# Animal Behavior An Evolutionary Approach



Victor S. Lamoureux Editor

## **ANIMAL BEHAVIOR** An Evolutionary Approach

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## INTRODUCTION

Human beings have been students of animal behavior from their earliest days. Understanding animals and what they did meant survival for humans, either through use of animals as food or avoidance of them as potential hazards. Certainly the understanding of animal behaviors and habits gave a selective edge to early humans who practiced the study. Further evidence for this appreciation of animal behavior can be seen in the many cave paintings depicting animals, while not depicting any of the other facets of the painters' lives. However, the modern study of animal behavior is rather recent and was first widely acknowledged with the awarding of the Nobel Prize in 1973 to Niko Tinbergen, Karl von Frisch, and Konrad Lorenz.

Animal behavior is technically known as ethology, which is considered the systematic study of the behavior of animals under natural conditions. Although historic distinctions existed between the fields of ethology and animal psychology, with the former being largely performed in natural settings and the latter in controlled laboratory settings, modern studies are frequently a combination of laboratory and field work. Ethology is highly integrative and draws on the fields of evolution, ecology, psychology, molecular biology, development, neurobiology, endocrinology, and mathematics. A student in an animal behavior course will typically already have a substantial background in math and sciences.

Tinbergen set the stage for the questions of animal behavior in his 1963 paper, "On Aims and Methods of Ethology". Now commonly referred to as "Tinbergen's Four Questions", he suggested four ways to answer the question "Why?" when referring to animal behavior: as a function of causation, evolution, function, and ontogeny. One aspect is no more important than another, but all four are needed to gather a clear picture of the role of a behavior. In most cases, behaviors should be considered adaptations and therefore subject to the pressures of natural selection.

The study of animal behavior has become a foundation to other biological disciplines, including neuroscience, behavioral genetics, ecology, and conservation biology. Although the study of animal behavior might seem only academic, or even esoteric, the reality is that ethology is applicable to many aspects of our modern lives. The comparative approach of studying animals and using them as models for ourselves leads to important discoveries on how the human brain and behaviors might work. Animal behavior studies are also useful to better the welfare of animals in a wide variety of situations, from agriculture to zoos.

One of the more recent areas in which animal behavior's importance has emerged is in the field of conservation biology. In fact, insights from animal behavior have become critical in the implementation of conservation strategies. The design of wildlife reserves and biological corridors linking reserves needs to be examined with a full knowledge of the wildlife utilizing such reserves. Animal behavior is also critical to the success of captive breeding programs and the subsequent release of these captive-reared individuals. Not fully understanding the behavioral repertoire of an endangered species will likely lead to ineffective conservation measures.

Hard work, long hours, and stiff competition await those who want to enter this field. Those with bachelor's degrees will have opportunities in research, pharmaceutical testing, animal training, and conservation. Specialization in animal behavior usually occurs at the graduate level with a PhD, or even as a veterinarian. Most positions will be in research with the government or research and teaching at colleges and universities. But for those who love animals and who want to delve into and discover new aspects of their behavior, the rewards are substantial. With the current worldwide decline in natural systems, it seems likely that a demand for people trained in animal behavior and conservation will continue into the future.

## ACKNOWLEDGMENTS AND HOW TO CITE

The chapters in this book were previously published in various places and in various formats. By bringing these chapters together in one place, we offer the reader a comprehensive perspective on recent investigations into this important field.

We wish to thank the authors who made their research available for this book, whether by granting permission individually or by releasing their research as open source articles or under a license that permits free use provided that attribution is made. When citing information contained within this book, please do the authors the courtesy of attributing them by name, referring back to their original articles, using the citations provided at the end of each chapter.

## Free-Ranging Macaque Mothers Exaggerate Tool-Using Behavior when Observed by Offspring

Nobuo Masataka, Hiroki Koda, Nontakorn Urasopon and Kunio Watanabe

### ABSTRACT

The population-level use of tools has been reported in various animals. Nonetheless, how tool use might spread throughout a population is still an open question. In order to answer that, we observed the behavior of inserting human hair or human-hair-like material between their teeth as if they were using dental floss in a group of long-tailed macaques (Macaca fascicularis) in Thailand. The observation was undertaken by video-recording the tooluse of 7 adult females who were rearing 1-year-old infants, using the focalanimal-sampling method. When the data recorded were analyzed separately according to the presence/absence of the infant of the target animal in the target animal's proximity, the pattern of the tool-using action of long-tailed adult female macaques under our observation changed in the presence of the infant as compared with that in the absence of the infant so that the stream of tool-using action was punctuated by more pauses, repeated more often, and performed for a longer period during each bout in the presence of the infant. We interpret this as evidence for the possibility that they exaggerate their action in tool-using so as to facilitate the learning of the action by their own infants.

