Huddling Behavior as Critical Phase Transition Triggered by Low Temperatures

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Received April 23, 2010; accepted November 8, 2010

Huddling is a grouping behavior where animals maintain close bodily contact and save energy. We tested the hypothesis that this thermoregulatory behavior behaves as a system with continuous (second-order phase) transition called critical when the environmental temperature (driving parameter) is near a critical value. To do so, we followed theoretical and experimental approaches, examining data from geometrical models, metabolic rate during huddling in small mammals, and also conducting an experiment on thermoregulatory huddling behavior with white mice. Our results support all predictions for systems under continuous-phase transition triggered by low temperatures, a phenomenon reported for the first time in a biological system. We suggest that huddling behavior in social animals, a recognized adaptive behavior, may be considered a self-organized system coupled with an external driving parameter. © 2011 Wiley Periodicals, Inc. Complexity 00: 000–000, 2011

Key Words: huddling; self-organization; energy

INTRODUCTION

Self-organization is central to the description of physical and biological systems and is likely to operate at all levels of integration, including social organization [1, 2]. Among social animals, for example, there are behavioral interactions producing group cohesion. These interactions are viewed as a network of nonlinear connections among the multiple components of the system [3–7]. The rules guiding group organization depend on several key factors: (i) the presence of leaders, (ii) group pattern building directed by a representation of the spatial or temporal relationships of the parts of a pattern (blueprints), (iii) recipes that each individual of the group possesses to build the whole pattern, and (iv) full-size mold that specifies the final pattern (templates) [8]. According to Camazine et al. [8], in terms of genetic coding all these alternatives are energetically costly. In contrast, a system in which a structure or pattern appears from the local interactions of the elements that make up the system, without central authorities or external elements imposing this pattern (i.e., a self-organized biological systems), be quite economical in both physiological and behavioral machinery; therefore, they are more likely to evolve through natural selection [8]. Also, self-organization may be an important mechanism allowing individ-
Huddling induces metabolic depression without hypothermia [28] and is mainly attributed to the reduced surface area/volume ratio of the huddling group (but see Ref. 31). Indeed, on the basis of geometrical approaches, previously, we [26] proposed a general model to account for the reduction of the relative exposed area of grouped animals and for the decrease in metabolic energy expenditure during huddling behavior in small rodents. Here, we propose that huddling in our animal model (white mice = *Mus musculus*; CF-1), a small rodent that usually show this behavior, exhibits characteristics of a critical state system and may emerge as an outstanding example of self-organization with continuous-phase transition in natural systems. If this is accurate, near the phase transition the system could be not sensitive to the constituents or the interactions operating between them, falling into one of a limited set of universal classes that are defined by the exponents in the power laws describing the phase transition. Consequently, to test if huddling behavior near a phase transition becomes critical, we studied social thermoregulation (huddling) in white mice through both theoretical and experimental approaches.

In the theoretical approach, we explored different geometrical arrangements of huddling behavior through changes in the number of grouped individuals (*n*) and their consequences on mass-specific metabolic decrease. We considered a Euclidean array if during huddling there is a tendency to a spherical form, and autosimilar if during huddling there is a tendency to conserve the individual form, that is, if a group can be viewed as composed of parts that are similar to the whole group. Thus, the metabolic response allows us to infer indirectly the geometric assembly used by the grouped animals. Second, we empirically studied huddling behavior in white mice and compared our results against our theoretical proposals by testing if they follow the self-organized system under the critical conditions [9].

**ANIMALS AND METHODS**

We studied thermoregulatory huddling in 15 female laboratory white-mice CF-1 [body mass = 35.1 ± 3.6 g (average ± standard deviation)] at eight environmental temperatures (*T*<sub>a</sub>): 35, 32, 28, 24, 20, 16, 12, and 5 °C. Each temperature was maintained 2 days. Previously, animals were maintained all together at room temperature and light:dark cycle = 12:12 with rat food pellet and water ad libitum.

