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# Neonatal Capsaicin Administration: Effects on Behavioral Development of the Rat

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CARILLO, P., J. MANZO, M. MARTINEZ-GOMEZ, M. SALAS AND P. PACHECO. *Neonatal capsaicin administration: Effects on behavioral development of the rat.* PHARMACOL BIOCHEM BEHAV 48(2) 447–452, 1994.—The effect of a single dose of capsaicin administered neonatally on the development of six motor behavioral patterns (scratching, rearing, grooming, searching, remaining still, and sniffing) was examined in Wistar rats. Treated animals exhibited a significant increment in scratching, rearing, grooming, and searching. Capsaicin also provoked precocious eye opening of one to two days and a reduction in remaining still, sniffing, and body weight. These effects were accompanied by pelage dishevelment and presence of skin ulcers in the region of the head, neck, and shoulders. The distribution of skin ulcers suggests that the fifth cranial nerve and roots innervating C1–C3 spinal cord segments contain a high proportion of capsaicin-sensitive "C" and Aδ fibers.

Neonatal capsaicin    Behavioral development    Scratching    C fibers    Aδ fibers    Eye opening

EVIDENCE is accumulating that transplacental passage (15,21) or continuous exposure to various components of the maternal diet can be associated with long-lasting brain deficiency (34). After birth, abnormal levels of circulating hormones (6,10,30), exposure to industrial solvents (16,37), poisons and drugs (14,32), and malnutrition (22,29), to name but a few, also represent noxious factors that may result in altered brain development.

Neonatal administration of capsaicin provokes irreversible neurotoxic effects (15), reported as a significant loss of unmyelinated "C" and myelinated Aδ fibers (23). Additionally, it has been found that the injury of primary sensory neurons produces alterations in chemogenic pain transmission (1,20, 33); reduction in the response to noxious thermal, mechanical, and chemical stimuli; and substance P depletion in dorsal root ganglia (12,13).

In spite of the widespread use of capsaicin as a neuroanatomical (17), functional (28), and even therapeutic probe (2), there is a lack of information concerning its short- and long-term effects upon behavioral development. Thus, the present study was designed to examine the effect of SC neonatal capsaicin administration on the development of six behavioral motor patterns in the rat. A longitudinal analysis of these patterns in both sham-injected and neonatally capsaicin-treated animals was performed.

## METHODS

### *Animals*

A group of 32 adult virgin female Wistar rats (*Rattus norvegicus*) was selectively mated so as to produce eight consecutive parturitions per day over a four-day period. Prior to parturition and throughout lactation the dams were housed individually in Plexiglas maternity cages (50 × 40 × 20 cm) containing nesting material (wood shavings). Cages were kept in a colony room at 22–24°C, 50% humidity, and a 12-h illumination cycle (light on 0700). Food (Purina chow) and water were available ad lib. At birth (day 0), 22 pups of both sexes were randomly allocated to capsaicin treatment in a group with three dams, so as to form two litters of 7 pups and one of 8 ($n$ = 22). The control group comprised three litters of 7 pups and one of 8 ($n$ = 29). This distribution ensured

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that possible genetic and prenatal biological differences were balanced between litters. In all cases the pups were weaned at day 21 of age and given free access to food and water.

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) (Sigma, St. Louis) dissolved in an emulsion with 10% ethanol, 10% Tween 80, and 80% saline (0.9%) was SC injected in a single dose of 50 mg/kg on day 2 of life. The dose was contained in a 0.01-ml volume. Controls were injected SC with a similar volume of emulsion without capsaicin.

### *Behavioral Testing*

Behavioral observations were performed using plastic translucent cages (50 × 40 × 20 cm) during the dark phase of the cycle (10–14 h) and under red light. The time-sampling technique previously described and validated by Gispen et al. (9) was used. Thus, behavioral measurements from a randomly selected pup started 30 s after its placement into the cage. Four animals were tested simultaneously. During a 30-min observation period the frequency of the following six motor patterns was recorded every 15 s: digging, including moving wood shavings with snout or legs; grooming, involving forepaw vibration, face washing, body or genital licking, or tail preening; rearing, with the rat standing on its hindlegs; scratching, including the body and head skin using ipsilateral fast or slow hindleg movements; searching, comprising horizontal ambulatory activity; remaining still, including lying on the floor in a nonexploratory attitude; and sniffing, consisting of movement of the head and nostrils directed towards the surroundings.