### Introduction

The population-level use of tools has been reported in various animals. One of the best known instances of this is the so-called "ant-fishing" by free-ranging chimpanzees (Pan troglodytes) [1]. Nonetheless, how tool use, including that of ant-fishing in chimpanzees, might spread throughout a population is still an open question [2]. There is some controversy as to whether the transfer of these cultural practices is accomplished across individuals by observational social learning or just by individual learning alone [3].

Although there is some disagreement about whether or not various forms of observational social learning play a role in the transmission, there is a general consensus among researchers that the recipient is solely responsible for the successful acquisition of the skill, and that the skill's donor does not have any active role in the transmission of cultural information. In the present paper, on the other hand, we present evidence which indicates the possibility that free-ranging adult long-tailed macaques (Macaca fascicularis) modify their action in tool-using so as to facilitate the learning of the action by their own infants. The behavior we observed was that of inserting human hair or human-hair-like material between their teeth as if they were using dental floss. We compared the pattern of the behavior in each of 7 adult females when her own infant was in her proximity and when any other group member was not in her proximity.

Our study of the tool-using behavior in a group of the macaques in Thailand started in 2004 and continues up to the present [4]. Whenever the material picked up by an animal is to be used as the tool, the animal subsequently grasps the hair taut between its two hands. Then, the animal inserts the taut hair between its open jaws, and the action ends when the animal closes its jaws to engage the taut hair, and pulls the hair sharply to one side by one hand and removes it from its mouth. Here a 'bout' of the tool-use is defined as starting at the moment of grasping the material with the hands and ending at the moment of completely removing it from the mouth. With this removing action, food, if present could be cleaned from between the teeth. Before removing the hair, the animal was often observed to repeatedly rapidly close and open ("snap") its jaw to engage (clamp) the taut hair between its teeth. When this occurred, the number of times the animal clamped on the hair could be counted, calling it the number of snaps. Subsequent to the occurrence of such snapping, moreover, the animal was often observed to remove the taut hair which was kept grasped between the two hands, to briefly look at it at about eve level, and to reinsert it in its mouth as before. When this was observed, it was defined as an occurrence of "reinsertion" in a given bout. "Reinsertion" might be repeated in that bout: after reinserting the hair, the animal might repeat the same action and take out the hair again while grasping it with two hands. That bout continued until the animal finally pulled out the reinserted hair to one side using one hand. In each such bout, the number of occurrences of reinsertion as well as the number of occurrences of snapping while the hair was inserted could be counted. The length of each bout could also be measured by counting the number of frames of the video which were required to record from the onset until the end of the bout. In addition, the number of occurrences of "removing of the hair from the mouth" was computed in each bout as attempts to clean the teeth. It could be counted as (X+1 (X = 0, 1, 2,...)) in a given bout when the number of occurrences of reinsertion was 'X' in the bout.

When a bout ended, perhaps on the completion of the cleaning of the teeth, the animal abandoned the material onto the ground on some occasions. If this was observed, the tool-use 'episode' ended, during which a single bout of the activity was undertaken. Alternatively, however, the animal again grasped the material with the two hands and began another bout with an interval of no more than 1 second or so. Then, that episode continued until the animal finally abandoned the stimulus. Thus, the number of 'bouts' in the episode could be counted. Also, the number of frames of the video which were required to film from the onset of the first bout until the end of the final bout was defined as the total duration of that episode. If only a single bout was included in a given episode, the duration of the episode coincided with the duration of the bout. In addition, the total number of occurrences of "removing the hair from the mouth" in the episode was computed as an index of the frequency of cleaning attempts in the episode.

### Results

Results of the analyses are summarized in Figure 1. When the average number of occurrences of reinsertion in a given bout of the tool-use was computed across subjects, a likelihood-ratio test revealed that the score when the infant was in the proximity of the target mother was greater than that when the infant was absent

 $(\chi 12 = 22.201, p < 0.0001)$ . Similarly, the average number of jaw snaps during each insertion of the stimulus was greater when the infant was present as compared to when the infant was absent ( $\chi$ 12 = 123.6, p<0.0001). The average duration of a given bout when the infant was present was longer that that when the infant was absent ( $\chi$ 12 = 44.51, p<0.0001). In a given bout, the number of occurrences of reinsertion was found to positively correlate with the number of jaw snaps during each insertion (Pearson's correlation = 0.232, n = 355, p<0.01). In a given bout, both the number of occurrences of reinsertion and the number of jaw snaps were found to positively correlate with the duration of the bout (Pearson's correlation = 0.770, p<0.001; 0.417, p<0.001; n = 355, respectively). The average duration of a given episode, on the other hand, did not differ significantly when the infant was present compared to when it was absent ( $\chi_2^1 = 1.592$ , p = 0.2071) because the average number of bouts in a given episode when the infant was absent was greater than that when the infant was present ( $\chi_{1}^{1} = 8.9008$ , p = 0.00285). The average number of occurrences of removal of the hair from the teeth in a given episode did not differ when the infant was present compared to when it was absent ( $\chi^1_{,2}$  = 1.0519, p = 0.3051, mean±95%CI =  $3.38\pm0.40$  when the infant was present, and 3.76±0.50 when the infant was absent).



