

## Chemosensory deficits are associated with reduced weight gain in newly hatched chicks

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**Abstract** — The influence of chemosensory perception on weight gain by newly hatched chicks was investigated. One day after hatching, 96 chicks were randomly assigned to 3 treatment conditions (32 chicks per condition): Both nares blocked with acrylic cement; 1 nare blocked; controls, with unblocked nares. Pairs of chicks in the same treatment condition were housed together with ad lib access to food and water; all chicks were weighed on the treatment day and 1, 2, 3 and 6 days later. Beginning one day post-treatment, and on each of the subsequent test days, chicks with both nares blocked weighed significantly less than those with only one blocked nare, or the controls. No significant differences were observed between chicks with one blocked nare vs. the controls. The relative weight gained between successive measures (proportion of initial body weight) by chicks with both nares blocked was less than that of the other conditions during the first 2 days following treatment, however, there were no differences between conditions after that time. In tests conducted to assess whether the treatment effectively disrupted chemosensory perception, control chicks and those with one blocked nare displayed overt responses when exposed to the odor of mint, but there was little reaction by chicks with both nares blocked. Reduced weight gain by chicks with olfactory deficits presumably reflects disrupted feeding behavior, however, after 2 days, those chicks compensated for their perceptual impairments. These results further illustrate the salience of chemical stimuli for newly hatched chicks.

**chicks / olfaction / chemosensory perception / weight gain / anosmia**

**Résumé** — Un déficit de l'olfaction est associé à une réduction du gain de poids chez les poussins nouveau-nés. L'influence de la perception olfactive sur le gain de poids de poussins nouveau-nés a été examinée. Un jour après l'éclosion, 96 poussins ont été aléatoirement assignés à 3 conditions de traitement (32 poussins par condition) : (i) les deux narines bloquées avec un ciment acrylique ;

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(ii) une seule narine bloquée ; (iii) les narines dégagées (témoin). Les poussins sont logés par paires d'un même traitement avec un accès continu à l'aliment et à l'eau. Tous les poussins ont été pesés le jour du traitement (à l'arrivée) et 1, 2, 3 et 6 jours plus tard. Dès le blocage des narines et pendant chacun des jours suivants du test, les poussins avec les deux narines bloquées pesaient significativement moins lourd que ceux avec seulement une narine bloquée, ou les témoins. Aucune différence significative n'a été observée entre les poussins avec une narine bloquée et les témoins. Le gain de poids relatif quotidien (gain de poids/poids initial) était moindre chez les poussins ayant les deux narines bloquées par rapport aux autres pendant les 2 premiers jours après le traitement. Cependant, il n'y avait aucune différence entre les traitements après ce temps. Dans des tests conduits pour évaluer si le traitement a efficacement perturbé la perception olfactive, les poussins témoins et ceux ayant eu une narine bloquée exprimaient des réponses significatives quand ils étaient exposés à une odeur de menthe, mais il y avait peu de réaction chez des poussins avec les deux narines bloquées. Le gain de poids réduit par des poussins avec un déficit olfactif reflète vraisemblablement un comportement alimentaire perturbé. Cependant, après 2 jours, ces poussins ont probablement compensé leur problème de perception. Ces résultats confirment l'importance des stimuli olfactifs chez le poussin nouveau-né.

### **poussin / olfaction / perception chimiosensorielle / gain de poids / anosmie**

## **1. INTRODUCTION**

In domestic fowl, the anatomical structures and physiological systems that mediate the detection of airborne odorants are functionally mature during the later stages of incubation [9, 12]. Approximately 2 days prior to hatching, the beak of the chick embryo pushes through the inner shell membrane into an airspace; the presentation of strong odorants at that time elicits behavioral (head-shaking, beak-clapping) and physiological (heart rate acceleration) responses [14]. The influence of odors on the behavior of newly hatched chicks has been demonstrated in numerous experiments, and feeding behavior appears to be particularly susceptible to olfactory stimuli [4]. For example, food intake and other aspects of chicks' feeding activity are disrupted when unfamiliar odorants are added to their feed [2, 10, 15], but the neophobic effects differ to some extent according to the particular scents that are used [5]. Moreover, chicks that became ill after ingesting scented food subsequently displayed specific aversion of feed treated with that same odor [15].