Occurrence of each behavior was scored as 1 point and the total observation period was 30 min. Rats of the control ($n$ = 29) and experimental ($n$ = 22) groups were tested on days 3, 7, 11, 15, 19, 23, 27, 35, 40, 50, 60, 70, 80, and 90 postnatally.

All subjects were checked for eye opening, and since capsaicin treatment may interfere with weight gain, daily body weight measurements were also obtained. A previously described test (33) was performed to assess the neurotoxic effectiveness of the capsaicin treatment. Thus, at day 95 of age control and experimental animals were exposed to a drop of 1% solution of capsaicin deposited in the right eye. The frequency of the eye-wiping response was scored for 30 s.

### *Statistical Analysis*

The differences in the scores obtained for all behavioral patterns were compared using a multivariate analysis with repeated measurements (36). In addition, differences in the scores for each of the behavioral patterns were compared in a 2 (Treatments) × 14 (Days) analysis of variance (ANOVA) with repeated measures, and post hoc Newman-Keuls tests were conducted when pertinent for testing significant differences between groups. Body weight differences between groups were compared using an ANOVA. A probability value of 0.05 or less for all statistical analyses was considered significant unless otherwise stated.

## RESULTS

The ANOVA performed on the weight measurements yielded significant differences between control and capsaicin-treated groups, $F(1, 703) = 13.083$, $p < 0.0001$. The ANOVA over days revealed that the body weight of capsaicin-treated subjects was only significantly lower than those of the controls on days 35, 40, and 70, resulting in $F(1,50) = 16.394$, $p < 0.001$; $F(1, 48) = 13.794$, $p < 0.001$; and $F(1, 48) = 5.037$, $p < 0.05$, respectively (Fig. 1). Capsaicin subjects also presented alteration in the development and care of the body pelage. Signs of disheveled fur, dirty skin, and sparse hair implantation and the appearance of several ulcers in the region of the head, neck, and shoulders were observed, starting at approximately 19 days of age. When compared to the controls (mean ± SE : 15.18 ± 0.73), capsaicin-treated pups also showed precocious eye opening of one to two days (13.79 ± 1.11).

Analysis of the mean digging scores of capsaicin-treated and control rats did not reveal significant differences (not shown here). However, a significant capsaicin Treatment × Days interaction, $F(13, 703) = 1.890$, $p < 0.05$, was observed. Until postnatal day 35 both experimental and control groups did not exhibit significant differences in grooming

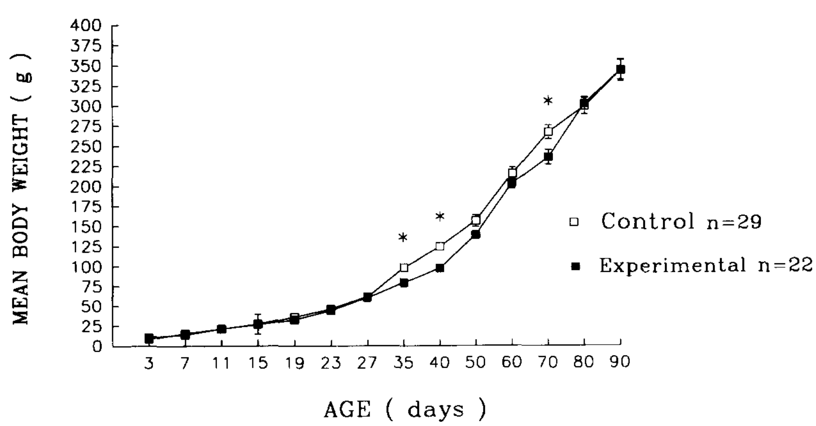


FIG. 1. Mean body weight measurements in control and neonatal capsaicin-treated rats during development. Before postnatal day 27 control and experimental rats followed a similar pattern of weight gain. Thereafter the body weight of experimental rats was only significantly reduced on days 35, 40, and 70 postnatally. Asterisks indicate days on which differences between groups were statistically significant.