Figure 1. Summary of results of the analyses. Average scores of number of occurrences of reinsertion in a given tool-using bout (Reinsertion), of number of occurrences of snapping during each insertion (Snap), of length of each bout (Length), of overall mean number of snaps during each insertion as a function of number of occurrences of reinsertion in a tool-using bout (Snap/Reinsertion), and of total duration of a given tool-using episode (Total Duration) are computed across target adult females when the infant was in her proximity and when the infant was absent.

## Discussion

Overall, once the long-tailed macaque mothers (the target animals) started to use the stimulus as a tool, they devoted a similar amount of time to the stimulus regardless of whether or not their infant was present. However, as shown in Figure 2, the pattern of their action changed in the presence of the infants as compared with that in the absence of the infants so that the stream of tool-using action was punctuated by more pauses, repeated more often, and performed for a longer period during each bout in the presence of the infants.



**Figure 2.** Typical sequences of the action of "flossing teeth." (P 1 to 6) When her infant was in the proximity of an adult female (With Infant; P-1: Grasp the hair taut, P-2: Insert, P-3: Snap, P-4: Look at the hair, P-5: Reinsert, P-6: Pull out). (A 1 to 3) When no animal was in the proximity of an adult female (Without Infant; A-1 Grasp the hair taut, A-2: Insert, A-3: Pull out).

As a possible factor affecting this difference, the activity of feeding by the animals per se is not considered likely because the present observations were undertaken at least 30 min after the end of the animal's final food-taking. Rather, it seems more likely that the behavioral difference is socially modulated, and influenced by the presence/absence of other animals in the proximity of the target

animals. In this regard, the fact should be noted that only their infants were situated within arm's range of the target animals. Although no overt social interactions (occurrences of any facial expression or communicative movement) were observed in either the mothers or the infants, the influence of the presence of other group members than the infants did not appear to be a variable affecting this change.

As a possible explanation, one might assume that the mothers were more distracted when the infants were present and thus took longer to clean their teeth than when they were alone. However, the average duration of a given tool-using episode did not increase significantly when the infants were present. More importantly, the mothers' attempts to clean their teeth (as assessed by the number of times they removed the hair per episode) did not increase either when the infants were present. Actually, the average number of hair removal per episode when the infants were present was even smaller than that when the infants were absent. Rather, the change of the pattern of tool-using should be interpreted as a behavioral modification produced by the presence itself of the infants who were watching the mothers.

Concerning human mother-infant interactions, a series of experiments have revealed the fact that strikingly similar parental modifications in their actions, called motionese, can help infants to detect the meaningful structure of the actions [5], [6]. On the basis of observations of 51 and 42 mothers, respectively, who were demonstrating novel objects to their own infants whose ages ranged from 6 months to 13 months, it was found that the mothers tended to modify their infant-directed actions in various ways. They were likely to repeat the actions, to put longer pauses between actions and to exaggerate actions themselves. Such magnification of the movement or 'looming' has been argued so far to play an important role in educating the attention of human infants by attracting their attention due to the occlusion of other sensory information [7].

Indeed, such reasoning is confirmed by an analysis subsequently undertaken from an infant-like viewpoint by applying a model of saliency-based visual attention to such parental action [8], [9]. That analysis was conducted by scientists specializing in robotics originally for the purpose of investigating how such modifications contribute to the infant's understanding of the action. The results of their analysis showed that the model does not suppose any a priori knowledge about actions or objects used in the actions. Instead, it is able to detect and gaze at salient locations, which stand out from the surroundings because of the primitive visual features, in a scene. The model thus demonstrates which low-level aspects of parental actions are highlighted in their action sequences and could attract the attention of young infants, and also robots. Actually, a more recent experimental study [10] demonstrated infants' preference for motionese compared to adult-directed actions by presenting videos of both types of movement to 6- to 13-month-old infants. In the study, the participants showed evidence of such preferences even when demonstrators' faces were blurred in the videos.

Concerning macaques, unlike humans, there is no evidence for imitation under controlled conditions [3]. If we define imitation as the reproduction of the behavior of a model by an observer [11], most empirical studies have failed to show its occurrence in social groups. This could also be the case for the behavior of the monkeys in the present study. In order to explain the spread of the behavior in the group, therefore, we are forced to assume that animals may learn new behaviors from each other through simpler mechanisms than imitation. A typical instance of such reasoning is that its recipient's attention may be drawn to the environment or an object by the presence or interest of the donor itself, even in the absence of any form of intervention of social learning, for the transmission of cultural information. Under such circumstances, again, the modification of the action by the donor is as crucial as it is in the case of imitation because it profoundly affects the likelihood of the recipient acquiring a new behavior, which must be worked out by the recipient itself. The chance that the recipient's resulting behavior comes to resemble the donor's due to environmental or object constraints appears to be facilitated effectively by such modification of the behavior as we report here, which would eventually result in the population-level phenomenon of that behavior.

