stinging [1,13]. The localized pain began immediately upon injection, peaked within a minute later,

then gradually declined. The durations of sensations produced by the highest doses were 10–15 min. In this study, mice scratched the site of an injection of capsaicin to the nape of the neck. Presumably, capsaicin injection in mice caused a localized, rapid onset, stinging, burning pain, as reported in humans. But one could imagine that such a pain might suggest the presence of a thorn, burr or stinging insect on the skin. Thus, scratching the nape of the neck in response to capsaicin could be interpreted as an attempt to rid the skin of the offending stimulus. Scratching with the hind limb is the only behavior available to the mouse for this purpose. However, when capsaicin was injected into the cheek, the true, painful, nature of the stimulus was revealed and wiping occurred. The vehicle for capsaicin evoked a small amount of scratching. But with the addition of a sufficiently high dose of capsaicin to the vehicle, the scratching was significantly reduced and replaced by a significant increase in wiping. The behavior of wiping suggested that the pain increased with a sufficiently high dose of capsaicin. Presumably, the increase in pain inhibited the itch of the vehicle.

In the original model of Kuraishi [5] in which the ddY strain of mouse was used, capsaicin did not evoke a response. The absence of a response was interpreted as indicating that painful substances did not provoke scratching and that the model was therefore specific for itch producing substances. However, histamine also did not evoke scratching in this strain of mouse. It may be that the ddY mouse used in these studies was not very responsive to either histamine [4] or, possibly, capsaicin.

It is a common observation that rodents and carnivores favor scratching the side of the face with the ipsilateral hindlimb rather than with the forelimb. Perhaps the forelimb delivers less force than the hindlimb and is less effective for alleviating itch on the face. Limited supination of the forelimbs may also reduce their utility for scratching the face. In contrast, primates have hands and can direct their hands with more range of motion, to most of the body.

When given histamine or capsaicin to the cheek, mice behaved in an appropriate manner that was consistent with the respective sensations reported by humans. The mice wiped their cheeks to a substance that produces pain in humans and scratched to a chemical that evokes itch. Thus, we conclude that by observing the behavior directed towards the cheek, one can differentiate between stimuli that are painful from those that elicit itch.

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Fig. 1. Line drawings of a mouse showing (a) wiping of the cheek with the forelimb, (b) grooming with forelimbs, and (c) scratching of the cheek with the hind limb. Although grooming involved wipes over the cheek, this behavior was not counted as a response to chemical application to the cheek. Cross-hatched area is the area of the cheek that was shaved prior to experiments.

Fig. 2.

Scratching directed toward the site of injection of capsaicin and histamine into the nape of the neck. (a) The time course of scratching before and after the injection (at time = 0). The mean number of bouts of scratching/min after an injection of 10 μ g of histamine ($n = 8$, solid circles) or capsaicin ($n = 12$, open squares). (b) Mean total number of bouts of scratching during the 20 min period after injection of vehicle and different doses of histamine ($n = 8$ for all groups, mean $\pm$ SEM). Each dose of histamine evoked significantly more bouts than the vehicle alone (* $p < 0.05$; ** $p < 0.01$). There were no significant differences in bouts among doses. (c) Mean total number of bouts of scratching/20 min evoked by vehicle and two doses of capsaicin. The

only significant difference in number of bouts was between the largest dose of capsaicin and the vehicle ($p < 0.05$; $n = 12$ for all groups, mean $\pm$ SEM).

Fig. 3.

The time course of scratching and wiping directed toward the site of injection of capsaicin and histamine into the cheek. Wiping and scratching were simultaneously recorded in responses to each stimulus. All injections occurred at time = 0. (a) and (b) The mean number of bouts of scratching/min (a) and mean wipes/min (b) after an injection of 50 μ g of histamine ($n = 8$). (c) and (d) The mean number of bouts of scratching/min (c) and mean wipes/min (d) after an injection of 40 μ g of capsaicin ($n = 8$).

Fig. 4.

Cumulative scratching and wiping directed toward the site of injection of capsaicin or histamine into the cheek. Scratching with the hind limb (left column) and wiping with the forelimb (right column) were simultaneously recorded in response to each stimulus. (a) and (b) The mean number of bouts of scratching (a) and mean number of wipes (b) occurring within 20 min after an injection of vehicle (saline) or different doses of histamine. Significant differences between means obtained for each dose and vehicle are indicated with asterisks (* $p < 0.05$; ** $p < 0.01$). (c) and (d) Mean number of bouts of scratching (c) and wipes (d) occurring during the 20 min after an injection of vehicle of tween-80 saline and different doses of capsaicin in vehicle.

Fig. 5.

Scatter gram that summarizes the cumulative scratching and wiping evoked in each mouse by each dose of histamine and capsaicin. These data were used to obtain the means in Fig. 3. For each dose of histamine (solid symbols) and capsaicin (open symbols), the total number of bouts of scratching/20 min evoked in each mouse are plotted against the total number of wipes/20 min. The different shaped symbols represent different doses. Solid circle = 10, solid square = 20, and solid triangle = 50 μ g histamine; open circle = 1, open square = 10, and open triangle = 40 μ g capsaicin.