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Social features of scent-donor mice modulate scent marking of C57BL/6J recipient males

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Abstract

Territorial male mice can form familiarity-dependent amicable relationships, suggesting that they manage their territorial aggressiveness based on individual recognition, which may be mediated by olfactory signals. The present study demonstrated modulatory effect of odorant cues from mice of different social/housing groups on territorial scent marking by C57BL/6J males. Pair-housed males deposited few scent marks in a novel situation without mouse odors, while singly-housed males marked more in the same situation (Experiment 1). However, when confronted by a conspecific, singly-housed males made fewer marks to pair-housed than to singly-housed stimulus males, while pair-housed males showed few marks to either stimulus animal (Experiment 2). Reduction in scent marking in singly-housed males was also seen on exposure to urine scent alone from a pair-housed male, indicating that the inhibitory cue is mediated by urinary odor (Experiment 3). This inhibitory odor was effective even when singly-housed males were placed in a no-odor environment following exposure to soiled bedding used by pair-housed males (Experiment 4). When singly-housed males were exposed to scent from subordinate males, they showed less marking than when the stimulus scent was from dominants or singly-housed males (Experiment 5). Scent marking was not influenced by the total amount of urine scent marks deposited on the substrates (Experiment 6), suggesting that it is a particular component in urinary odor rather than the magnitude of the odor, that plays a significant role in recipient behavior. Together, these experiments indicate that conspecific male odor signals modulate territorial scent marking behavior in mice.

Keywords

Urinary scent; odorant communication; housing effect; dominance; C57BL/6J mice; amicable relationships; chemosignals

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1. Introduction

Mice are a territorial but aggregative species capable of forming complex social relationships: They readily seek social contact [21,77–79]. An important system of communication between mice is through scent emanating from the body, particularly through urinary scent marks deposited on the substrate [7,13,30,34–35,50]. Male mice use urinary scent to mark territory and maintain dominance-subordinate relationships [29–30,35,72]. Territorial males can be highly aggressive towards unfamiliar males in their territories [21,75]. Dominant males, more than subordinates, deposit urine marks around their territory to advertise their dominance over the area, and use the presence of their own odor to recognize the area that they defend [22, 35,73]. Thus, they are more aggressive towards unfamiliar males when surrounded by their own odor cues [41,46] and they avoid areas spotted with urine from foreign males [42–43, 45,47]. This suggests that urinary odor is used for territorial boundary marking [31,33,72].

Urinary odor modulates social behaviors and organization of mice [86]. Male mice deposit scent marks when they find urine odor from other genetically different males but show only baseline levels of marking if they cannot detect any foreign odor in a familiar territory [36, 38,40,73]. Although both dominant and subordinate males investigate scent marks of other males to a similar extent [35,37], they tend to show more investigation of the urinary odor of subordinate males than that of dominants [35,48,66]. Subordinates mark more to subordinate odor than dominant odor while dominants show equal amounts of countermarking toward subordinates and dominants [40]. However, subordinates make fewer urine marks than do dominants [22,24,42],

Several lines of evidence support a view that the urine of male mice has aggression or avoidance–eliciting potency [15,57–58,61,64–65]. The odor of adult male mice sprayed on juvenile or female mice, to which male mice are generally less aggressive, has been found to induce aggression in adult male opponents [55–56]. Odor in the home cages can also modulate behavior of recipient animals; social investigation and aggression of male mice were both significantly higher in dirty cages which contained feces and urine, compared to clean cages [32]. Complete removal of all olfactory cues in the cage reduced aggression between males while transfer of olfactory cues such as feces and urine in sawdust bedding elicited robust aggression [32,50].