### Methods

The study group was inhabiting a small city, Lopburi, 154 km north of Bangkok, Thailand. In the center of the city stands the old Buddhist shrine of Prang Sam Yot in an open sandy area of approximately  $50 \times 50$  m surrounded by three 20m-wide roads and a railway. The present experiment was undertaken there. The area is included in the home range of the study group, which consisted of roughly 200 animals when the study was conducted in February, 2008. Because tourists often visit the shrine when it is open (between 9 a.m. and 5 p.m.), most of the group members were likely to stay there during this period. However, the study group does not spend night there, but in other woody areas at least 1 km away from the shrine. When the research started in 2004, we confirmed the tool-use in 9 adult female monkeys, who rode on the head of female tourists, pulled out their hair, and used it to "floss" their teeth [4]. Since then, the number of animals in which we have confirmed similar behavior has increased up to 50, all of which are adults.

During the study period, 7 females were rearing their approximately 1-yearold infants (3 males and 4 females). We chose all of these 7 females as target animals for the present study. The observation was undertaken by video-recording (30 frames per second) the tool-use of the adults in the area of the shrine. In order to control the variability of the material for the tool-use, we used hairs from a single type of human hairpiece. To provide the stimuli, on each day of observation, we scattered numerous hairs (approximately 20 cm long) that had been dissociated from the hairpieces around the study area early in the morning and waited for the target animals.

The data collection was undertaken using the focal-animal-sampling method. The collection starts with a focal animal, at least 30 min after than the final foodintake of that animal. When using the stimulus as a tool, the animal at first picks it up from the ground. Whenever such behavior is observed, our video-recording is started. When finishing the tool-use, on the other hand, the animal abandons the stimulus onto the ground, and we operationally defined this sequence of handling activity with the stimulus as the material for the teeth-flossing as an 'episode' of the tool-use.

In order to investigate whether the tool-using activity of a target animal was affected by the presence of other group members who were particularly naïve to the activity, we attempted to record the tool-using 'episodes' of the animal when her infant was present in her proximity and when no other animals were present in her proximity. The criterion was solely whether her infant alone remained present within arm's range as well as within the visual range of the target animal throughout a given episode, both animals being situated in a face-to-face position, or whether no animals remained present within such range throughout another given episode. In all, we were able to record 50 episodes where just her infant remained in the target animal's proximity and 50 episodes where no animals remained in the target animal's proximity. In addition, we recorded another 21 episodes during the study period. In these 21, however, animals other than the infant of the target animal entered into proximity with her during the tool-using activity (18 episodes), or the infant was not visually oriented toward the target animal (3 episodes). Thus, data concerning these cases were not included in further analyses.

The video-recording was performed using two video cameras. One of the two filmed the frontal view of the target animal. The tool-using behavior recorded by the videos was coded online by two highly trained coders independently from one another. They were not told the purpose of the present study. The detailed coding schema was essentially the same as that used in our previous study [12]. Overall interrater agreement was 97%. The other camera monitored the area proximal to of the animal. When the infant of the target animal was present in the proximity, the camera filmed its frontal view so that, by analyzing the videos recorded by this second camera and the camera monitoring the target animal, any occurrence of facial expressions and gestural movements could be recorded in both the infant and of the target animal. The occurrences were assessed again by the two raters. However, none of them reported any occurrence of such communicative behavior in the target animal or in the infant during any episode.

The research methodology complied with protocols approved by the guidelines (Guide for the Care and Use of Laboratory Primates, Second Edition) of Primate Research Institute, Kyoto University, Japan and the legal requirements of Thailand.

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## Authors' Contributions

Conceived and designed the experiments: NM HK NU KW. Performed the experiments: NM HK. Analyzed the data: NM HK. Wrote the paper: NM.

