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Grooming, sequencing, and beyond: how it all began

M. FRANCES STILWELL AND JOHN C. FENTRESS

Summary

This chapter has taken two distinctive but complementary approaches to mouse grooming. The first is based upon Frances Stilwell's intuitive perceptions in the 1970s of previously unappreciated order in grooming sequences. An important principle here is that early stages in research depend upon sensitivity to what our animals can show us. Premature narrowing of observational perspective can limit the richness of analytical questions that are initially hidden from view. In the second part of the chapter, John Fentress outlines some of the richness of subsequent research that sensitive descriptions have led up to. Mouse grooming has led to a host of studies in behavioral genetics, development, brain mechanisms, and motivational models including stress.

Introduction

This chapter is intentionally divided into two parts. The first part, by Frances Stilwell, outlines the discovery of rules underlying order in the rich patterning of mouse grooming. As Stilwell discovered in the early 1970s, there is indeed syntax, perhaps even a grammar, in these rodent movements. One of the lessons here is to look closely at rules of order in seemingly inconsequential action patterns of the animals around us. They are rich in their structure. Mouse grooming has led to a number of important insights about brain and behavior. Furthermore, Stilwell's comments are not only refreshingly personal, but also important

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as a picture of how research sometimes actually progresses. This reminds us of the insights early ethologists, such as N. Tinbergen, came up with by just watching. Tinbergen's point was well made: sometimes we just need to open our eyes and have our minds ready to receive what nature offers.

In the second part of the chapter, John Fentress takes Stilwell's insights on the discovery of the order in mouse grooming and outlines how it opened up areas of research that were never before appreciated. Others, including the authors of this volume, have taken this richness in movement and shown conclusively how it opens up a wide range of brain/behavior issues that extend far beyond mice, or grooming. It is a rich story that continues to invite explorations from many channels.

Finally, there is an important footnote this chapter offers. The original *Nature* article on mouse grooming "grammars" listed John Fentress as first author (Fentress and Stilwell 1973). There is something uncomfortable, even wrong, if the impression was that Fentress led the discovery of order in mouse grooming. It was Frances Stilwell who discovered the order while in the lab of John Fentress. It is important to make that point of the story clear.

How the study of mouse face-grooming sequences began (M. Frances Stilwell)

Wherein the beauty of the behavior is honored, the value of the nonconscious is confirmed, and a record is clarified.

My job at the University of Oregon Chemistry Department lab ended abruptly three weeks after my arrival from Ohio in September, 1969. It never crossed my mind to go back to Ohio. Instead, I responded by driving from Eugene to British Columbia with drawings from college botany classes to show professors and doctors, hoping to sell myself as a scientific illustrator. Just before Christmas, I took my drawings to a veterinarian's office in nearby Springfield. It just so happened I was taking a course entitled introductory biology, in order to satisfy the state of Oregon's very specific teaching requirements.

The vet said, "Why don't you go to the University? A professor there has wolves and he may want you to illustrate them." I said, "Is his name Fentress?" He said, "Yes!" I said, "I'm taking a course from him and doing very well!" When I approached Dr. John Fentress about illustrating his projects, I introduced myself by saying that I was in his lecture class. He said, "Oh, yeah? What did you think of my test? Some of the students were complaining about it. They said there were trick questions."

In my opinion, the questions were not tricky although they did exact quite a bit of the focused attention required for a Graduate Records Exam to answer them. I

equivocated, "Oh it was fine." He smiled, mumbled, and said, "Tsk. Tsk. Students these days." Then, when he looked up my score on the test, his tone and attitude changed. In a lower voice, sounding as if speaking just between you and me, he said, "As a matter of fact I am waiting to hear about a grant. Why don't you come back after the first of the year. I do need clerical help. How do you feel about working with mice?"

After that conversation, on New Year's Day, the *Eugene Register Guard* newspaper reported on its front page that an unnamed university professor in the Departments of Biology and Psychology had just been awarded a huge federal grant. The next day I called Dr. Fentress. "Could that professor be you?" I asked. "Yes," he said, "why don't you come in tomorrow?"

Dr. Fentress was interested in the role of sensory feedback in mouse behavior. His approach in studying this was to interrupt the route for the information from a mouse's front paw back to the brain, or to "deafferent" the limb, by surgery, and then note the behaviors affected. A postdoctoral staff member, Dr. Maria Rosdolsky, MD, had previously performed the surgeries for him. She had also devised simple tests to determine any effects on their activities. Dr. Fentress wanted me to continue her surgeries and testing work with the mice as well as perform clerical duties as an educational project aide.