Aggression and scent marking toward conspecifics declines as mice become more familiar with each other and social amicable relationships are established [6–7,72]. This may also be modulated by odorant signaling. The odors of subordinates on substrate are important in maintaining tolerance between familiar males, as the removal of substrate odor results in increased aggression against subordinates by dominants [36,81–82]. Because mice keep their nests clean of urine and feces, the nesting area predominantly contains odors originating from plantar and other body glands rather than odors from elimination products. The transfer of used nesting material from an old cage to a new cage reduced aggression between cage mates, while sawdust soiled with urine and/or feces appeared to intensify aggression [81]. This suggests that male mice may use olfactory cues to both suppress and enhance agonistic interaction among familiar conspecifics to modulate established social relationships.

Isolation rearing induces increased aggression in male mice [8,19,68]. This manipulation may also influence scent marking behavior; singly-housed mice deposit more scent marks on the substrate of a novel environment compared to pair-housed mice, suggestive of enhanced territory formation [5–6]. However, a possible alternative explanation for the housing effect on odorant communication may be that pair-housed animals release inhibitory cues in their cages to maintain amicable relationships, and these are responsible for the reduction in territorial scent marking for group-housed animals. The present study investigated whether

olfactory cues influenced by housing condition modulate social behavior and amicable relationships among male mice, examining whether isolation rearing induces aggression-promoting and/or territory-protecting cues, or, whether pair-housing releases aggression-inhibiting and/or territorial behavior-inhibiting cues. To clarify olfactory modulation of territorial scent marking, the present experiments were designed with combinations of housing condition (singly or pairs) in stimulus animals and in recipient animals. In Experiment 1, the baseline expression of scent marking in a novel environment was assessed in singly- and pair-housed male mice. Experiment 2 explored the effect of presence of singly- or pair-housed conspecific stimuli on scent marking responses in recipient males. In Experiment 3, odor stimulus effects were investigated using three odor conditions (no odor, urinary scent, or a living, pair-housed, animal). In Experiment 4, male mice were exposed to three different conditions (clean sawdust, or soiled sawdust used by singly- or pair-housed males) prior to the test, to evaluate whether pre-exposure of odor cues influences territorial scent marking behavior in a novel, no-odor environment. In Experiment 5, male mice were confronted with soiled substrates from mice that were singly-housed, or, were dominants or subordinates. Experiment 6 examined whether scent marking response of male mice was influenced by the amount of scent marking on substrates, rather than differences in housing condition of the stimulus animals. All of these experiments utilized C57BL/6J (B6) mice, an inbred strain often used as a background for transgenic mice and in studies of social behavior [4,12,20,80] as subjects.

2. Materials and methods

2.1. Animals and housing conditions

Male C57BL/6J mice bred from stock obtained from the Jackson Laboratory (Bar Harbor, ME), were used as the subjects. All subjects were weaned at 24–26 days of age, and then housed, in groups of 2–3 same sex littermates, in standard polypropylene cages, 26.5 × 17 × 11.5 (H) cm, under 12L:12D cycle (lights on 06:00) in a temperature- (22±2 °C) and humidity- (60 %) controlled room at the University of Hawaii Laboratory Animal Services. In all experiments, animals in singly-housed groups were housed individually for 1 week prior to the beginning of the test trials, while those in the pair-housed group were housed in pairs with a same-sex littermate for 1 week prior to the test. All animals were allowed free access to food and water in their home cages. All protocols and animal handling and treatment were approved by the Institutional Animal Care and Use Committee at the University of Hawaii.

Experiment 1—Twenty four B6 mice (25–30 g), 14–16 weeks of age, were used as the subjects. They were assigned into two groups (singly- and pair-housed) (each N=12).

Experiment 2—Twenty four B6 mice (26–30 g), 14–16 weeks of age, were used as the subjects and stimulus animals as well. They were assigned into two groups; singly- or pair-housed (each N=12).

Experiment 3—Twenty four B6 mice (25–28 g), 13–14 weeks of age, were used as the subjects and assigned into two groups; singly- or pair-housed (each N=12). Ten B6 mice (26–29 g), 13 weeks of age, were used as the stimulus animals.