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## Mouse Cognition-Related Behavior in the Open-Field: Emergence of Places of Attraction

Anna Dvorkin, Yoav Benjamini and Ilan Golani

### ABSTRACT

Spatial memory is often studied in the Morris Water Maze, where the animal's spatial orientation has been shown to be mainly shaped by distal visual cues. Cognition-related behavior has also been described along "well-trodden paths"—spatial habits established by animals in the wild and in captivity reflecting a form of spatial memory. In the present study we combine the study of Open Field behavior with the study of behavior on well-trodden paths, revealing a form of locational memory that appears to correlate with spatial memory. The tracked path of the mouse is used to examine the dynamics of visiting behavior to locations. A visit is defined as either progressing through a location or stopping there, where progressing and stopping are computationally defined. We then estimate the probability of stopping at a location as a function of the number of previous visits to that location, i.e., we measure the effect of visiting history to a location on stopping in it. This can be regarded as an estimate of the familiarity of the mouse with locations. The recently wild-derived inbred strain CZECHII shows the highest effect of visiting history on stopping, C57 inbred mice show a lower effect, and DBA mice show no effect. We employ a rarely used, bottom-to-top computational approach, starting from simple kinematics of movement and gradually building our way up until we end with (emergent) locational memory. The effect of visiting history to a location on stopping in it can be regarded as an estimate of the familiarity of the mouse with locations, implying memory of these locations. We show that the magnitude of this estimate is strain-specific, implying a genetic influence. The dynamics of this process reveal that locations along the mouse's trodden path gradually become places of attraction, where the mouse stops habitually.

## **Author Summary**

Spatially guided behavior and spatial memory are central subjects in behavioral neuroscience. Many tasks have been developed for laboratory investigations of these subjects since no single task can reveal their full richness. Here we turn to the simplest and oldest "task," which involves no task at all: introducing a mouse into a large arena and tracking its free behavior. Traditionally, the test is used for studying emotionality and locomotor behavior, using simple summaries of the mouse's path such as its length and the percent of time spent away from walls. More sophisticated computational analysis of the dynamics of the path enables us to separate visiting behavior at locations into stops and passings. Using this distinction, the mouse's path reveals quantifiable locational memory: the mouse's decision to stop in a location is based on its visiting history there. In some strains of mice, the visited locations gradually become places of attraction where the mouse stops habitually. In other strains, the phenomenon is not evident at all. Such quantifiable characterization of locational memory now enables further exploration of the senses that mediate this type of memory and allows measurement and comparisons across mouse strains and across genetic and pharmacological preparations.

## Introduction

In the present study we ask how can a kinematic description of Open-Field behavior lead to an understanding of a mouse's higher cognitive functions. We use the organization of elementary patterns for revealing memory-related phenomena.

Low-level kinematic features such as the animal's instantaneous location and speed are extracted from the tracked paths by using special smoothing algorithms [1]. These have been used to statistically partition the mouse's trajectory into intrinsically defined segments of progression and of staying-in-place (stops, lingering episodes; [2]). In previous work on rats, examination of the spatial distribution of stops revealed the home base-the most preferred place in the environment [3]. The home-base is used by the animal as a reference around which it performs structured roundtrips [4], [5]. The home-base also exerts a constraint on the number of stops per roundtrip: the probability of returning to the home-base is an increasing function of the number of stops already performed by the animal in that roundtrip [6]. The home-base acts as an attractor in 2 ways: first, in the vast majority of cases the animal stops in this place upon visiting it, and second, within a roundtrip, this place exerts a gradually increasing attraction on the rat to return to it. Both forms of attraction imply recognition and memory of home-base location. In the present study, starting with the same trajectory data, we approach the issue of recognition and memory of places in a different way, by examining stopping behavior across all locations in the periphery of the open field.

We accomplish this aim by establishing the history of visits to locations all around the periphery of the arena, where visits are classified as stops or passings. We then determine whether the number of previous visits to a location affects the animal's decision to stop in it. An effect of visiting history on the probability of stopping would imply recognition and therefore locational memory.

We used two inbred strains commonly contrasted for their spatial memory— C57BL/6, which is considered to have good spatial memory, and DBA/2, whose performance is poor (e.g., [7]–[9]; see however [10]), and as a third strain, the recently wild-derived strain CZECHII whose spatial behavior might be less affected by domestication.

This study, which has been part of an ethological analysis of mouse exploratory behavior [11]–[14], provides a high throughput test for locational memory.

### Results

Since most activity takes place at the periphery of the circular arena (see Methods), we moved to polar coordinates description of the smoothed path (with (0,0) at the center of the circular arena). As illustrated in Figure 1A, the polar projection of the mouse's path as a function of time was punctuated by stops (black dots) in an apparently sporadic manner. While the mouse's decision to stop at a specific location upon traversing it could be taken randomly we wanted to take a closer look at the possibility that it still depended on the history of visits to that location. For that purpose we first established a record of visits in reference to a location, classifying each visit as a stop or a passing through. We then studied jointly records for all locations, and calculated the probability of stopping during a specific visit to a location as a function of the ordinal number of that visit. A change in this probability across visits would have implied that the decision to stop was influenced by visiting history.