On my second day at the lab, which was Dr. Rosdolsky's last day, she showed me the procedures, emphasizing how important it is not to tear the motor nerve or damage a muscle. One of the mice she had worked on carried its desensitized arm flat on its chest, as useless as a polio victim's, which was the result of such a tearing mishap. On previously deafferented mice, and control mice, she demonstrated the battery of tests she'd devised noting whether the deafferented arm adducted or abducted, rolling these two words off her tongue as if they were pablum. I could see there was much ahead for me to learn. These words meant move the limb toward the body ("adduct") or away from it ("abduct"). Her list included a test for response from her pinching the mouse's paw. Another was to hang the mouse by its tail to see if it would grab the wire grill on its cage top with its deafferented arm. A third specified dumping the unsuspecting mouse in a pan of water to watch how it would swim. At some point during the testing protocol a mouse began flapping its arms in all directions. It appeared to launch into a juggling act without a ball. And I said, "What's it doing?" She said, "It is grooming."

The following day when I came in, I lightly chloroformed a mouse, as Maria had shown me, so it would lie cooperatively on its back during the injection of anesthetic. Then I injected the mouse below its rib cage. I can still feel the resistance to the needle prick, which was too low. The mouse squeaked loudly in protest, through its chloroform, with a sound worse than a cat whose tail has been stepped on. The mouse was so vulnerable and trusting in my care. Hearing its cry, I began to cry. Mixed in with my crying for the mouse was the realization that I'd botched

my first surgery. Dr. Fentress, who was talking with a graduate student, John Mates, across the room, interrupted their conversation to ask, "What's wrong?" "It squeaked," I said. "Why don't you go home for the day?" he suggested.

This experience resulted in a change in my mouse responsibilities, which sent me down a different route. That change ultimately produced a much more interesting result both for animal behavior and for myself. I don't recall whether that mouse died, but I never attempted any more surgeries. With Dr. Fentress' agreement, I was now charged just with testing the control mice and the mice whose arms Maria had already deafferented. I particularly remember the swimming tests. I would dump each mouse in the water on one side of a plastic pan and watch it swim frantically across the eight inches to the other side. The idea was to note each mouse's limb activity to check for two responses, apparent through the clear plastic walls. Firstly, to see if it used its experimental paw to swim as a normal mouse would, and secondly, to see whether it reached out that paw to touch the home-free side, its goal.

So on my fourth day of work, I began the battery of tests. I looked at both desensitized and control mice. Once each mouse had reached the opposite side of its water-filled cage, I lifted it out and let it recover on the counter top before its next test. I recorded my results. I would then wait patiently while the mouse shook off all its water droplets and calmed down from its experience. Once that test was done, I lifted each one back into its proper home cage or proceeded to the next test. I only vaguely watched each mouse as it sat on the counter top between tests, but it was always in my field of view. Though I faced my subject, my attention, and probably my eyeballs, drifted away from the mouse and back.

Between the fourth and the tenth mouse something about the mouse being tested made me focus on it. The mouse had inexplicably grabbed my attention. Even then I focused for less than an instant. It was the strangest passing of information from mouse to human! I said to myself reflexively, in an offhand way, "Oh, yeah, that's what they do." I have described it later as having my body pick up the information through its pores and that only later did the information rise up into my brain. Actually, my mouth knew it before my brain did.

I was talking to myself not to anyone else. It wasn't until my ears heard the content of my phrase that I was aware of what I knew. There was order. Another graduate student, Doug McDonald, heard me from his nearby desk. "What was that?" he said. "When the mice groom," (I'd heard this word from Maria, here I was referring to grooming of their heads) "they do it in a particular way each time." John Mates came across the room from his desk. "What was that?" he asked. After I told him, he and Doug looked at each other. One of the graduate students said, "This would fit very well into John's research," referring to our supervisor

Dr. John Fentress. I did not know what they meant by that statement, but when I heard it, I realized there was something here that could make me very valuable at my new job, so I set out to identify this order in the grooming.

Obviously, if I were to identify what this order was, I would have to describe what the mice were doing. I decided that the first approach would be to count the arm movements. The next time a mouse groomed after swimming, I tried. But the action was much too fast. It was not possible to keep up, much less to be able to speak the count out loud with the resources at hand.