Experiment 4—Twenty four B6 mice (26–29 g), 15–17 weeks of age, were used as the subjects. They were assigned into singly-housed or pair-housed groups (each N=12).

Experiment 5—Twelve B6 mice (25–29 g), 16 weeks of age, were used as the subjects. They were singly-housed for 1 week prior to the test session. Twenty six B6 mice (26–30 g), 15–18 weeks age, were used as the stimulus animals. They were assigned into three groups; singly-

housed, dominants, or subordinates. The stimulus animals in the singly-housed group (N= 6) were maintained for 1 week. The animals comprising the dominant and subordinate groups had been housed in pairs with a same-sex unfamiliar mouse for 1 week prior to the test after which they were identified as dominant or subordinate based on their body weight differences and coat conditions. In C57BL/6 mice, chronic social stress has been shown to induce weight loss [85], while deterioration in coat quality is a standard measure of responsivity to chronic mild stress. A criterion of a minimum 10% difference between the two animals in a pair-cage, plus concordance of coat quality differences, was required before the larger animal, with better coat quality, was designated the dominant, and the remaining animal was designated as subordinate. Animals from pairs not reaching this criterion were not used as stimuli. This criterion does not reflect direct observations of agonistic behaviors, but it does require agreement between two independent measures of validated stress-linked behavior changes in a social situation, presumably reflecting asymmetries in the stress associated with social interactions.

Experiment 6—Twelve B6 mice (26–29 g), 15 weeks of age, were used as the subjects and kept in singly-housed conditions.

2.2 Apparatus

Testing of urine marking was conducted in Polycarbonate cages (46 × 24 × 21 cm) placed upside-down on a coarse gradient parchment (457 × 365 mm, Rough Newsprint paper, Bienfang) substrate. The cage was divided into two equal-sized compartments by a wire mesh screen (1cm squares) that prevented direct physical contact between the subject and the stimulus, but it allowed for olfactory, visual, and auditory cues to be received.

2.3. Procedure

All test trials were conducted during the light phase of the light/dark cycle under dimly lit condition. Previous studies have confirmed that B6 males consistently show scent marking depending on their situation under such condition [5–7]. In general, twenty minutes prior to testing, subjects were moved from the holding room to the experiment room in their home cages. Animals were placed in one compartment of the test chamber cage, the bottom of which was covered by a rough paper. All tests were 20 min in length. For those tests using marked paper substrates, the marked papers were obtained by placing stimulus male mice into the stimulus side of the standard test chambers for 20 min. The paper substrates were left on the floor of the stimulus compartment of the test chamber in order to expose them to the subjects. Between trials, the entire apparatus was cleaned with 15% alcohol and dried with paper towels, and the paper substrate was replaced.

After each trial, urine on the rough paper substrate was fixed by Ninhydrin spray (LC-NIN-16, Criminal Research Products, LLC). After 24 hours of drying, the number of urine marks was measured by placing a transparent grid sheet over the substrate paper and counting the number of grids (each 10 × 10 mm) containing urine marks (maximum: 552 squares).

Experiment 1: Baseline testing of housing effect on territorial scent marking behavior—To clarify the simple effect of housing (singly vs. pair-housed), scent marking was compared for animals that were singly-housed or in pairs and tested in an empty chamber.

Experiment 2: Modulation of scent marking response by opponents' housing condition—To examine the effect of the presence of the conspecifics that were singly- or pair-housed, experiment 2 was designed with a between-subjects factor (subjects: singly or pair-housed) and a within-subject factor (stimulus animals: singly or pair-housed). Each subject (singly or pair-housed) was placed into a test chamber with a stimulus mouse (singly or pair-

housed: randomly selected except for equal numbers) on the other side of the screen. Three days after the first exposure to the test chamber, each subject was exposed to stimulus animals with the opposite housing profile from the previous stimulus animals (cf. if previously exposed to a singly-housed stimulus animal, then to a pair-housed stimulus animal in the second test). The order of the trials with different stimuli was counterbalanced.