**Figure 1.** Establishing a Record of Visits in Reference to a Location. (A) CZECHII mouse's polar angles across the first 25 min of a session. Lines represent progression segments and dots represent lingering episodes. The path near the wall is shown in black and the path in the center—in gray. (B) The extraction of a sequence of passings and stops from a time-series of the mouse's polar angles during the first 2.5 min. The horizontal line denotes a specific polar location for which the sequence of visits is extracted, and the numerals printed within squares indicate the ordinal numbers of the visits, white squares for passings, and black—for stops. Only the path near the wall (in black) is used for scoring. The enumerated squares construct, from bottom to top, the column on the right, which depicts the sequence of passings and stops in the selected location.

The procedure of establishing a record of visits in reference to a location is illustrated in Figure 1B: angular position 270° is represented by a straight line parallel to the x-axis. By following the line one can see that upon visiting this location the mouse did not stop in it during the first 3 visits, stopped in it during the 4th and 5th visit, and then again passed through it without stopping during the 6th visit, etc. This sequence of discrete events, consisting of 3 successive passings, 2 stops, 1 passing, and another 2 stops, is presented from bottom to top in the right column of Figure 1B. Similar sequences of passings and stops were obtained for all 120 locations defined by the grid superimposed on the periphery of the arena (see Methods).

The sequences of passings and stops obtained for all locations in 3 representative mouse-sessions are shown in the graphs of Figure 2, left panel. An overview of these graphs reveals that the stops appeared to be distributed evenly throughout the sequences in the DBA mouse, but occurred mostly during later visits in the CZECHII mouse. The increase in stopping frequency across visits was also present in the C57 mouse, but in a milder form. These tendencies appeared to characterize the 3 strains.



**Figure 2.** The History of Visits to Peripheral Locations in the Arena. (Left) History of visits to all peripheral locations during a 30-min session of 3 mice belonging to 3 different inbred strains. White squares represent passings, and black squares—stops. (Right) Probability of stopping as a function of the ordinal number of visits. Each horizontal bar represents the proportion of stops performed during the nth visit to a location, by summing up the stops and passings belonging to the corresponding row plotted in the left panel. The black portion of the bar represents the percentage of stops performed during the nth visit to all locations in which such visit occurred (the white portion represents the complementary percentage of passings). As illustrated, the probability of stopping increased as a function of the ordinal number of a visit in the CZECHII and C57 mice, and did not change in the DBA mouse.

We estimated the probability (pn) of stopping during a visit to a location as a function of the ordinal number of that visit in the following way. With Vn being the number of such n-th visits, Sn out of the Vn visits had been classified as stops. The proportion Pn = Sn/Vn was the desired estimator of the probability of interest pn (see Methods). As shown in the right panel of Figure 2, the probability of stopping increased as a function of the ordinal number of a visit in the CZECHII and C57 mice, and did not change in the DBA mouse. In other words, in these CZECHII and C57 mice, the decision to stop in a location was influenced by the number of previous visits paid to that location, whereas in the DBA mouse, visiting history did not affect this decision.

To quantify the rate of change in the probability of stopping, we fitted a linear function of n to the logit-transformed pn in the form (Figure 3):

$$Log\left(\frac{p_n}{1-p_n}\right) = \beta_0 + \beta_1 n,$$

for each mouse. The estimated slopes for all 3 strains are presented in Figure 4. All mice of the CZECHII showed an increase in the probability of stopping as the number of visits increased, so did the trends of all mice of the C57 strain, though the trends were closer to 0. In contrast, DBA showed mixed trends, 21 increasing and 14 decreasing trends. See Figure 4 for the summary of the individual mice trends per each strain and laboratory. Pooling across laboratories using fixed model ANOVA we found that the trend for CZECHII and C57 was significantly positive (p<.0001 and p = .009 respectively) while for the DBA it was not (p = .28) (all results are deposited in the database of the Mouse Phenome Project, [15]).



**Figure 3.** Rate of Change in the Probability of Stopping as a Function of Number of Visits. 3 examples of a linear regression fitted to the normalized probabilities of stopping data. The graphs are similar to the graphs in Figure 2, right panel. Each vertical bar represents the percentage of stops performed during the nth visit to all locations in which such visit occurred. Gray level of bars denotes the weight assigned to the probability value used for the calculation of the linear regression. The data are transformed in order to allow the fitted regression to be linear (see Methods). The black line depicts the regression. The rate of change in the probability of stopping as a function of the ordinal number of a visit was indicated by the slope of the fitted linear function, which reflected a significantly positive trend in CZECHII and C57 mice, and no significant trend in the DBA mouse.



**Figure 4.** Rate of Change in the Probability of Stopping at a Location. Boxplot summaries of the rate of change in the probability of stopping at a location as a function of the number of previous visits to that location, in 3 strains and across 3 laboratories. Results obtained in NIDA (N), MPRC (M), and TAU (T) are shown, respectively, in light, medium, and dark gray. The trend of the rate of change in the probability of stopping at a location for CZECHII and C57 was significantly positive (p<.0001 and p = .009 respectively) while for the DBA it was not (p = .28).