I said to myself, "I need slow-motion photography." When Dr. Fentress came back to the lab, I told him what I'd noticed. He procured a slow-motion movie camera and a stop-action projector for me to use. From then on my focus at the job, when clerical activity was not required, was to film the mice, to attempt to track down this illusive order in the movements, and to document my work.

There was no question about its being there, the only question was "What is it?" This was uncharted territory for me. I was not conscious of any traditional scientific approach used to accomplish this analysis. I pretty much went as if I were a hound dog sniffing a scent or as if I were untangling a ball of yarn. Since in science, the approach is to test hypotheses, to try to eliminate them not to prove them, one might be tempted to say the notion of order was hypothesis – but I had no question about the order's existence. I just wasn't aware of what it was. I knew that the order would eventually pop out of my chronology of what turned out to be the two seconds or more of face-grooming activity. I felt everyone believed I was going to find that order so they all patiently awaited my result. Occasionally I got inklings that perhaps Dr. Fentress might not be as convinced as the others that I was going to find order or even that it was there in the first place. But, I encouraged myself, "I will find it and when I do will he ever be surprised!"

Step 1. I set up an old, cracked glass aquarium on a lab desk, and cut up a plastic cage. Then I fabricated a mouse-size plastic cage with one side open at the top and one side open where it could be taped to the inside of the aquarium. I faced the camera through the aquarium glass into the plastic cage. Then I would drop either a control or desensitized mouse by its tail into the cage, focus the camera and, with my finger on the shutter, wait and watch. Very soon, in its new surroundings the mouse would begin to groom. It took me a few tries to learn when to start the camera whirring so as to catch the whole or most of the routine. Sometimes the mouse would not be facing the camera when I started so I turned it around with a pencil while it was still grooming. I realized later that in only a certain part of the face grooming, which I called "single-stroking," could I do this without interrupting the grooming activity.

Step 2. To analyze the film frame by frame, I set up the projector in the lab to describe the action. I had to get information out there on paper to see what the

order was. I remember the first grooming activity I tried to describe. My inadequate words almost immediately gave way to what came more naturally to me, and I resorted to making drawings of the arm movements along the head. This generally meant tracing the paws' trajectory with respect to the snout. As I analyzed the film, I became aware of categories of movements, which I referred to as strokes. My first such drawing of a stroke was of a half-moon trajectory. I saw that all the movements fell out quite naturally into strokes of repeated forms, which invited my naming them, which simplified my note taking. I labeled the strokes as follows:

1. Parallel was a half-moon trajectory along the snout with the paws duplicating each other's form.
2. Circling reminded me of a cheerleader introducing her cheer at a high-school basketball game, by moving her forearms in an irregular-looking way.
3. Licking was the horizontal movements back and forth beneath the lower jaw. (Occasionally I would see a tongue come out. This stroke had two versions: (a) short-licking, which was slower and of short duration and (b) long-licking, which was faster and of longer duration.)
4. Overhand reminded me of an overhand smash stroke in tennis.
5. Single-stroking was a series of ten flat strokes with the arms alternating left and right perfectly as to which made the greater excursion. It was almost a staccato percussion movement as rigid as wooden soldiers.
6. Shimmy, a blur of the whole body in every frame at 32 frames per second, reminded me of a hula dancer.
7. Pause was when both paws were held still somewhere below the chin or at chest height. It still seemed to be part of the grooming activity.

I dutifully recorded frame numbers involved for all the strokes and briefly illustrated some of them. I particularly remember drawing the meandering paths of the overhands and that I included the frame number for each point along the route. Out of curiosity at the end of the analysis of my first routine, I added up the strokes, and I was gratified but astounded to realize how many there were. The mouse had performed twenty strokes in two seconds, including the ten in single-strokes. No wonder I couldn't count them! Dr. Fentress eventually found me a desk space for my analyses in a dark closet full of photographic equipment. There I could better see the contrasts in the blacks, whites, and grays when the film was projected.

Since my work with mice was second in priority to my clerical duties around the lab, I could not focus full time on answering my question, "What is the order?" However, I was never hesitant or concerned about finding it, nor did anyone rush me. Analyzing the grooming steps was time consuming. However, I loved watching

the mice do their thing, especially in slow motion. It was like witnessing a ballet. I liked working with the DBAs (the little gray mice) the most, as they seemed to thrive on grooming. The overhand strokes particularly were executed with robustness, verve, sensuousness, and pride.

The whole phenomenon of my filming and studying mouse face-grooming made some other people curious. One day I became aware that one of the professors in the neurobiology group was watching me from about five feet away as I concentrated on filming a mouse. When I finished with the filming, I looked up to see what he wanted. When he kept on looking forward in a kind of daze, someone said, "He's watching Frances watch the mice."