Experiment 3: The impact of odor-donors' housing condition on scent marking response—To determine which features of the opponent stimulus animals that were pair-housed influence scent marking response (viz. odor cues, or specific behavior and/or acoustic cues), singly- or pair-housed subjects were exposed to three conditions; No odor (no stimulus mouse); Odor (scent marked papers from male B6 mice housed in pairs); and Pair-housed B6 (a mouse that was housed in pair with a littermate). Note that data was compared only within subjects, to the different stimulus conditions. Trials with different stimuli were conducted daily for successive 3 days, and the order of stimuli that were exposed was counterbalanced in a Latin Square design.

Experiment 4: Modulation of scent marking behavior by pre-exposure of conspecific odor—To determine if there is a residual effect of odor from conspecifics on scent marking, the subjects were placed for 20 min just prior to the test in rearing cages with bedding in which other mice had been housed. There were three condition of cages with bedding with the within-subject factorial design; Clean (clean sawdust bedding); Singly-housed bedding (S-cage, with sawdust from a B6 male mouse that had been singly-housed for 7 days); and Pair-housed bedding (P-cage with sawdust from two male B6 mice pair-housed that was kept unchanged for 3 days). The sawdust bedding in cages where two mice were housed together (pair-housed) were maintained for 3 days prior to the test, while those for singly-housed mice were kept for 7 days, to minimize differences in dirtiness of soiled bedding since a robust aggression was observed in male mice following exposure to dirty cages [32]. These different schedules for the pair- and single-housed stimulus mice were designed to equalize the quantity of scent in the bedding for the singly and paired groups. Immediately after exposure to the rearing cages, the subjects were placed into the test situation with without conspecific odor. The order of the trials with different stimuli was counterbalanced in a Latin Square design. Inter-trial interval was 3 days.

Experiment 5: The impact of social dominance of odor donors on scent marking response—Individual dominant, or subordinate B6 mice (based on the criteria of weight differences within pairs and coat condition, as described under Procedures) or singly-housed mice, were placed into an empty, novel test chamber for 20 min, and then removed. B6 subjects were immediately placed on the other side of the compartment, opposite to the side with paper sheets marked by the stimulus mice, for a 20-min test session.

Experiment 6: The impact of scent magnitude on scent marking response—To investigate the relationship between the quantity of stimulus odor marks and the intensity of scent marking response, singly-housed mice were exposed to a full- or a half-sheet of rough paper scent-marked by a singly-housed male B6 mouse; with within-subject design. For the half sheet condition, the paper substrate was cut perpendicular to the wire mesh screen. The order of the trials (exposures of a full- or a half-, scent-marked sheet) was counterbalanced. Inter-trial-interval was 3 days.

2.4. Statistical analysis

For Experiment 1 and 6, data were analyzed by a Welch's t-test (housed singly vs. in pairs for exp. 1, and full size vs. half size paper for exp. 6). For Experiment 2, a two-way analysis of variance (ANOVA) with a between-subject factor of rearing condition (singly- or pair-housed)

and within-subject stimulus factor (to singly-housed or pair-housed animal) was conducted. For Experiment 3, a two-way ANOVA was used to evaluate between-subject factor of rearing condition (singly- or pair-housed) and within-subject stimulus factors (no odor, odor, or pair-housed B6). In Experiment 4, a two-way ANOVA involved a between-subjects factor (single- or pair-housing) and a within-subjects factor (stimulus: clean, singly-housed, or pair-housed cage). For Experiment 5, a one-way between-subjects ANOVA was used for analysis of the number of scent marks made by singly-housed, dominant, or subordinate animals, on papers that were to be used as stimuli in the study. A within-subjects ANOVA was used to analyze the marking data from the singly-housed stimulus animals exposed to these marks. Post hoc comparisons used the Bonferroni test for within-subject factors and the Tukey's HSD test for between-subject factors. A probability level of $p < .05$ was adopted as the level of statistical significance for all analyses.