Putting the result through a more stringent test for replicability, by using the mixed model ANOVA where laboratories were treated as random as well as their interaction with strains [16], we found that the difference in slopes across strains was highly statistically significant (p<.0001). Furthermore, 95% confidence interval for the slope for CZECHII was (.053, .132), for C57 is (-.001, .069) and for DBA was (-.026, .045) giving similar results to those of the fixed effect.

To rule out the possibility that changes in the probability of stopping reflect the level of activity of the animal per session, the Pearson Correlation Test was performed on Distance Traveled near the wall and the slope value obtained from each animal. The correlation was small, r = -.2 and not statistically significant at the .5 level.

The visiting sequences used for the computation of the slopes of regression described the order of visits to the same location; they did not provide the time of the visits' occurrence. The increase in the probability of stopping at locations could, therefore, merely reflect an increase in the frequency of stopping across the session. To examine this possibility we scored the number of stops per sliding time window (3-min time bins with an overlap of 1 min) across the session, fitted a linear regression to the obtained values, and computed the slope of the line. As can be seen in Figure 5, the slopes of all strains in all laboratories were either parallel to the x-axis or negative, implying that the frequency of stopping did not

increase across the session. Therefore, changes in the frequency of stopping across time could not explain the change in the probability of stopping at a location with increasing number of visits.



**Figure 5.** Rate of Change in the Frequency of Stopping across Time. Boxplot summaries of the rate of change in the frequency of stopping across time, in 3 strains tested simultaneously in 3 laboratories. Results, obtained from NIDA (N), MPRC (M), and TAU (T), are shown in light, medium, and dark gray, respectively. The slopes of all strains in all laboratories were either parallel to the x–axis or negative, implying that the frequency of stopping did not increase across the session.

Having ruled out the possibility that the frequency of stopping increases across time, and having shown in the previous section the replicability of the results in 3 laboratories, we concluded that the rate of change in the probability of stopping as a function of visiting history was a reliable measure of mouse locational memory in the open-field.

#### Dynamics of Stopping in Specific Locations

The changes in the probability of stopping (Figure 4) were computed by pooling the data across all locations at the periphery. Therefore, the results presented so far applied to all locations in a general way, ignoring changes at specific locations. Further investigation of the data collected in TAU revealed 3 types of locations: those in which the probability of stopping increased, those in which it decreased, and those in which it stayed unchanged (see Methods). The locations showing an increase appeared in clusters and so did the locations showing a decrease (see Figure 6). In order to distinguish between the arbitrarily defined single locations, and their clusters, which were revealed by our analysis, we termed the clusters places (it should be noted that the minimal number of locations in a cluster is 3, reflecting our measurement resolution; see Methods).



**Figure 6.** Places Marked by an Increasing or Decreasing Probability of Stopping. History of visits to all peripheral locations across 30-min sessions of 3 mice. White squares: passings, black squares: stops. White stripes mark places in which the probability of stopping increases; gray stripes mark places in which the probability of stopping decreases. The CZECHII mouse was characterized by having only places where the probability of stopping increased or stayed unchanged; in contrast, the C57 and DBA mice were characterized by having all 3 types of places.

As shown in Figure 6 in 3 examples, the CZECHII mouse was characterized by having only places where the probability of stopping increased or stayed unchanged; in contrast, the C57 and DBA mice were characterized by having all 3 types of places; finally, the DBA mouse was characterized by having the highest number of places where the probability of stopping stayed unchanged. Those strain differences prevailed in all the mice tested in TAU.

## Discussion

### Intrinsic Constraints on Stopping Behavior Imply Locational Memory

In this study we show that in the open field, visiting history to a location influences stopping behavior in that location; the magnitude of this influence is strain specific. In 2 out of 3 examined strains, the higher the ordinal number of a visit to a location, the higher is the probability of stopping in that location. In the third strain, the ordinal number of a visit to a location appears to be irrelevant for the decision whether to pass through the location or stop in it. In the strains that show increased probability of stopping with consecutive visits to a location, this is not due to a general tendency of the mice to stop more frequently with time. On the contrary—the tendency to stop either decreases or stays unchanged across the session in all mice and strains (Figure 5). Because the phenomenon depends on the ordinal number of visits, it implies some type of memory, and because it describes behavior in specific locations, it is spatial. Taken together, it indicates a locational memory.