An alternative method to study the possible role of olfaction in the behavior and

growth of animals involves experimental disruption of odor perception. Rat pups that lose their sense of smell as the result of olfactory bulbectomy or peripheral destruction of the olfactory epithelium exhibit deficits in weight gain and heightened mortality rates [1, 11]. Bulbectomized pups were unable to locate and attach to a nipple from a very short distance [7, 13]. Following olfactory bulbectomy, chickens exhibited increased ingestion of food that was not associated with a marked gain in weight [8]. However, these results appear to reflect neurophysiological effects of the lesions (i.e. heightened thyrotropic activity) rather than anosmia per se. The capacity to perceive airborne odors can be more easily manipulated in birds by reducing access to the olfactory epithelium. Thus, chick embryos reacted noticeably to the odors of amyl acetate and cineole when their nasal openings were uncovered, but not when they were plugged with wax [14]. In a similar manner, intact chicks were attracted to a familiar scent in a novel environment, but such odor preferences were not evident in chicks whose nares were covered with dental cement [3].

The present study is an initial attempt to assess whether chemosensory input is

important for normal physical growth of newly hatched chicks. More specifically, weight gain during the first week after hatching was compared between control chicks and those whose external nasal openings were blocked to disrupt the perception of environmental odors.

## 2. MATERIALS AND METHODS

### 2.1. Subjects

The subject population consisted of 96 male domestic fowl chicks (Label breed) obtained from a commercial hatchery.

### 2.2. Treatment

On the day of hatching (Day 1), the chicks were housed in groups of 10–12 individuals, with ad libitum access to water; food was not available during that time. The next morning (Day 2), the chicks were randomly assigned to the following treatment conditions.

– Both nares blocked ( $n = 32$  chicks): the 2 external nare openings on the beak of each chick were completely blocked with a layer of rapid-drying acrylic cement applied with a small spatula. The chick was held loosely while the cement hardened (approximately 1 min). The chicks were then housed in pairs, in individual mesh cages measuring  $45 \times 45$  cm, for the remainder of the experiment. Food (chick “starter” feed) was continuously available in a rectangular hopper ( $9 \times 10$  cm) and water from an automatic dispenser. The ambient temperature was maintained at  $31\text{--}33$  °C, and there was constant illumination. In each pair, the two chicks were individually identifiable by a small (felt crayon) mark on the top of the head.

– One nare blocked ( $n = 32$  chicks): sixteen of the chicks in this condition had their right nare blocked with acrylic cement; the left nare remained unblocked. For the re-

maining 16 chicks, only the left nare was blocked. After the cement was applied, the chicks were housed in 16 pairs, each made up of one chick with the left nare blocked, and one chick whose right nare was blocked. Housing and maintenance were otherwise the same as described for the previous condition.

– Controls ( $n = 32$  chicks): the 16 pairs of chicks in the control condition were also treated with acrylic cement, but it was applied to the base of the beak, avoiding contact with the nares. The control chicks were then maintained like those in the other two conditions.

### 2.3. Weight measures

Each chick was weighed on a digital balance on Day 2 (immediately after being treated with the acrylic cement), and Days 3, 4, 5 and 8.

### 2.4. Assessment of odor perception

To verify whether blocking the external nares with acrylic cement indeed impaired odor perception, the responsiveness of the chicks in all 3 conditions to an airborne odorant was assessed using procedures similar to those recently described by Porter et al. [6]. Essential oil of mint (Monot; Lyon, France) was selected as the olfactory stimulus because this aroma elicited strong behavioral responses by chicks in the earlier experiment; water was used as an odorless control.

The tests were conducted after the weight measure on Day 5. At that time, individual chicks were held loosely by hand, under a 100-W lamp. When the chick became immobile (usually within 90-s.), a 10-mL soft plastic squeeze bottle containing  $\sim 0.03$  mL of essential oil of mint, or water, was held with its open tip 3–4 mm from the chick’s beak/nares and gently squeezed 15 times, during a period of approximately 10 s. The chick’s response during the

odor exposure trial was given a single score ranging from 0–2 on the following magnitude scale: 0 = no observable response to the stimulus; 1 = slight, slow movement of the head; beak clapping; 2 = abrupt shaking or jerking of the head, sometimes accompanied by shrill peeps. In this manner, each chick was tested successively with the 2 stimuli (mint and water). For each treatment condition, 16 chicks were first tested with mint, then with water; the order of presentation of the 2 stimuli was reversed for the remaining 16 chicks. There was a minimum interval of 10 s between the 2 test trials, during which the score on the first trial was recorded.