3. Results

3.1. Experiment 1: Baseline testing of housing effect on territorial scent marking behavior

Figure 1 shows the mean squares with urine marks for each group of male B6 mice when exposed to the empty chamber for 20 min. Singly-housed mice deposited significantly more scent marks in the empty chamber than those housed in pairs, $t(11) = 6.572$, $p < .001$.

3.2. Experiment 2: Modulation of scent marking response by opponents' housing condition

Figure 2 shows the mean squares with scent marks that were deposited by singly- or pair-housed mice, when exposed to a stimulus animal that was singly- or pair-housed. A two-way ANOVA conducted on these scores revealed a significant main effect of rearing condition, $F(1,22) = 11.94$, $p < .01$, and of stimulus animals, $F(1, 22) = 6.40$, $p < .05$, and a significant interaction between rearing condition and stimulus animals, $F(1,22) = 4.62$, $p < .05$. Subsequent analysis indicated that singly-housed mice showed significantly more scent marking than pair-housed mice in both stimulus conditions, with pair-housed subjects showing lower scent marking toward either singly- or pair-housed mice. Singly-housed mice showed lower scent marking toward pair-housed mice than toward singly-housed mice.

3.3. Experiment 3: The impact of odor-donors' housing condition on scent marking response

Figure 3 depicts the mean squares with scent marks which were deposited by singly- or pair-housed mice, under each of three conditions; no odor, odor, or a pair-housed B6 male mouse. Note that the stimulus odor for the odor condition was the marked paper from a pair-housed B6 mouse. A two-way ANOVA conducted on the scores for singly- and pair-housed mice found a significant housing effect, $F(1,18) = 27.94$, $p < .001$, and stimulus odor type effect, $F(2,36) = 11.70$, $p < .001$. The interaction between housing and odor type was also significant, $F(2,36) = 6.59$, $p < .01$. For singly-housed mice, both exposure to scent marks from a pair-housed B6 and a pair-housed B6 mouse induced a decrement of scent marking compared to the empty chamber condition (no odor) ($p < .01$). For pair-housed mice, no significant stimulus effect was found. Singly-housed mice showed greater scent marks than pair-housed mice when exposed to scent marks from a pair-housed B6 or to the empty chamber (no odor) ($p < .05$). These data indicated that odor scent from a pair-housed B6 also induced a reduction of scent marking in recipient B6 males that were singly-housed, but not when these were pair-housed.

3.4. Experiment 4: Modulation of scent marking behavior by pre-exposure of conspecific odor

Figure 4 displays the mean squares with scent marks deposited by singly- or pair-housed mice, in a no-odor chamber following exposure to each of three conditions. A two-way ANOVA found significant main effects of stimuli, $F(2,44) = 10.908$, $p < .001$, and rearing condition, F

(1,22) = 17.151, $p < .001$. The interaction between stimuli and rearing condition was also significant, $F(2,44) = 6.619$, $p < .01$. Subsequent analyses indicated that singly-housed mice deposited lower scent marks following exposure to the cages containing soiled sawdust occupied by pair-housed mice than either following exposure to clean sawdust cages or soiled cages occupied by other singly-housed mice. Pair-housed mice, on the other hand, showed no differences in scent marking following pre-exposures to these conditions.

The result indicated that exposure to a cage in which pairs of B6 males were housed together prior to the test elicited a decrement of scent marking in singly-housed B6s, while this was not observed in pair-housed B6s.

3.5. Experiment 5: The impact of social dominance of odor donors on scent marking response

Figure 5a depicts the mean squares with scent marks for each group of stimulus animals; singly-housed, dominants, or subordinates, in a novel, no-odor environment. These are presented to demonstrate the quantity of scent marks deposited by the stimulus animals, as these same scent marks, immediately after being collected, were used as stimuli for subjects of Experiment 5. An ANOVA found significant differences between groups, $F(2,15) = 4.980$, $p < .05$. Both singly-housed and dominant mice deposited more scent marks in the empty chamber than subordinate mice.