The spirit of inquiry was alive in what we called the ethology lab, the original European term for animal behavior. I loved being a part of it. Dr. Fentress would toss out an idea and then challenge his own thinking. I felt appreciated, and thought I'd found my niche in the whole field of animal behavior, which had more engaging stories than those in my previous academic fields of concentration, botany, and biophysics. Often while pipetting in the chemistry department I had looked longingly out of the window at the veterinary office across the boulevard and wished I were working with animals. The goodness of life had come through for me.

Dr. Fentress had a way of encouraging people in their work. He suggested that I take his course on animal behavior. I bought the thick blue textbook written by his mentor Robert Hinde at Cambridge University in England and began to consider a PhD to pursue a career in ethology. Years afterwards I met a student from that class, who said Dr. Fentress was the most inspiring professor he had ever had. Professor Robert Hinde's presence was felt in the lab long before he came to the University of Oregon for an invited lecture. In a sense he was mentor to us all.

Dr. Fentress pretty much left me on my own. I filled pages and pages of long sheets of newsprint with my penciled observation notes. I planned to describe 20 sequences before presenting the findings to him. As I worked I began to develop an impression of which strokes were associated with which. Eventually I called these associations "units." I also began to be convinced of the rhythm of the order of the units as they proceeded through the "sequence."

In addition to regaling us in the laboratory about his motor scooter ride to interview Carl Jung, Dr. Fentress often repeated his version of quotes of famous people. Of relevance to what I was doing was a quote he attributed to Nikko Tinbergen, Noble Prize winner, "I let the animals ask the questions. And when they do, I listen, for they ask very good questions." The mice had indicated to me, "We groom our faces in an order, do you know what it is?" I was searching for the answer to their question.

One day Dr. Fentress stopped by the viewing closet. That was the only occasion when we discussed my grooming work until I presented him with the results. He asked, "How's the closet working out?" "Fine." I said. "Imagine that – they do twenty strokes in two seconds! No wonder I couldn't count them!" Then, by way of conversation I continued, "For some reason I know when to start the camera," which I said as if accepting one of life's wonders. He said, "Perhaps now is the time for introspection."

Introspection meant two things to me. First, was to scavenge around internally to ask, do I feel this or do I feel that? Second, was to be open to what I found. I chose to emphasize the second. Very soon, probably at my next filming of a mouse, with my mind open to possibility, I suddenly saw that the mouse wiggled its body all over fast before the start of its routine! I'd seen this motion before, mixed in with other strokes and had called it "shimmy." This shimmy was the signal at the start of their routine for me to start the camera.

For 16 months the routine of my job was to perform my clerical duties, then when a break occurred to go to the grooming work. In April, 1971 Dr. Fentress was to give a talk at the Annual Biology Colloquium at Oregon State University in Corvallis. It seemed to me that he might be interested in the results of my grooming research to include in his speech. I had 16 sequences completed, including 3 from the deafferented mice. My observations by then had developed into a logical process I believed in concerning the flow of strokes. Generally this was it: at the start, there would be a shimmy, then a variety of stroke types would occur. Then the mice began slow-licking, which suddenly shifted to fast-licking. Then single-stroking blasted forth followed by a series of overhands, which was followed by a variety of stroke types again.

I summarized the sequences on one long sheet of newsprint, by letter for each stroke with the numbers of frames spent on each. I left it on Dr. Fentress' desk one evening after he had gone home. It felt redundant to say, "These are the units in the grooming sequences," but I did. Although they were obvious to me, for emphasis I put brackets around the units and numbered them. The order within the sequences, including the composition of the units, started to be apparent to me as I was taking notes on the 16 mice. However, I don't believe I was cognizant of it until I forced myself to write the information down. The next morning I found the list of pencil markings on my desk with his comment in ink "Excellent!"

Dr. Fentress reacted further to my breakthrough by saying: "This is the first time since birdsong, such complex stereotyped behavior has been found in vertebrates. It is the only such behavior known in mammals." He heralded the discovery as being a second example of hierarchical organization in vertebrates. His word, "hierarchy," brought to my mind a royal line, which would be an elegant and appropriate connotation for these behaviors in my mice. It also brought to mind

a family tree flattened on paper, which I felt really ought to be three-dimensional with lives of children going all directions. Such a concept could better be represented by a mobile, which shifts with directional breezes but remains in balance.