(Fig. 2A). Grooming follows a progressive increment initiating with erratic face-washing followed by forepaw, fur, and genital licking (26,29). From postnatal day 40 capsaicin-treated rats increased their grooming values, although significant differences were only observed on days 50 and 80 postnatally (Fig. 2A). The ANOVA indicates a significant increment of grooming in the capsaicin-treated group, $F(1, 702) = 6.837$, $p < 0.05$, and a significant capsaicin Treatment × Days interaction, $F(13, 703) = 1.980$, $p < 0.05$. Rearing of experimental and control groups was sparing until 11 days of age. Afterwards, it gradually increased in values, with the capsaicin-treated subjects showing increased significant rearing in most of the ages (Fig. 2B). Analysis of the mean rearing scores revealed a significant increment in capsaicin-treated rats, $F(1, 702) = 26.193$, $p < 0.0001$, and a significant capsaicin Treatment × Days interaction, $F(13, 703) = 1.803$, $p < 0.05$. Scratching in both experimental groups was rarely seen before the first three days of age. Thereafter, a consistent significant increase in the scratching of capsaicin-treated rats, $F(1, 702) = 87.707$, $p < 0.05$, indicated that these animals spent more time in this activity than in others studied here (Fig. 2C). Moreover, the capsaicin Treatment × Days interaction was highly significant, $F(13, 703) = 3.680$, $p < 0.0001$. Searching was poorly developed during the first week of age in either group, followed by a progressive increment until day 15 postnatally, and their values were reduced for the rest of the experiment (Fig. 2D). In capsaicin-treated rats searching scores had a tendency to maintain higher values compared to controls. However, the statistical comparisons indicated that capasaicin-treated animals exhibited higher significant values when compared to controls, $F(1, 702) = 6.629$, $p < 0.05$. Moreover, the capsaicin Treatment × Days interaction was also significant, $F(13, 703) = 2.662$, $p < 0.0001$.

During the first week of age in both groups of rats scanty sniffing movements were detected. Afterwards, an abrupt increase followed by a plateau of sniffing scores was observed (Fig. 3A). The mean frequency analysis of sniffing revealed a reduction in the capsaicin-treated group, $F(1, 702) = 4.516$, $p < 0.05$. No significant interaction was found. Remaining-still measurements showed a progressive declining in their values throughout the experimental period. In most of the ages the scores of the capsaicin-treated rats had a tendency or were significantly reduced compared to controls (Fig. 3B), $F(1, 702) = 19.513$, $p < 0.0001$. Additionally, a significant capsaicin Treatment × Days interaction was also detected, $F(13, 703) = 1.906$, $p < 0.05$.

The multivariated statistical analysis of the six behavioral measurements and ages revealed that the experimental group was significantly different from the controls (Wilkes lambda = 0.804 and $F = 24.057$, $p < 0.0001$). However, this analy-