Figure 5b shows the mean squares with scent marks for mice confronted with the paper substrates described just above, containing urinary scents of singly-housed, dominant, or subordinate males. An ANOVA found that mice confronted with urine scent from singly-housed mice and dominant males deposited more scent marks compared to those exposed to urine scent from subordinate males; $F(2,22) = 10.771$, $p < .001$.

The results indicate that subordinate males showed lower marking than either singly-housed or dominant males in a novel no odor environment. Singly-housed males responded to exposure of scent marks depending on the characteristics of the odor donors; they revealed a reduction of scent marking response to urine from subordinates compared to those from dominants or singly-housed males.

3.6. Experiment 6: The impact of stimulus scent magnitude on scent marking response

Figure 6a shows the total number of squares with scent marks on the substrates used as the full size or half size paper stimuli, confirming that the half size paper did indeed contain about half the scent marks of the full size paper. Figure 6b shows the mean number of squares with scent marks made by singly-housed mice in response to these stimulus papers. There were no significant differences in marking to full or half size paper substrates, $t(11) = 0.1776$, n.s. These findings indicate that the amount of scent marks deposited by singly-housed males on substrate did not determine the magnitude of scent marking response to these substrates in singly-housed subject (recipient) mice.

Through all experiments, the size of urine pools and the number of fecal boli for each group did not show significant differences for any subject or stimulus conditions, or interactions of these.

4. Discussion

In the present experiments, B6 male mice were allowed to scent mark on one side of a wire mesh barrier with a stimulus mouse or its odor cues on the other side. In these conditions, subjects could smell volatile chemical signals and/or contact stimulus animals through the wire mesh, but could not engage in full physical contact with these stimuli. In experiment 1 singly-

housed mice showed robust scent marking of the novel, no-odor, situation, while pair-housed mice displayed less, consonant with the importance of cage mates in territorial behavior in a novel situation; e.g. isolation-induced territorial aggression in male mice [e.g. 8,19,68]. Experiment 2 showed that pair-housed mice deposited few scent marks to either singly- or pair-housed stimulus animals, while singly-housed mice displayed substantial scent marking to singly-housed male mice with reduced marking to pair-housed males. This suggests that pair-housing of male mice reduces their ability, as stimuli, to elicit scent marks in the opponents. However, the result showing that singly-housed mice marked substantially both in the no-odor situation and to singly-housed stimulus males, suggests that odor-related cues of pair-housed mice may have an inhibitory effect on scent marking response in odor-recipient males.

Experiment 3 demonstrated that the inhibition of scent marking associated with pair-housed stimulus animals was mediated by odorant scent from body glands or urine. A pair-housed B6 mouse, or the urinary scent marks of a pair-housed B6 mouse induced reductions in scent marking of recipient, singly-housed mice, indicating consistent inhibitory effects of these stimuli. That odorant scent from pair-housed males strongly reduces scent marking is consonant with previous findings of aggression-inhibitory pheromones [cf. 23,56,59–61,81]. Male B6 subjects were prevented from direct contact to scent odor of stimulus animals by wire-mesh screen, suggesting that volatile components released from the urinary scent odor of pair-housed stimulus animals play a signaling role in this case. Notably, the pair-housed B6 males in Experiment 2 showed no such marking increment toward singly-housed B6 males, while a previous study [5] showed that pair-housed B6 males scent marked substantially to the presence of a CD-1 mouse that was singly housed. Such differential responses in pair-housed mice depending on the opponents' mouse strain, indicates that for inbred same-strain, stimulus animals housing conditions may play a less significant role in stimulation of scent marking. Since each inbred mouse strain is genetically homogenous, members of the same strain and sex have identical type of the major histocompatibility complex (MHC) [69,83] and express the same pattern of major urinary proteins (MUPs), important mediators of chemical messages between male mice [16,50,67,74]. Genetic differences in patterns of non-volatile urinary chemicals are known to influence counter-marking from competitive males [33,37,39]. The present experiments demonstrated that volatile odor scent from pair-housed males inhibits scent marking in recipients, and this inhibition does not require genetic differences in odorant signatures.