Animals were exposed to experimental conditions and videotaped (JVC GR-D350U) for 60 min from the top of the setup. All experiments started at 18:00 h. We used a circular black-painted open field (0.85 m<sup>2</sup>), which was maintained inside a thermoregulated climatic chamber. Recordings were made every 2 days. To avoid the analysis...
of early exploratory behavior, only the last 30 min of each tape record was analyzed through random selection of five frames per test. An $8 \times 8$ grid was superposed on each picture, and the number of grouped and nongrouped individuals was counted (Figure 1). Groups were all aggregations where individuals exhibited physical contact. Thus, group size varied between 2 and 15. We measured the size of each group and counted the number of individuals within each grid cell. The variance-mean coefficient (CVM) of the number of individuals in each grid cell was defined as an index of huddling, an index that detected a random location versus an aggregation pattern. As a direct measurement, we determined the distance ($d$) by the following four rules: (i) $d$ between two individuals was the minimum distance without the tail, (ii) $d$ of one individual to a group was the minimum distance of the individual to the entire group, (iii) $d$ between individuals of the same group was 0, and (iv) $d$ between one individual within the group and an external one was considered as case ii. In each picture, we measured the distance between pairs of individuals and calculated the mean distance ($D$), allowing us to obtain five values for each temperature. Also, we estimated the fractal dimension of the edge (=surrounding area) of the white-black silhouettes of each group ($f_d$) by the box-counting method (five per test), implemented with the

**Figure 1**

Huddling behavior of *Mus musculus* in the open field. Animals were exposed to different environmental temperatures. (A) 28°C, (B) 20°C, (C) 16°C, and (D) 5°C (see text).
software BENOIT® 1.2. A fractal is characterized by a fractional $f_a$, where the more convoluted geometry, the greater their $f_a$. For example, a convoluted line could have $f_a = 1.93$, approaching the dimension of a surface ($f_d = 2$). Thus, $f_a$ allows us to estimate directly the convolution of the geometry of the huddling group.

Because we followed a repeated measurement design, CVM of the number of individuals as well as $f_a$ parameters among temperatures were compared with a Friedman test (five measurements per temperature). As an attempt to search for the critical temperature ($T_c$) and independent of the fundamental measurement ($D$), we conducted a piecewise linear regression between CVM of the number of individuals and $T$. CVM $= (b_0 + b_1 T_d)$ ($T_a > T_d$) with the Levenberg-Marquardt algorithm implemented in the STATISTICA® software, determining the $T_d$ value at which the coefficient of determination ($R^2$) was maximum. Based on the power law $D = k (|T_a - T_c| / T_c)^n = k r^n$, which was proposed for temperature-dependent physical-state transitions [9, 32], we performed a linear regression of log ($D$) on log ($r$) and obtained the critical exponent $\alpha$. We performed a sensibility analysis through changes in $T_d$ and the number of points in the regression from five to three points nearly $T_c$.

**RESULTS**

**Theoretical Test**

Metabolic rate (MR) is related to the surface area of an animal ($A$) and is represented by a power law MR = $m A^\alpha$; this equation also represents the MR of grouped individuals (MR<sub>g</sub>): MR<sub>g</sub> = $m_A A_A^\alpha$, where $A_a$ is the exposed area during huddling; $m$ and $m_A$ are arbitrary constants for grouped and non-grouped individuals [26]. Thus, the ratio MR<sub>g</sub>/MR = $R$, where $R = (m_A / m) R_a$, $R_a$ being the area ratio of grouped versus non-grouped animals. The exponent $\alpha$ may be derived from the empirical relationships between body mass (mb) and the thermal conductance [25–27]. As (i) MR at low temperatures follows the relationship MR = $C (T_b - T_d)$, with $C$ = thermal conductance, $T_b$ = body temperature, and $T_d$ = ambient temperature, (ii) $C$ follows an allometric relationship with body mass of 3.4 mb<sup>0.5</sup> [33, 34], and (iii) assuming that body density is 1, then mb is related to area following mb = $K_m A_d^{3/2}$, with $K_m$ the Meh constant, a parameter that depends on the animal form [35]. Combining (i–iii), Canals et al. [26] proposed that MR = $C (T_b - T_d)$ = 3.4 mb<sup>0.5</sup> ($T_b - T_d$) = $b_o$ mb<sup>0.5</sup> = $b_o$ ($A_d^{3/2}$) = $b_o$ $A_d^{0.75}$, where $b_o$ is a constant. Thus, the exponent $\alpha$ is 0.75, and $R = (m_A / m) R_a^{0.75}$.

**Autosimilar (Fractal) Solution**

As the area of one individual ($A_i$) is $A_i = K_m V_i^{2/3}$, where $V_i$ is the volume of one individual, the area of $n$ non-grouped similar individuals is $A_n = n \cdot K_m V_i^{2/3}$ ($K_m$ is the mammalian Meh constant ($\approx$10]). Assuming our autosimilar argument (and also see Ref. [36]), $n$ grouped individuals may be considered as one large individual made up of $n$ similar individuals; thus, the area of $n$ grouped individuals ($A_n$) is $A_n = K_m \cdot (n V_i)^{2/3}$, and the area ratio ($R_n$) is as follows:

$$R_n = \frac{K_m \cdot n^{2/3} \cdot V_i^{2/3}}{n \cdot K_m \cdot V_i^{2/3}} = n^{-1/3}.$$  

Finally, $R_n = (m_A / m)(n^{-1/3})^{0.75} = c \cdot n^{-1/4}$, with the initial condition $n = 1$, then $R_n = 1$, and consequently $c = 1$. Thus, in an autosimilar arrangement of huddling behavior, we expect that $R_n = n^{-1/4}$.