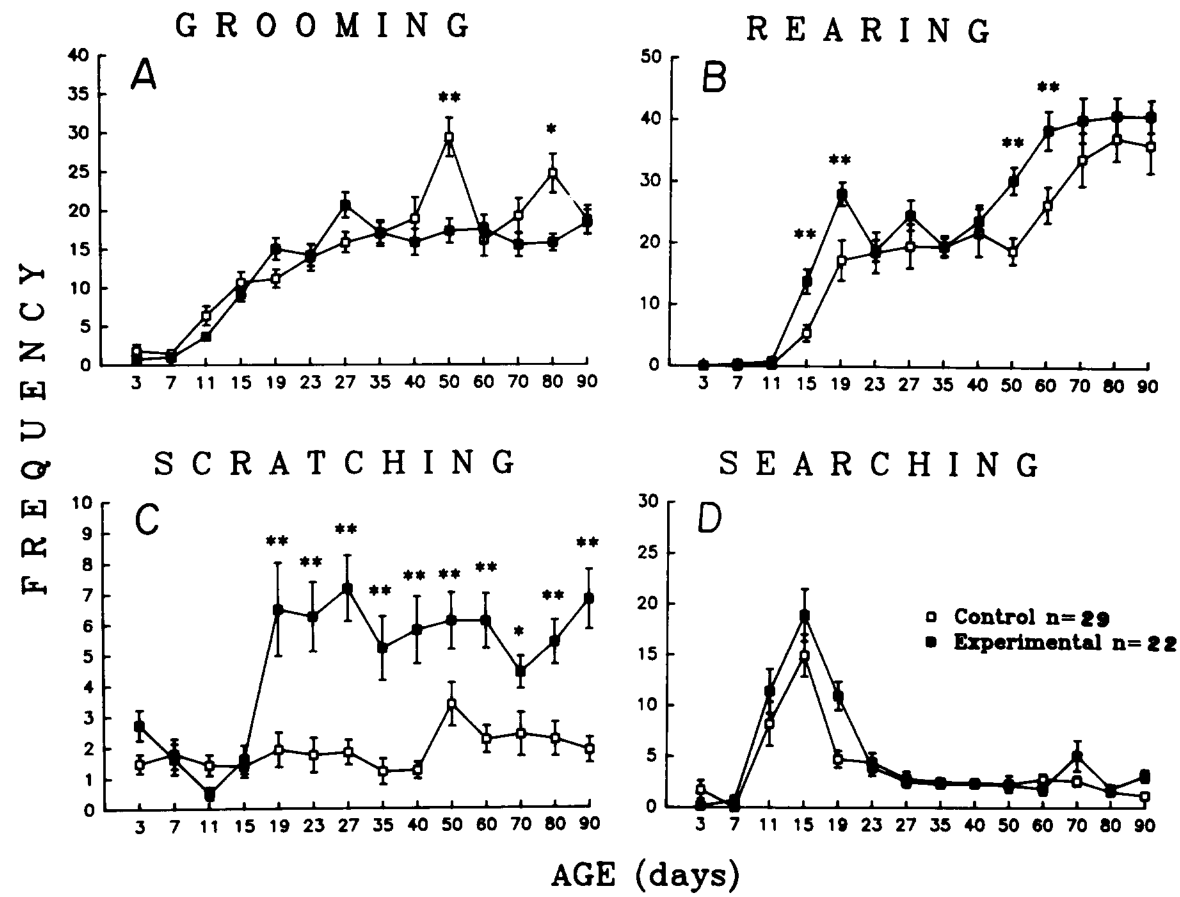


FIG. 2. Temporal sequence of behavioral maturation in control (□) and capsaicin-treated rats (■). Note the progressive increment in frequency of the various behavioral patterns and how grooming, scratching, and rearing after 15 days of age in capsaicin-treated subjects significantly increase their values (asterisks).

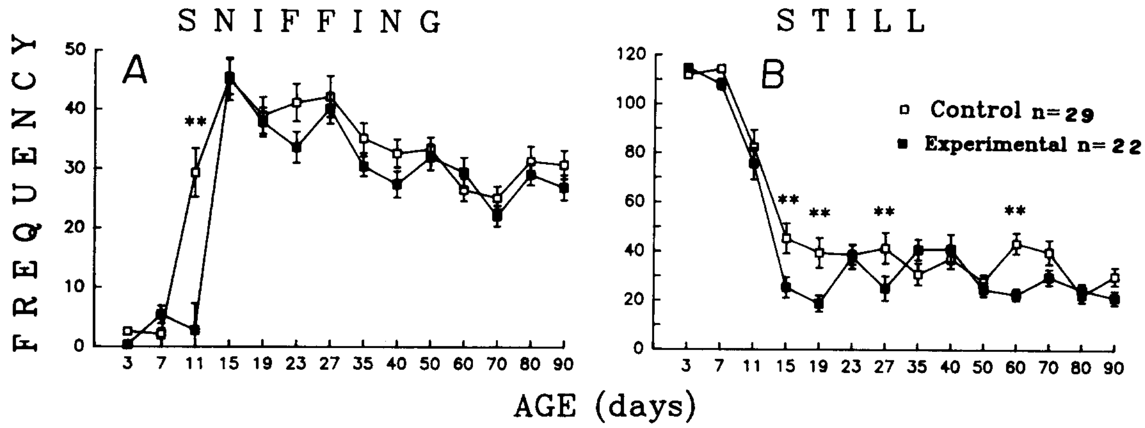


FIG. 3. Temporal sequence of sniffing and remaining-still maturation in control (□) and capsaicin-treated rats (■). Both behavioral patterns had a tendency to be lower or were significantly reduced (asterisks) in their frequency with increasing age.

sis did not provide information as to which of the six individual variables contributed more to the group difference, nor did it show which behavioral pattern in the capsaicin-treated rats was greater for any measure.