About then, Dr. Fentress also suggested that the discovery was a grammar of movement. I never liked that metaphor since grammar implied such rigidity to me. It seemed inappropriate for such a lovely dance as the filmed mouse grooming, performed with a looseness in order left somewhat to the discretion of the mouse performer. This grammar metaphor gave impetus to a remark by Loren Northrup, another graduate student, who, after my results were revealed, had begun studying the grooming sequences in his neurological mutant mice. His comment was, "Do you want good grammar or good grooming?"

Dr. Fentress also straightaway applied statistics to what to me were obvious results. I thought he must have been one who ascribed to Lord Kelvin's point of view, which is essentially that "nothing is known until there are numbers on it." The data did provide a good opportunity for a statistician. The statistics were consistent with my observational findings. Actually, the mice's movement reminded me of the progression of orchestral music. I described the grooming as being like watching an orchestra with players tuning their instruments before a concert. The conductor raises his baton with circling, the symphony begins with licking, which suddenly increases in intensity and passion bursting, exploding into cymbals with single-stroking and finally, with violins, the overhands draw out the final ecstasy.

Shortly after I summarized the order, I recognized it in hamsters as well as mice. Then I saw it in gerbils, then rats of course, then in a film of golden-mantled ground squirrels. Each time the results of my pure research reminded me, "There really is order in the rodent universe!" In fact the order seemed to be a marker for rodents. Then a highly strung Sminthopsis, a marsupial rodent from Australia, arrived briefly in the lab. It groomed in long bouts and under similar circumstances as the mice but its movements, which appeared much faster than in the mice, did not look as if they would be so graceful in slow motion.

After I had listed the sequences, Dr. Fentress and I talked about whether the strokes and the ordered connections between the strokes could be detected in infant mice so I began a series of films on them, too. One time Dr. Fentress was in the lab as I filmed the infant mice. Someone asked him a question. I knew from his answer what the question concerned, because he replied, "I'm impressed with her because I wouldn't be able to do what she did." At the time I wondered whether he was impressed that I could sense the order of such a lightning-fast behavior before formal analysis, or was it that I had persisted in taking all those notes, or was it that I could spot the order from the notes? Upon reflection I continue to believe that each of us has much inside sending us signals that we don't ever

take advantage of. I truly believe that children musing over their pet gerbils are probably as aware of some kind of order as I was when beginning the work with the mice. But children don't often have the circumstances that would lead them to filming.

I saw the order and defined it, but it took a different kind of mind and experience to know where the order in mouse grooming fitted into the then-known body of scientific knowledge, and a different sort of personality to promote the new information. This is where our teamwork became truly effective.

During September that year, Dr. Fentress was to present a talk at the International Ethology Conference in Edinburgh, Scotland. I elected to take my vacation in Great Britain during that time, and then hear him present my work in the capital of Scotland. When Dr. Fentress gave his talk, I was proud to have the results of my research on order in face-grooming sequences presented at an international gathering. What a wonderful culmination that trip was. It was a celebration of my joy in unknotting the order I knew was there in the mouse face-grooming sequences. A year later I wrote to Nikko Tinbergen inquiring about research positions in his lab. At the request of Professor Tinbergen, Richard Dawkins, in his research group at Oxford University, answered the letter: "I think your work on mouse grammar is fascinating, and I am sorry you want to give it up! However, I am afraid that in any case we have no money at present to employ you, much as we should like to." I also wrote to Konrad Lorenz who returned a nice note saying he was "going emerit" at the end of the year and so could not promise a guest research position in his department.

The publication from Dr. Fentress' Corvallis Colloquium at Oregon State University (Fentress 1972) described the strokes and order quite adequately, but didn't have a wide readership. In those days the proceedings of an International Ethology Conference were not published. For the discovery's real debut, therefore, we wanted to publish in a very prominent journal. Because I had wanted to publish in *Nature* ever since my MS in botany–biophysics days, we agreed we would submit an article, "The grammar of a movement sequence in inbred mice," to that journal. I had learned during my graduate studies that the first author of two was considered to be the originator of the research, actually the lead contributor, and I looked forward to a first authorship in what might become my new field of animal behavior.

So we composed the first part of the article. Dr. Fentress then said, "I will add some statistics." I accepted by then that statistics are necessary to quantify and add credibility to observational data. When I saw Dr. Fentress' name listed first in our submission to *Nature*, I was stunned. If I understood the protocol correctly, readers could conclude from his being listed as the primary author that he had had my insight and had also done the follow-up work. When I asked