**Euclidean Solution**

However, the problem with an autosimilar solution is that it predicts a large surface with a limited volume, whereas animals in cold environments attempt to minimize their surface. As animals are unable to change their volume, we suggest that $A_n = K_c \cdot (n V_i)^{2/3}$, where $K_c = 4.83597$ is the sphere-Meh constant. Then,

$$R_n = \frac{K_c n^{2/3} \cdot V_i^{2/3}}{n \cdot K_m \cdot V_i^{2/3} \cdot (K_c / K_m) \cdot n^{-1/3}} = 0.483597 \cdot n^{-1/3} \approx 1/2 \cdot n^{-1/3},$$

and the Euclidean solution is:

$$R_n = (m_A / m) (1/2 n^{-1/3})^{0.75} = 0.58 \cdot c \cdot n^{-1/4} = 0.58 \cdot n^{-1/4}.$$  

In this case, we note that when $n = 1$, $R_n = 0.58$, which means that a geometric change to a sphere-like geometry could lead to a 42% individual metabolic energy saving. That could be, for example, the case of isolated small rodents and birds that exposed to cold tend to a sphere morphology, respectively, bristling fur and feathers.

**Small Mammal Solution**

We previously proved [25, 26] that $R_n$ in grouped deformable spheres and rigid prisms follows $R_n = (\phi / n + (1 - \phi))$, where $\phi$ is a deformation factor $\phi = 2a / A_1$, twice the ratio between the area $a$ lost during grouping behavior and the area of one individual ($A_1$) [26]. Also, empirically we showed that this is true in deformable cylinders and that the area ratio, when included to explain the behavior of $R_n$, fully adjusts to the observed decrease of $R_n$ in several small mammals [26, 27]. These relationships allow arriving at the “small mammal solution”:

$$R_n = (m_A / m) \cdot (\phi \cdot n^{-1} + (1 - \phi))^{3/4} = c \cdot (\phi \cdot n^{-1} + (1 - \phi))^{3/4},$$

Rearranging, this yields:
unable to break their own geometrical constraints, and this solution because during huddling small mammals are expected for an autosimilar arrangement. As an example, the relative reduction in MR during huddling of small mammal solution and the autosimilar solution varies as the exponent of the fractal as well as the Euclidean solution. The minimum values are always obtained at low values of \( n \), between 2 and 8. The function intersects with the fractal solution at \( n^* \) = 5, 10, and 30 for \( \phi \) values of 0.5, 0.6, and 0.7, respectively (Figure 2). The relative reduction in MR during huddling of small mammals is within the range described by the autosimilar and the Euclidean solutions, respectively (see text). Squares, rhomboids, and triangles represent the small mammal solution for different usual \( \phi \) values.

Thus, the function \( F(n) = [n+(1-\phi)]^{1/4} \) represents deviations of the fractal as well as the Euclidean solution. If \( F(n) \) is close to 0.59, it will be more Euclidean, and if close to 1 it will be more autosimilar. This is a cubic-type function which for values of \( \phi \) ranging from 0.5 to 0.8 decreases to a minimum of 0.68, but does not converge to the Euclidean solution. The minimum values are always obtained at low values of \( n \), between 2 and 8.

The piecewise determination coefficient \( (R^2) \) was maximum, \( R^2 = 0.947 \) \((P < 0.05)\) for a breakpoint \( T_c = 16^\circ C \), but was also high at 20 \(^\circ C\): \( R^2 = 0.859 \) \((P < 0.05)\) and at 12 \(^\circ C\): \( R^2 = 0.878 \), but in this last case it was not statistically significant. Considering \( T_c = 16^\circ C \) and the five points close to the breakpoint, the regression equation between log (D) and log (\( T \)) was as follows: log (D) = 1.672 + (0.272 ± 0.038) log (\( T \)); \( F_{1,2} = 50.43; P = 0.006; R^2 = 0.925 \) (Figure 4). The sensitivity analysis (Table 2) showed that at \( T_c = 16^\circ C \), the variability in the exponent was small: \( \alpha = 0.302 ± 0.044 \), but considering all significant regressions the exponent “\( \alpha \)” ranged from 0.153 ± 0.011 at 15 \(^\circ C\) (five