### Locational Memory and Spatial Memory

Future studies would tell us to what extent locational memory utilizes the various sensory modalities. Hippocampus-guided spatial memory is, for example, commonly demonstrated by showing that manipulation of distal visual cues is followed by corresponding adjustments in the animal's spatial orientation [17]. In real life situations, spatial orientation may also be supported by the processing of cues belonging to the other sensory modalities, including proprioception derived from self movement [18], yet the term spatial memory became mainly identified with visual processing. The locational memory highlighted in the present study implies spatial recognition and familiarity, and therefore also reflects spatial memory, but the particular contribution of each of the sensory modalities to the mouse's orientation is not known. Support for visual guidance by distal cues is indicated by the consistency of our results with those obtained for visually guided tests of spatial memory in 2 of the strains (CZECHII mice have not been tested yet for spatial memory). Thus, as with locational memory, good spatial memory is exhibited in C57 in various spatial tasks [9], [19]–[21]. The absence of a locational memory in DBA/2 mice similarly corresponds to the lack of spatial memory reported in most studies performed on this strain [7]–[9], known to suffer from hippocampal dysfunction [22]–[24]. These parallel findings support the hypothesis that the memory described in this paper is also guided visually. The hypothesis that a change in the probability of stopping in locations across visits is mediated by the accumulation of olfactory cues, which are in turn accumulated across visits is untenable, as it would require a mechanism explaining why scent accumulation has no influence on stopping in DBA/2 (Figure 6, lower panel), a strain gifted with a more sensitive olfactory sense than C57 [25], [26], does influence stopping, but in 2 opposite ways, in C57 mice (Figure 6, middle panel), and only increases stopping in the CZECHII mice (Figure 6, upper panel). Estimating locational memory in open field behavior recorded in full darkness would tell us to what extent this construct is supported by information derived from self movement. Finally, dependence on the hippocampus can be investigated by using lesions or temporary inactivation of this structure, and the role played by memory on this phenomenon, although not specific to spatial memory only, can be investigated by using pharmacological disruption that is predictive of memory loss. Whatever the underlying mechanisms, locational memory, which has been shown to be strain-specific can now be compared across strains and preparations.

## The Relationship between Locational Memory and the Level of Activity

Since our measure is based on locomotor behavior, there is a concern that this measure is influenced by the animal's level of activity. To rule out this possibility we examined the correlation between distance traveled per mouse-session and the corresponding rate of change in the probability of stopping as a function of the number of visits. The correlation was small and not statistically significant (r = -.2, p<.05), implying that within the range of values obtained in this study, the level of activity does not influence our measure.

Examination of pharmacological preparations exhibiting hyperactivity (e.g., [27],[28]) could further elucidate the issue of the influence of activity on the measure of locational memory. In 3 previously performed studies on the effect of dopamine-stimulants on locomotor behavior in general, and on stopping in locations in particular, all 3 drugs induced hyperactivity, but had 3 distinct effects on stopping in locations. (+)-amphetamine-induced hyperactivity was associated with a consolidation of stereotypic stopping in a limited number of locations in a relatively fixed order [29]; quinpirole- induced hyperactivity was associated with the performance of stopping in 2 fixed and several varying locations between them [30]; and apomorphine-induced hyperactivity was dissociated from

stopping in fixed locations, showing no organization in relation to the environment [31]. Since under the influence of the first 2 drugs the probability of stopping in specific locations increases, locational memory is implied, and our measure would have reflected it. The absence of an increase in the probability of stopping under the influence of the 3rd drug would have resulted in a near-zero rate of change in the probability of stopping implying no locational memory.

#### Newly Derived versus Classical Inbred Strains

CZECHII mice show a significantly higher rate of change in stopping probability than C57, implying even better spatial abilities. Some researchers consider the behavior observed in classic inbred strains to be dull and "degenerate" [32], whereas wild-mouse behavior is expected to exceed the behavior of these strains [33],[34] in terms of repertoire richness [35], and magnitude of parameters [11],[36]. CZECHII mice are a relatively new wild-derived strain, perhaps less affected by the domestication process; the enhanced spatial performance of this strain could be ascribed to its relative wildness.

## A Bottom-Up Approach to Higher Cognition-Related Constructs

The bottom-up approach employed by us aims at revealing higher-level phenomena, as they emerge out of low-level kinematic properties. In the present study, assigning visiting records to locations, and characterizing the sequences constituting these records, reveals locational memory. This phenomenon adds up to a list, reviewed below, of previously described higher-level phenomena also uncovered by the bottom-up approach.

Noting where rats stop, and for how long, highlighted the home-base-the s most preferred place in the arena [3]. Using this place as a reference for measuring kinematic properties of the rat's trajectory revealed several features of the rat's operational world. Partitioning the rats trajectory into roundtrips performed from the home-base highlighted a gradual lengthening of these roundtrips. This lengthening was correlated with an increasing amount of exposure to the arena. It defined, therefore, the animal's increasing familiarity with the environment [5]. A high level of familiarity ( = exposure) was also indicated by a reversal of speed differences in relation to the home-base: in a novel environment, the outbound portion of a trip was characterized by lower speeds, and the inbound portion–by higher speeds; in a well-trodden environment the speed difference was shown to be reversed. These speed differences together with the amount of exposure defined "inbound" and "outbound" directions from the rat's point of view [5],[37],[38].