The finding that pair-housed male mice showed little scent marking even in a novel, no odor environment (Experiment 1) suggests that an odorant cue in their home cage might be capable of inhibiting scent marking in a no-odor environment to which the mouse was successively exposed. In Experiment 4, singly- or pair-housed male mice were exposed to urinary odors from pair- or singly-housed male mice prior to testing. Pair-housed mice, with low baseline levels of marking, showed no changes in scent marking with pre-exposure to odor compounds. However, singly-housed males showed high baseline marking, which declined following exposure to a cage containing odor from pair-housed, but not singly-housed, mice. The selective reduction of scent marking in singly-housed mouse subjects indicates that odors in cages in which two adult males had been housed can provide an inhibitory cue for territorial scent marking of male conspecifics in a no-odor environment. It suggests that recent experience of odors from cage mates may be responsible for suppression of scent marking in pair-housed mice in no-odor tests.

Several lines of evidence indicate that behavioral characteristics of pair-housed animals that show unclear social dominancy between cage mates, such as the littermate pair-housed males of the present studies, are likely to be similar to those of subordinates compared to dominants [2–3,11,26]. Recently, Pohorecky [70] demonstrated that housing condition (singly or grouped) and social ranks (dominants, subdominants, and subordinates) have significantly

different effects on the composition of volatile compounds in preputial glands of male rats. This finding suggests that such social ranks also influence the properties of functional chemosignals in urinary scent. In Experiment 5, dominant stimulus males scent marked substantially in a novel, no odor environment, as did singly-housed mice, while subordinates showed reduced marking. Dominant males produce a large number of tiny urine spots and distribute them widely [22,24,72] as do singly-housed mice. In contrast, subordinates tend to deposit a few large urine pools, usually at the edge of a test arena [22,40], similar to the scent marks of pair-housed mice. Although the findings of Experiment 5 are compatible with a view that dominant and subordinate mice show consistent differences in both scent-marking and in response of other mice to their scent marks, these designations were based on indirect, rather than direct measures of social dominance, suggesting the value of additional work providing more direct measures of within-pair agonistic behaviors in this context.

Present findings that subordinate and pair-housed mouse show reduced marking, and also that their scent mark stimuli may elicit less marking from a recipient mouse compared to scent stimuli from dominants or singly-housed males (Experiment 5) suggests that the quantity of the stimulus marks may determine the magnitude of the elicited response. However, the finding of Experiment 6 that singly-housed mice deposited substantial and similar quantities of scent marks in response to conspecific marks varying in amount (approximately 2:1) suggests that qualitative, rather than quantitative differences in scent mark stimuli are the major determinants of scent marking responses to these stimuli. In this study, all pair-housed subject males were housed with their littermates from weaning, and there was no evidence that clear dominance relationships between was formed by cage mates. This argues against a view that subordination-induced suppression of scent marking is responsible for the present finding of little scent marking in pair-housed males. The present result indicating that subordinate and pair-housed mice may selectively release an odor signal that inhibits territorial scent marking in odor-recipient conspecifics (an amicable signal?) is compatible with findings that subordinate mice, but not dominant or singly-housed mice, show a conditioned place preference for a chamber associated with their own bedding odors [27]. Such a view may be consonant with the classic finding that group-housed males are less aggressive than singly-housed males regardless of establishment of their social dominancy [12,17,19].