### TABLE 1

Characteristics of Huddling at Different Environmental Temperatures (\( T \))

<table>
<thead>
<tr>
<th>( T ) ((^\circ C))</th>
<th>( P ) (%)</th>
<th>( S )</th>
<th>( I_d )</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>32</td>
<td>2.6</td>
<td>1.25 ± 0.09(^a)</td>
</tr>
<tr>
<td>32</td>
<td>51</td>
<td>2.6</td>
<td>1.29 ± 0.07(^b)</td>
</tr>
<tr>
<td>28</td>
<td>48</td>
<td>3.0</td>
<td>1.37 ± 0.03(^c)</td>
</tr>
<tr>
<td>24</td>
<td>48</td>
<td>5.8</td>
<td>1.34 ± 0.03(^c)</td>
</tr>
<tr>
<td>20</td>
<td>45</td>
<td>3.5</td>
<td>1.34 ± 0.07(^c)</td>
</tr>
<tr>
<td>16</td>
<td>67</td>
<td>9.3</td>
<td>1.41 ± 0.06(^b)</td>
</tr>
<tr>
<td>12</td>
<td>76</td>
<td>8.0</td>
<td>1.46 ± 0.03(^a)</td>
</tr>
<tr>
<td>5</td>
<td>91</td>
<td>14.5</td>
<td>1.52 ± 0.05(^a)</td>
</tr>
</tbody>
</table>

Proportions \((P)\) of individuals recorded during huddling, values of the median size of each group (\( S \)), and fractal dimensions \((I_d)\) of white-black silhouettes of groups (mean ± 1 standard deviation). Similar letters indicate similar values after nonparametric multiple comparisons.

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DOI 10.1002/cplx
points) to 0.303 ± 0.039 at 16°C (four points) with a mean value $\alpha = 0.240 \pm 0.066$. This suggests that independently of the critical temperature and of the number of points considered, the exponent that characterizes the phase transition exhibited low variability.

**DISCUSSION**

In the theoretical approach, we followed previous studies which considered that the reduction in surface volume is the principal factor accounting for the reduction in energy expenditure during huddling. Nevertheless, there are alternative explanations for this reduction. In fact, the increase in ambient temperature caused by huddling itself [14, 31], and adjustments in body temperature during huddling and psychophysiological effects [37, 38] have been proposed as mechanistic explanations for MR reductions during huddling. Although Hayes et al. [31] found that the increase in local temperature accounted for 55% of the energetic benefit during huddling in the vole Microtus agrestis, Gilbert et al. [14] reported that in the emperor penguin Aptenodytes forsteri at least two-thirds of energy saving was attributable to the reduction in cold-exposed body surfaces and one-third to the mild microclimate created within the groups. Adjustments in body temperatures and psychophysiological effects appear to have minor effects [36]. Thus, temperature-induced microenvironmental changes by individuals inside a group may effectively affect energy saving during huddling; however, these thermal changes seem to affect locally exposed surface areas of the neighbors, which finally decreases the area exposed to environmental temperature. In other words, temperature-induced microenvironmental changes during huddling seem to act through a reduction of the area exposed to cold, which is extremely relevant among large groups of endotherms such as penguins [13, 14], or during huddling in nests or inside burrows [21].

Although we did not conduct a dynamic analysis of the emergence of huddling, there is evidence that this behavior is a self-organized process. Indeed, Schank and Alberts [12] showed that huddling—as an aggregative behavior—can emerge as a self-organizing process from autonomous individuals following simple sensorimotor rules. Among

<table>
<thead>
<tr>
<th>$T_c$ (°C)</th>
<th>$n = 5$</th>
<th>$n = 4$</th>
<th>$n = 3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.153 ± 0.011*</td>
<td>0.163 ± 0.006*</td>
<td>0.164 ± 0.01*</td>
</tr>
<tr>
<td>16</td>
<td>0.272 ± 0.038*</td>
<td>0.303 ± 0.039*</td>
<td>0.331 ± 0.059</td>
</tr>
<tr>
<td>17</td>
<td>0.238 ± 0.029*</td>
<td>0.261 ± 0.026*</td>
<td>0.280 ± 0.043</td>
</tr>
</tbody>
</table>

Asterisks indicate significant regressions.
rodent species, huddling became spontaneous at low $T_a$ with groups of two to three individuals in close contact without any, already reported, group leader or preestablished configurations, because each group is different.

