Patterns of Variation in the Behavioral Responses of Rats to Irritants After Neonatal Capsaicin Treatment

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Introduction

Administration of capsaicin to neonatal rat pups has previously been shown to produce adults with decreased trigeminal sensitivity to many irritants. This observed loss in chemosensitivity presumably occurs through the specific elimination of the vanilloid receptor-expressive (VR1) cell population of the trigeminal ganglion, making capsaicin-desensitized animals a valuable tool in studying the functional organization of chemosensitive trigeminal afferents.

We currently utilize capsaicin-desensitized rats in both electrophysiological and immunohistochemical studies, under the assumption that a standardized capsaicin injection regimen leads to uniformity in trigeminal desensitization. In the present experiment, a behavioral screen was used to test the reliability and efficacy of neonatal capsaicin treatment in producing a desensitized adult state. Adult rats injected with either capsaicin or a control solution as neonates were presented with a series of eight irritating/non-irritating chemical stimuli. Each rat's response within the first few seconds of encounter with the stimulus source was then scored using a scale to measure aversive and/or favorable responses. Neonatal capsaicin treatment was found to result in varying degrees and differing patterns of desensitization to the five irritants tested, with no observed effect on responses to non-irritating stimuli.

Most of the capsaicin-injected rats displayed overall diminished aversion reactions when compared to control animals, but a few were found to be more sensitive than the least sensitive control animal. This observed degree of inter-animal variability shows that standard neonatal administration of synthetic capsaicin does not always guarantee a desensitized-adult state, and results in varying degrees of desensitization – a factor which must be taken into consideration when using neonatally capsaicin-injected rats in electrophysiological or immunohistochemical experiments.

Methods

Litters from 2 Sprague-Dawley rats were used in this experiment. A total of 19 one-day old pups were subcutaneously injected in the dorsal aspect of the neck with 0.1 ml of either a 1% capsaicin solution (14 pups) or the vehicle (5 pups). Control pups were marked with a cut in their tail-tips. The capsaicin solution was prepared by dissolving synthetic capsaicin in a mixture of ethanol, Tween 80, and 0.9% saline (1:1:8). Before the injection, the pups were wrapped in gauze and placed on ice for approximately one minute in order to minimize the pain produced by the injections. Capsaicin injected pups were observed for 15-30 minutes post-injection, and in some cases required manual stimulation to restore a regular respiratory cycle.

For behavioral testing, adult rats were placed in a sterilized clear polypropylene cage and allowed approximately 2 minutes to acclimate. Following this initial acclimation period, the cotton tip of an applicator stick was placed approximately 2 cm from an opening in the cage wall and the rat was allowed to acclimate to its presence for an additional 1-2 minutes. Using different applicators, rats were presented with a series of 8 different olfactory or trigeminal stimuli, plus water as a control. The stimulus-saturated cotton tips of the applicators were placed in front of the cage opening and each rat's response within the first few seconds of encountering the stimulus was scored using a behavioral scale ranging from –2 to +2. To reduce subjectivity in the behavioral scoring method, a simple 5-level scale was used, with (–2) corresponding to a display of a trigeminal reflex, and (+2) corresponding to attempted manipulation of the applicator tip or attempted feeding behavior. A score of zero corresponded to no response to the presence of the applicator.

Acetic acid, (-)-nicotine, cyclohexanone, ethanol, and amyl acetate were chosen as trigeminal stimuli and presented randomly in three repetitions. After each behavioral score was marked, the applicator was removed from the vicinity of the cage and the rat was allowed a 2-minute rest period before the next stimulus presentation. To decrease the possibility of learned aversion behavior in response to the presentation of applicator sticks, presentation of a trigeminal stimulus was always followed by the presentation of a favorable olfactory stimulus or water, also presented randomly and in three repetitions. This presentation scheme insured that a trigeminal stimulus was always followed by a favorable stimulus.

The three favorable olfactory stimuli used were wheat flour, chocolate powder, and fresh home-cage bedding extract. The wheat stimulus was prepared by covering the water-saturated cotton tip of an applicator with whole-wheat flour. The chocolate stimulus was prepared similarly by using Hershey's brand chocolate powder. To prepare the bedding extract, fresh cage bedding wood chips were boiled in water for approximately one hour. The mixture was then cooled and filtered, leaving a concentrated bedding extract. For each stimulus, mean response scores from the control and capsaicin-desensitized rats were compared using Mann-Whitney *U*-test (two-tailed, $p < 0.05$).

