Response to Comment on “Molecular and neural basis of contagious itch behavior in mice”

Devin M. Barry,1,2 Yao-Qing Yu,1,2,5 Yan Hao,1,2† Xue-Ting Liu,1,2† Zhou-Feng Chen1,2,3,4†

Liljencrantz et al. report the failure of observing contagious itch behavior using mice injected with histamine as the demonstrators. Analysis of their results shows that the histamine model is limited by inadequate frequency and duration of scratching bouts required for contagious itch test. To streamline the contagious itch test, the screen paradigm is highly recommended.

Although we (1) and Liljencrantz et al. (2) used the same strain of mice, their experimental design was different from ours in several important aspects. Limiting factors complicate the detection of imitative scratch behavior in mice. The most notable constraint is the low frequency of look behaviors by the observer toward the demonstrator. In 1-hour home-cage or screen paradigm, the average number of look behaviors is ~20, which is similar to that of spontaneous scratches. The low frequency of looks and spontaneous scratches makes the coincidental occurrence of the two behaviors, which cannot be differentiated from imitative scratches, very rare. For example, out of eight control observers in the home-cage paradigm, only one coincidental “look and scratch” behavior was observed (1) (Fig. 1A). A lack of “look and scratch” behavior in the control mice therefore provides a solid foundation for detection of imitative scratches in a contagious itch test (1).

The frequency of imitative scratches typically is about 5 to 10 times in 1 hour, much lower than the number of looks (1). This discrepancy can be explained as follows. First, our assessment of look behavior according to the head movement of the observer is conjecture in nature, because one cannot be certain whether a mouse may appear to look but not pay attention to or see the demonstrator. Second, even though the observer does see the demonstrator, the latter may not be in the act of scratching for the former to imitate. Scratching is merely a part of a series of rapid movements of the hind limb, ranging from lifting of the hind limb to returning the paw to the floor (3).

To obtain the number of imitative scratches sufficient for statistical analysis between the groups, it is crucial to maximize the chance for the free-moving observer to see the scratching motion. We achieved this goal by using BRAFNav1.8 mice, a genetically engineered mouse model for chronic itch (4), as the demonstrators, because the majority of pruritogens elicit only short-lasting scratching behaviors (within 30 min) (3). There is limitation to the imitative scratching because it is dependent on the look behavior of the observer. Our studies suggest that ~200 scratches per hour in the home cage is the minimal number necessary for the observer to imitate to a degree that can be measured by a statistical method. In a screen paradigm, the frequency of scratching bouts was further adjusted to 30 per min or 1800 per hour (1). Equally important is to perform the test for 1 hour so that the results are more statistically tractable, given the low frequency of imitative scratches.

Using histamine-induced scratching behavior as described by Liljencrantz et al. is problematic. Histamine-induced scratching typically peaks at 10 min and recedes within 20 min, with most scratching bouts occurring between 0 and 15 min (Fig. 1B). Considering that the observers showed little or no imitative scratching within the first 10 min (Fig. 1A), this severely narrows the time window for the observers to see scratching action. Although scratching induced by compounds 48/80, PAR2 agonist SLIGRI-NH2, and chloroquine exhibit slower onset, peak at about 15 to 20 min, and recede at about 30 min (3, 5), they also lack the duration required for the observer to imitate, given the fact that on average mice imitate 1 to 2 times every 10 min. Considering the low number of imitative scratches, it is not meaningful to compare the total number of scratches between the two groups. A comparison of the frequency and duration between the histamine model—which shows scratching bouts similar to what Liljencrantz et al. showed—and

Fig. 1. Imitative scratching behavior in the home cage requires robust and long-lasting scratching by the demonstrator. (A) Raster plots for look, spontaneous scratch, or look-and-scratch behaviors from representative observers to control or scratching demonstrators in the home-cage paradigm. (B) Time course showing the comparison of mean number of scratching bouts in 5-min intervals between histamine (200 μg in 50 μL, sigma)—induced scratches in C57/B6 mice and spontaneous scratches in BRAFNav1.8 mice, n = 8.
the home-cage paradigm using the chronic itch model illustrates the marked difference, either in intensity or in duration of scratching bouts (Fig. 1B). Although histamine is a relatively weak pruritogen in mice, one cannot exclude the possibility that other pruritogens, which induce more robust scratching, may be suitable for contagious itch tests. Other minor differences are also noted. Liljencrantz et al., used two boxes side by side instead of the home cage, and mice were habituated several times before the experiment. Moreover, Liljencrantz et al. kept two mice in one cage and used one as the demonstrator and the other as the observer. Mouse familiars may show fewer look behaviors than strangers, perhaps due to lack of curiosity. In contrast, we used naïve strangers as the observers. Last, our test is always conducted in the morning between 8 and 10 a.m., when mice are less likely to rest or fall asleep. We observed that mice tend to be less active and more restful later in the day.

In short, neither frequency nor duration of scratching bouts of mice injected with histamine as described by Liljencrantz et al. are adequate for a contagious itch test. Given a great range of variability in the frequency of scratching behavior among mice with chronic itch, the screen paradigm is highly recommended.

REFERENCES AND NOTES

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