The main environmental trigger of this behavior seems to be low $T_a$; nevertheless, in rats there are some reports that other nonthermal cues could elicit huddling [39]. Our results suggest that critical temperature is round about 16 and 20°C, as all statistical analyses of the relationships CVM versus $T_a$ and the evolution of group size showed that at 16°C there was a change in these variables and also in the fractal dimension. In fact, between 20 and 28°C, the median group size was about 4, whereas at 16°C it increased to 9. These $T_a$ values seem reasonable, as the lower limit of thermoneutrality in mice is 25–30°C [40]. Interestingly, and considering that in mice $\phi = 0.58$ [26], the group size observed at temperatures of 16°C or less ($S = 9.3, 8.0$, and 14.5) is near the intersection between autosimilar and small mammal solutions (Figure 2), showing that at these group size the geometrical assemble of the group is roughly autosimilar, which is one of the main characteristics of a fractal geometry. Also, the fractal dimension changed in the transition zone from 1.3 to nearly 1.45, as expected in a spontaneous fractal organization. The actual fractal dimension of the group can be estimated as the calculated dimension plus one ($D_f = D_a + 1$; see Ref. 41) because silhouettes were a two-dimensional section of the group, but the group is three dimensional. In the huddling dynamics, there was a transition from a situation mainly governed by dimensions near the topological dimension of a planar surface (dimension = 2), for example, $D_f = 2.2$ at 35°C, to a more complex figure with fractal-like groups, for example, $D_f = 2.52$ at 5°C. This is surprising because fractals exhibit larger area for a given volume, exactly the opposite of the biological function of huddling. However, as our models shown, at a given group size, animals in groups cannot break their own geometrical constraints, showing a fractal-like geometry instead of a Euclidean geometry.

As expected for a second-order phase transition of a thermodynamic system, the variance-mean coefficient of the individuals in each grid cell followed a sigmoid curve. Nevertheless, we chose to estimate the critical temperature from the relationships CVM versus $T_a$ because it is an independent criterion of our main function of interest, which is the distance between individuals. This has the advantage of being a continuous variable that, under our conditions, is 0 when individuals are in contact. Also, the power law obtained showed an adequate data adjustment for a critical temperature of 16°C. Nevertheless, the sensitivity analysis also showed a significant adjustment at critical temperatures between 14 and 17°C without a large variation in the $\alpha$ exponent. The same analysis revealed that the variability in the $\alpha$ exponent was low when changing the number of points near $T_a$, which is relevant because the critical exponent should be estimated only near the critical temperature.

In summary, we tested the hypothesis that thermoregulatory huddling behavior, a self-organized phenomenon, behaves as a system with continuous (second-order)-phase transition called critical when the environmental temperature (driving parameter) is near a critical value. Our results support all predictions for critical systems under phase transition [9]: (1) A transition from nongrouped individuals at high $T_a$ to huddling at low $T_a$s. This change was represented by a sigmoid change in CVM of the number of individuals in each grid cell, as expected for that continuous-phase transition; (2) the main descriptor of huddling behavior ($D$) followed a power law as a function of $T_a$ near the critical environmental temperature, and (3) in the transition-phase zone, the group size did not differ from autosimilar behavior and became spontaneously organized in fractals. This suggests that huddling behavior may be considered as a transition between two states (nonordered at high temperature and ordered at low temperature). Each state has their own thermodynamics characteristics, where the system falls into one of universal classes defined by its critical exponent. Near the continuous-phase transition the system could be not very sensitive to the nature of the constituents or the details of the interactions subsisting among the individuals during the huddling dynamics. We predict that huddling dynamics in other species will be represented by the same exponent (i.e., falling in the same universal class).

During the last few years, there is increasing evidence that self-organization plays an important role in the behavior and development of biological systems [2]. Also, self-organization acting in concert with natural selection may be part of the whole evolutionary process [2, 42–45]. The importance of abiotic factors, mainly the thermal environment, in determining behavioral traits has been a central issue of discussion [46–48]. Physiological and environmental constraints are determinant in the relationship between abiotic variables and the spatial distribution of individuals and populations, but the processes driving such patterns remain poorly understood. We believe that our results help to visualize behavioral social thermoregulation, a recognized adaptive behavior as a self-organized second-order thermodynamic system coupled with an external driving, which to our knowledge has not been reported in biological systems.

Acknowledgments
This work was supported by grants FONDAP 1501-0001, Program 1 (FB) and a FONDECYT 1080038 (MC). L. Eaton provided useful commentaries.
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