### 3. RESULTS

#### 3.1. Weight gain

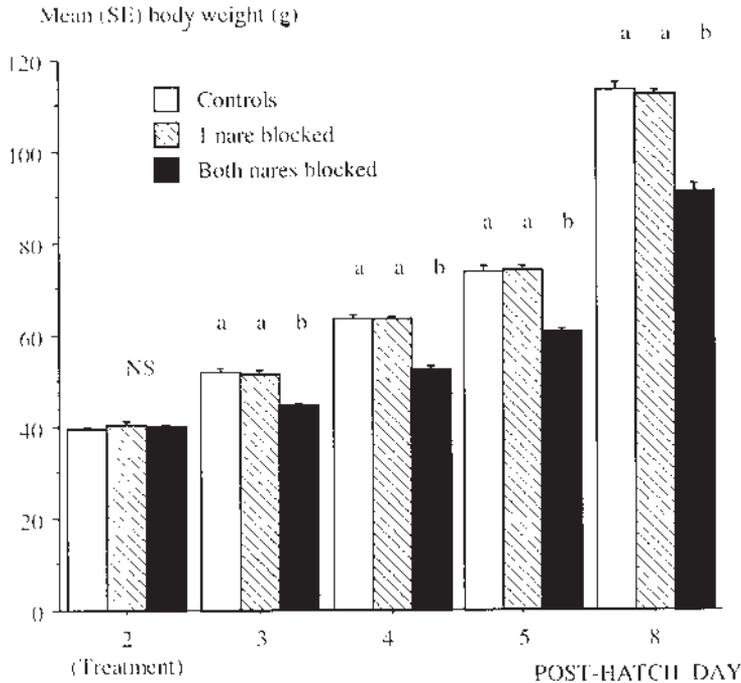
Mean (SE) daily body weights of chicks in each of the three treatment conditions are presented in Figure 1. In the one blocked-nare condition, weights of the chicks with the left vs. right nare blocked were not significantly different on any day; therefore, these 2 sub-groups were combined for the analyses. At the initial weighing performed immediately following the application of acrylic cement (Day 2), there were no significant differences between the weights of chicks in the three conditions (ANOVA,  $F = 1.25$ ,  $df 2/93$ ). However, on the day following the treatment (Day 3), and on all subsequent weighing days, one-way ANOVAs revealed statistically significant differences in body weights between treatment conditions. A posteriori comparisons of the differences between all pairs of means (Scheffé test) [16] further indicate that the chicks that had both nares blocked weighed significantly less than the control chicks and those with one nare blocked on Days 3, 4, 5 and 8 ( $P < 0.001$ , in each instance). There were no reliable differences in the body weights of chicks with one blocked nare versus the control chicks on any day.

Additional analyses assessed the absolute amount of weight gained between successive measurements. Beginning on Day 3, and for each assessment day throughout the remainder of the experiment, there was a significant difference between conditions in the absolute amount of weight gained since the preceding weighing trial (i.e. between condition comparisons for absolute weight gain over Days 2–3, 3–4, 4–5 and 5–8; in each instance,  $P < 0.0001$ , ANOVA). Over each of these intervals, the chicks with both nares blocked gained significantly less weight than did either the control chicks or those with one blocked nare ( $P < 0.01$ , Scheffé test); there were no significant differences in weight gain between the latter two conditions.

A final set of comparisons considered the relative gain in weight across treatment conditions; i.e. increase in body weight during the interval between 2 successive weighings/initial weight. Significant between-treatment differences in relative weight gain were found over Days 2–3 and 3–4 (ANOVA,  $P < 0.001$ ), but not between Days 4–5, or 5–8 (see Fig. 2). Between Days 2–3, as well as Days 3–4, the increase in relative weight was significantly less for chicks with both nares blocked compared to chicks with one blocked nare or those in the control condition ( $P < 0.01$ , Scheffé test); no significant differences in relative weight gain were found between the latter 2 conditions.

#### 3.2. Odor perception test

The responses of chicks in all 3 conditions during the odor perception tests are summarized in Figure 3. The results of one chick in the control condition were excluded because the squeeze bottle containing mint extract accidentally came into contact with the chick's beak during the test trial. As indicated in Figure 3, the magnitude of the response to mint was significantly greater than that observed during the



**Figure 1.** Mean (SE) daily body weight (g) of chicks in each of the 3 treatment conditions: controls; one-nare blocked; both nares blocked. For a given treatment day, means with different superscripts differ significantly ( $P < 0.001$ , Scheffé test).