An amicable odor-mediated signal is also compatible with results of a number of other studies of odor effects on mouse sociality. Individual scents within a group of mice may contribute to maintaining group familiarity due to scent transfer between group members that may occur through contact with each other and shared substrate scents [1]. Familiarity of scent cues is a major factor influencing aggression and scent marking behavior between male mice [6,17, 54]. Male mice can maintain stable groups so long as each member of the group continues to contribute to the shared substrate odor [36], in which the familiarity of shared scent cues among inbred mice contributes to increased tolerance and reduced aggression [38,62–63].

Mouse inbred and outbred strains show substantial variation in aggressiveness toward conspecifics [9–10,12,26] and individual housing is well known to prompt territorial aggression in mice [19,68]. Nonetheless, the high adaptability of mice to complex social situations such as emigration and dispersal [14,28], flexible group formation [51–52,84], and performance of communal nesting [49,53,76], suggests the existence of a mechanism for maintaining familiarity-based reciprocal relationships in mice. This high adaptability to social changes, shown also in a number of findings in the present study, may be mediated by odorant signals released in urine scent marks. The present finding additionally suggests that odorant signals reflecting such conditions as pair-housing and subordination are not primarily mediated by quantitative changes in amount of scent marks, but may involve a qualitative change in odor cues, serving as a territorial scent marking - inhibiting signal.

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In no-odor situation

Figure 1.

The mean number of squares with scent marks on the substrates in no-odor chamber for each group of singly- or pair-housed mice. Data are expressed as mean $\pm$ S.E.M. Significant differences between groups; ** $p < .01$.

Figure 2.

The mean number of squares with scent marks on the substrates for each group of singly- or pair-housed males, when confronted with a stimulus animal of singly- (to S-st) or pair-housed (to P-st) via wire mesh. Data are expressed as mean $\pm$ S.E.M. Significant post hoc differences between groups; * $p < .05$, ** $p < .01$.

a) Singly-housed males **b) Pair-housed males** **Figure 3.**

The mean number of squares with scent marks made by singly-housed (a) or pair-housed (b) males. They were confronted with each of three stimulus conditions; No odor (subjects alone), Odor (scent marks deposited on the paper substrate by a pair-housed B6), and pair-housed B6 (A stimulus B6 mouse that was pair-housed). Data are expressed as mean $\pm$ S.E.M. Significant post hoc differences between groups compared to No-odor condition; * $p < .05$.

a) Singly-housed males **b) Pair-housed males** **Figure 4.**

The mean number of squares with scent marks made by singly-housed (a) or pair-housed (b) males. They were exposed to an empty chamber immediately following a 20-min placement into one of three different cages; Clean (cages with clean sawdust), S-cage (cages with soiled sawdust occupied by a singly-housed mouse prior to the test), and P-cage (cages with soiled sawdust in which two mice were housed together). Data are expressed as mean $\pm$ S.E.M. Significant post hoc differences between groups compared to Clean cage condition; * $p < .05$.

a) Stimulus odor **b) Singly-housed males** **Figure 5.**

The mean number of squares with scent marks on the substrate for each group of (a) stimulus animals and (b) the subjects. Stimulus animals were divided into three conditions; Singly (A singly-housed male), Dominants, or Subordinates, were placed in a novel, no-odor environment for 20 min. After urinary scent was collected from the stimulus animals, singly-housed subjects were exposed to each of these urinary scents on the other side of a wire mesh for 20 min. Data are expressed as mean $\pm$ S.E.M. Significant post hoc differences between groups compared to Singly condition; * $p < .05$.

a) Stimulus odor **b) Singly-housed males** **Figure 6.**

The mean number of squares with scent marks for each group of (a) the stimulus animals or (b) subjects. The paper substrates from singly-housed stimulus animals were cut in half for the half size group and were not cut for the full size group. Each of these paper substrates was exposed to a singly-housed subject for 20 min. Data are expressed as mean $\pm$ S.E.M. Significant differences between groups; * $p < .05$.