Methods

Figure 1. Neonatal Capsaicin Treatment

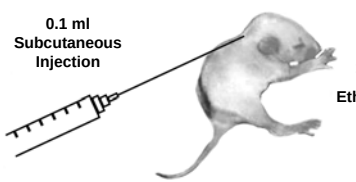
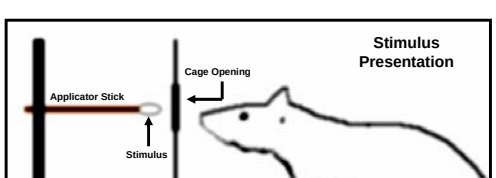


Figure 2. Stimulus Presentation During Behavioral Study



Behavioral Response Scale

-2	**Trigeminal Reflex: Reflexive Withdrawal Movement**
-1	**Aversive Response: Head Turn or Rejection Movement**
0	**Neutral Response: No Response or Lack of Interest**
+1	**Favorable Response: Inspiratory Sniffing & Investigation**
+2	**Highly Favorable Response: Attempted Feeding Behavior**

Figure 1. Neonatal Capsaicin Treatment. A total of 19 one-day old rat pups were subcutaneously injected in the dorsal aspect of the neck with 0.1 ml of either a 1% capsaicin solution (14 pups) or the vehicle (5 pups). Control pups were marked with a cut in their tail-tips. The capsaicin solution was prepared by dissolving synthetic capsaicin in a mixture of ethanol, Tween 80, and 0.9% saline (1:1:8).
Figure 2. Stimulus Presentation During Behavioral Study. Adult rats were presented with various olfactory/trigeminal stimuli, utilizing applicator sticks placed in the vicinity of the cage. Each rat's response within the first few seconds of encountering the stimuli was scored using a scale ranging from -2 to +2 as described above. Stimuli were presented randomly, with three repetitions per stimulus.

Results

Figure 3. Behavioral Response in Individual Rats

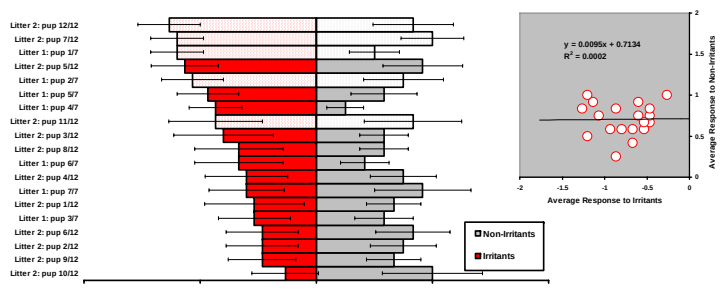


Figure 4. Mean Behavioral Response Scores

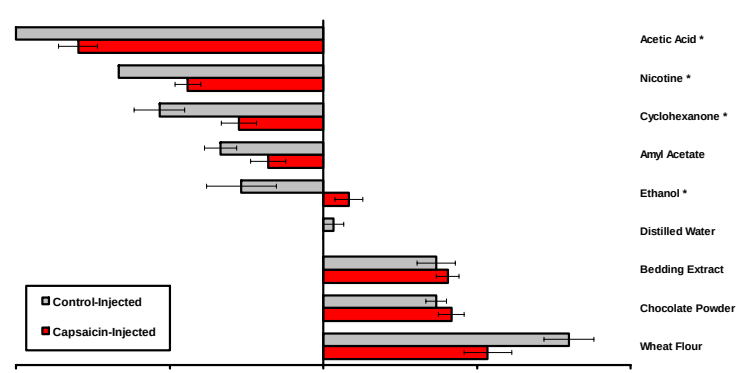


Figure 3. Behavioral response in individual rats. Mean behavioral score values (X-axis) for all 19 rats tested. Data are sorted in order of the mean observed responses to irritants, placing the most desensitized animals lowest on the Y-axis. At least 3 of the capsaicin-injected rats displayed more overall sensitivity to irritants than the least sensitive of the control-injected rats, represented by the patterned bars. Error bars indicate one standard error unit. No pattern or relationship was observed between the level of desensitization and response to non-irritants, as shown by regression analysis (inset): Mean behavioral response score to non-irritants is plotted as a function of the response score to irritants for each individual (represented by white circles).
Figure 4. Mean behavioral response scores. Mean behavioral score values for each individual stimulus, plus control (water). Capsaicin-injected rats displayed significantly diminished aversive behavior in response to acetic acid, nicotine, cyclohexanone, and ethanol (two-tailed Mann-Whitney *U*-test, $p < 0.05$). No statistically significant differences were found between the behavioral scores of control- and capsaicin-injected rats in response to non-irritants. Error bars indicate one standard error unit.

Conclusions

Neonatal capsaicin treatment does not necessarily result in the production of uniformly desensitized adult rats.

Approximately 20% of neonatally capsaicin-injected rats were found to be more overall sensitive than the least sensitive control animal in this experiment.

The extent or degree of desensitization to irritating chemical stimuli does not seem to be correlated to the behavioral response to non-irritants.

On average, neonatally capsaicin-injected rats – including those found to be more sensitive than the least sensitive control animal – displayed significantly diminished aversion responses to acetic acid, nicotine, cyclohexanone, and ethanol.

No differences were found between control- and capsaicin-injected rats in their mean behavioral response to non-irritants.

Final Conclusion: Neonatal administration of synthetic capsaicin does not guarantee a desensitized adult state, but instead may result in varying degrees of desensitization.

Literature List

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