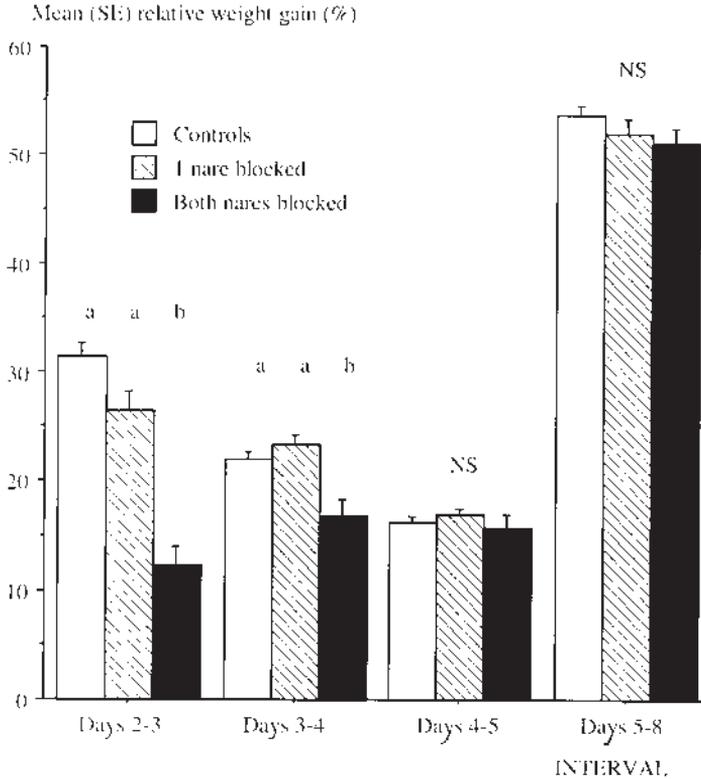
water exposure trials for the chicks with one blocked nostril, as well as the control chicks. In contrast, the behavior of chicks that had both nares blocked did not differ as a function of the exposure stimulus, i.e. the majority of chicks in this condition displayed no overt reaction to either mint (score = 0 for 26/32 chicks) or water (score = 0 for 30/32 chicks).

Comparisons across the 3 conditions revealed significant differences for the responses to mint (Kruskal-Wallis test,  $H = 18.70$ ,  $n = 95$ ,  $P < 0.0001$ ). The results of further analyses (Mann-Whitney U test) indicate that the magnitude of the response to mint was significantly lower for chicks with both nares blocked than for either the chicks with 1 blocked nare ( $Z = 3.46$ ,  $n = 32/32$ ,  $P < 0.001$ ) or those in the control condition ( $Z = 4.01$ ,  $n = 32/31$ ,  $P < 0.0001$ ).

The strength of the responses to mint did not differ between the latter 2 conditions ( $Z = 1.26$ , ns). Similar between-condition comparisons found no statistically significant differences for the water-exposure trials (Kruskal-Wallis test,  $H = 2.10$ ,  $n = 95$ ). Responses by chicks that had their left nare blocked were similar to those with the right nare blocked (one nare blocked condition) when exposed to mint and to water.

#### 4. DISCUSSION

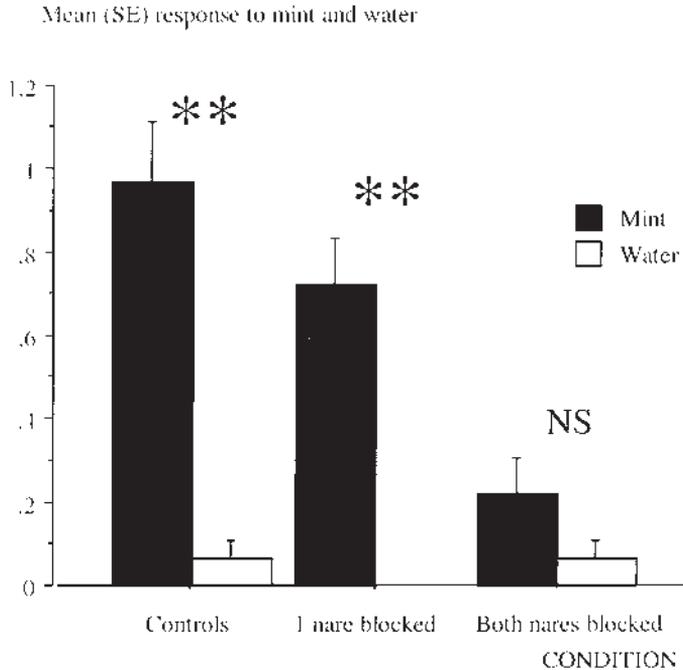
Application of acrylic cement over the external nasal openings of one-day old chicks had a negative effect on subsequent weight gain. On the day following the treatment, chicks that had both nares blocked weighed significantly less than either the



**Figure 2.** Mean (SE) relative weight gain (%) for chicks in each of the 3 conditions during the intervals between successive measures. For a given interval, means with different superscripts differ significantly ( $P < 0.01$ , Scheffé test).

control chicks or those with 1 blocked nare, and this weight difference was still evident when the final weight measure was recorded six days later (post-hatch day 8). Throughout the experiment, chicks with 2 blocked nares gained less absolute weight between successive assessments, than did chicks in the other 2 conditions. Similar deficits in relative weight gain by chicks with 2 blocked nares were observed on Days 3 and 4 (i.e. one and two days after treatment), but were no longer evident on Days 5 and 8. Thus, the relative weight gain by chicks in this condition was at control levels 3 days after both nares were blocked; even though their absolute body weight remained lower.