The canonical discriminant analysis resulted in significant differences between the experimental and control treatments ($p < 0.0001$). This analysis showed that scratching, rearing, grooming, and remaining still, which had a standardized coefficient greater than 0.3, contributed more to the behavioral differences seen between groups (Table 1).

Capsaicin-treated rats did not exhibit increased values for protective wiping movements in response to capsaicin applied to the eye (mean ± SE : 3.34 ± 0.6) compared to controls (29.0 ± 1.09).

## DISCUSSION

The neonatally administered SC single dose of capsaicin used here was associated with a variety of effects, some of them already described, but others not. A large body of data regarding neurotoxic effects of capsaicin on C and Aδ fibers (1,13) has been widely correlated with chemogenic pain (20,33) and altered thermal, mechanical, and chemical transmission (11–13,24). However, little is known about its effect upon behavioral development (1,3).

In the present study capsaicin exposure resulted in a reduction of protective wiping movements in response to the application of a solution of capsaicin to the eye, indicating its neurotoxic effectiveness (33).

TABLE 1

CANONICAL DISCRIMINANT ANALYSIS: BEHAVIORAL MOTOR PATTERNS WITH STANDARDIZED COEFFICIENTS

| Canonical Factors | Standardized Coefficients |
|---|---|
| Scratching | 0.795 |
| Rearing | 0.448 |
| Grooming | −0.320 |
| Still | −0.309 |
| Sniffing | −0.258 |
| Searching | 0.158 |
| Digging | 0.062 |

Alteration in the development of various behavioral motor patterns was one of the more striking effects observed in the present study. An increase in the frequency of scratching, rearing, grooming, and searching together with a significant reduction in remaining still and sniffing was observed during the development of capsaicin-treated pups. Recently, we proposed that C and/or Aδ fibers participate in an inhibitory feedback mechanism regulating motoneuronal activity during scratching (19). These fibers can activate modulatory circuits that modify the nociceptive transmission produced by scratching. This modulatory effect was revealed when, in absence of C and/or Aδ fibers, itching that provokes scratching produced a significantly larger or more frequent response than that expected in the presence of modulation. If so, this can be the cause of the scratching increment in the desensitized capsaicin rats.

Another interesting point derived from the present study concerns the C and Aδ fiber innervation of the cephalic extremity versus the innervation of the rest of the body. The presence of skin ulcers, which has been reported in other studies (18), was analyzed here and a regional distribution was clearly observed by the end of the third postnatal week. Head, neck, and shoulders innervated by the fifth and C1–C3 spinal nerves were the areas principally affected, and ulcers in other body areas were not observed. Skin ulcers may result as a consequence of a trophic effect (4,18), altered skin sensitivity (27), or excess of scratching and/or increased grooming (see Fig. 2A). Scratching achieves adult characteristics by the end of the third postnatal week (26,29). This probably determines the time for the development of the skin lesions in capsaicin-treated pups.

Before postnatal day 27 control and experimental rats followed a similar pattern of weight gain (Fig. 1). Thereafter, the weights of capsaicin-treated animals were lower. These findings are in line with previous data in neonatally capsaicin-treated rats (8,35), although the mechanisms underlying this effect are still under investigation.

The neonatal pup typically relies on sensory modalities such as olfaction for nipple attachment and touch for maintaining contact with the dam. Our findings showed a precocious eye opening in capsaicin-treated rats for which at present we do not have a satisfactory explanation. However, it might be taken into consideration that this phenomenon is concurrent with an impoverishment in olfactory (25), tactile (7,11,27), trigeminal (5,31), and visual (1) sensory avenues and altered scratching, rearing, searching, and sniffing motor behaviors. If the precocious eye opening is a functional adaptation or a compensatory effect in response to an unbalanced sensory maturation, it requires further experimental analysis. These alterations in the behavioral maturation may be critical for the development of adaptative behaviors in response to the environmental demands.

Finally, present data suggest that the use of neonatal capsaicin may be helpful in investigating the paramount role of the C and Aδ fibers upon the cephalic extremity in initiating feeding, exploration of the environment, mother recognition, gaining experience, neuronal growth, and the acquisition of new cognitive processes.

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