Because the chicks with one blocked nare did not differ from the controls, it is unlikely that the reduced weight gain by chicks that had both nares blocked reflects pain or discomfort caused by direct contact of the acrylic cement with the nasal openings. It is possible, however, that altered breathing patterns by the chicks with 2 blocked nares could have affected food and water intake to some extent. Since they were unable to breath through the beak, chicks in this condition had to breath through the mouth, which could have initially interfered with their feeding and drinking behavior. Nevertheless, the results of the odor perception test suggest that the observed weight differences between the



**Figure 3.** Mean (SE) responses (0–2 magnitude scale) to Mint and Water by chicks in the 3 treatment conditions (\*\*  $P < 0.001$ , Wilcoxon test).

treatment conditions may have been mediated at least partially by olfactory deficits suffered by chicks that had both nares blocked. That is, the response to mint odor stimulation was significantly lower in this condition as compared to that displayed by chicks with 1 blocked nare or the control group. Moreover, while mint elicited greater responses than did water exposure by chicks in the latter 2 conditions, there were no differential responses to these two stimuli by chicks that had both nares blocked. Since chicks in all 3 conditions displayed little overt reaction during the water-exposure trials, the reduced responsiveness to mint by chicks with 2 blocked nares cannot be explained by impaired sensitivity to airflow directed to the beak during the stimulus presentations. It can therefore be concluded that odor perception was disrupted when both nares were cov-

ered with cement plugs, but not when only one nare was blocked. This treatment was not 100% effective, however, since a small number of chicks in this condition responded to mint odor on Day 5 (score > 0). It is likely that gaps or cracks occurred in the cement plugs as the chicks grew (for all conditions, median body weight increased at least twofold during the experiment), thereby allowing passage of air through the nasal openings. Although the olfactory perception test used in this experiment revealed clear differences between the chicks with both nostrils blocked and those in the other 2 conditions, it is possible that the manual restraint may have induced tonic immobility in some individuals. This might account, in part, for the moderate (mean) magnitude of response to mint odor displayed by the control chicks and those with one blocked nare.

Perhaps the most plausible means by which olfactory perception could ultimately influence chicks' body weight involves the role of that sensory modality in the development of feeding behavior. As discussed above, several authors have reported that the introduction of novel scents has a negative impact on feeding activity and food consumption [2, 10, 15]. Moreover, there is evidence that chicks are preferentially attracted to feeders scented with a familiar odorant [15]. It is also possible that odor familiarity may account (at least partially) for the observed weight differences between the treatment conditions in the present experiment. For chicks with intact chemosensory systems (controls and chicks with one nares blocked), the odor of their feed would become increasingly familiar as a function of continual exposure to that diet. In contrast, chicks with an impaired olfactory sense (those with both nares blocked) would be unable to recognize the characteristic scent of their feed and therefore less readily become familiarized with their diet. Decreased weight gain by the chicks with 2 blocked nares might then reflect reduced intake of feed perceived as somewhat novel.

Although the chicks with both nares blocked evidenced reduced chemosensory capabilities on Day 5, their rate of relative weight gain was not different from that of control chicks or those with one blocked nares, at that time. Thus, 3 days after the treatment, they were able to compensate effectively for their continuing sensory deficits. It should also be noted that the chicks with 2 blocked nares gained weight throughout the experiment despite the negative effect of this treatment on relative weight gain (compared to the other two conditions) during the first 3 days. It is therefore evident that fully functional chemosensory processes are not necessary for food intake by chicks, even though these modalities are implicated in normal feeding behavior.

The results of the present experiment are further evidence of the biological significance of the sense of smell for newly hatched chicks. In practice, however, this sensory modality is typically ignored by poultry breeders. Aside from olfactory influences on growth rate and feeding behavior, ambient odors may be "reassuring" or elicit fearfulness, depending upon their degree of novelty/familiarity [4]. Greater appreciation of the importance of chemosensory perception might therefore have a positive impact on chick breeding and welfare.

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