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Motivation and Arousal Influence Sound-induced Maternal Pup-retrieving Behavior in Lactating House Mice

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With 3 figures

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Abstract and Summary

1. Influences of sounds, short-term motivation and arousal (alertness) on sound-induced pup-retrieving behavior of female house mice were studied in choice tests.

2. The females respond significantly faster to key-stimuli (preferred ultrasonic call models) compared with non-preferred ultrasounds, given the choice between these two alternatives. In addition, response latency depends on the pre-response behavior, being shortest when the females are orienting and longest when they are nursing their pups (intermediate latencies after "active" broodcare, self-directed behavior, and warming the pups).

3. Key-stimuli are significantly more often chosen after broodcaring activities than after other behaviors.

4. Retrieval time is generally longer after broodcaring behavior than after other activities. Retrieval time is not influenced by the presence or absence of sound during retrieval.

5. Duration of control runs is longer when sound (key-stimuli or non-preferred ultrasound) is present compared with a no-sound situation.

6. The behavior after the control runs (at the end of the tests) depends on pre-response behavior.

7. The results are summarized and interpreted in a flow-diagram which semi-quantitatively describes the release and course of the behavioral sequence of pup searching and retrieving behavior in house mice.

Introduction

Ultrasonic calls (USC) of mouse pups are effective releasers of maternal activities in lactating female house mice (ZIPPELIUS and SCHLEIDT 1956; NOIROT 1964 b, 1966 a and b, 1972; SMOTHERMAN et al. 1974; SMITH and SALES

1980; HAACK et al. 1983). When the source of USC radiation is outside the nest area, e.g. a pup displaced from the nest is calling, the mother is activated by the USC to leave the nest and is guided by the sound to the lost pup. Normally the pup will then be carried back to the rest of the litter. In this situation the function of the pup's ultrasound seems twofold: a) to release maternal behavior (searching for the lost pup), and b) to serve as a cue for the pup's location.

In a previous series of experiments (EHRET and HAACK 1981, 1982) we characterized the configuration of the ultrasound necessary for recognition by the females. In a two-alternative forced choice test the females showed their preference for one or the other stimulus by moving out from the nest toward the respective sound source. In successive tests we determined the numbers of choices for either alternative in order to separate preferred and non-preferred stimulus configurations and thus to measure the "key-stimulus" configuration for recognition. Despite the fact that a certain class of sounds (the preferred ones) were significantly more effective in directing the females toward the sound source, others (the non-preferred ones) were also capable of at least activating the females to search for lost pups. Sound stimuli in general seemed to increase the level of arousal or of general motivation for a motor activity in the females, which then became specifically goal-directed in the presence of preferred USC. The sequence of maternal behavior — being activated for leaving the nest, approaching a pup, retrieving it to the nest, and settling again — obviously is influenced by the quality of external stimuli as well as by internal factors.

Since we know the sound stimulus conditions for initiating pup searching behavior (e.g. pure tones between 20 and 60 kHz, bands of noise with frequency components between 20 and 60 kHz) and for selectively guiding a female to the lost pup (only preferred USC models which shall be called key-stimuli: pure tones between 40 and 60 kHz, noise bands of limited bandwidths, e.g. a 40—60 kHz band) we now want to quantify 1. the influence of sound stimuli on the whole sequence of maternal behavior mentioned, and 2. the influence on that behavior of internal (motivational) tendencies different from those for searching and retrieving. Finally we shall combine external and internal influences on pup searching and retrieving behavior in a diagram giving an idea of the weight of the different factors mentioned in this behavioral sequence.

Materials and Methods

The behavioral data presented here were obtained from the same mice (primiparous lactating house mice, *Mus musculus*, outbred strain NMRI, aged 3—5 months, with litters 1—7 days old) as in the previous study (EHRET and HAACK 1982). In general, aspects of the following behavioral sequence were quantified: A female is occupied by a certain behavior in the nest; then, in response to external stimulation, leaves the nest and searches for a lost pup, finds it and carries it back to the nest, makes control runs on the running board outside the nest, and finally settles again in the nest and returns to a certain behavior. The measures of interest were:

- a) The kind of behavior shown by the female before she left the nest in response to external stimulation.
- b) The response latency from stimulus onset to response onset (the latter being when she just left the nest area).
- c) The duration of pup retrieval (retrieval time between leaving the nest and putting down the retrieved pup in the nest).
- d) The duration of control runs (control run time between the deposit of a retrieved pup and the end of moving around on the running board (the latter being defined as the instant when she was settled again in the nest for at least 30 s, whereby the 30 s were not included in the duration of control runs).
- e) The kind of behavior shown by the females in the nest immediately after retrieval and control runs were finished.

The measurements were made in two test situations always under very weak illumination (≤ 1 lx) in a sound-proof and anechoic room.

1. Measurements in Situation A

Response latencies from stimulus onset to response onset were taken in the choice tests for determination of the key-stimulus configuration (see EHRET and HAACK 1982 for a detailed description). In short: At least 6 h before the measurements began a female with her litter (together with some wood shavings as nest material) was placed in a nest depression (10 × 10 cm) in the middle of a running board (length 110 cm, width 8 cm). A water bottle which was removed during the tests was suspended at one corner of the nest. Synthesized models of ultrasonic pup calls (pure tones and noise bands of 80 ms duration, 10 ms rise and fall times, 3 calls per s) were delivered through two identical ultrasonic speakers mounted independently from the running board, one at each end, 65 cm from the nest depression. Each speaker (a or b) emitted one stimulus alternative (A or B) in such a way that the females heard a series of alternating stimuli (e.g. $A_a, B_b, A_a, B_b, \dots$). Each female responded at most 6 times to a given stimulus configuration and to no more than two different configurations presented on different days.

Before a test series started the females were motivated by letting them carry 4–6 pups, randomly distributed on the running board, back to the nest. This pre-motivating, which increased the arousal and alertness to a defined and very similar level in all females, was done for the following reasons:

- a) To reduce interindividual variability of response latencies and of response durations. Since we intended to measure subtle influences of external stimuli and of short-term motivation other than that for pup searching and retrieving on the behavioral sequence described above, the setting of a common baseline seemed to be necessary. From this baseline modulations in terms of shifts of response latency and duration can be easily detected and treated statistically.
- b) To indicate to the otherwise undisturbed and uncaged animals that a test series was started during which pups could get lost. Thus we initiated the natural behavior of search and retrieval which we wanted to study and took measurements when the behavioral sequence had stabilized. By that we intended to avoid variability by measurements during the "warming up" period just as we tried to reduce sequential effects by running only maximally 6 tests in a sequence.
- c) To keep observation times within reasonable limits. For example, females which nurse their pups and sleep do not at all respond to ultrasounds from outside the nest (HAACK 1978).

After pre-motivating, when the female was settled once more in the nest, the speakers were switched on simultaneously for the first test. The latency of the response was measured and the sound stimulus which attracted the female was noted. When the female had settled back in the nest for at least 30 s, the speakers were switched on for the second test, etc. Sometimes a female had to be re-motivated before 6 tests were finished, because she was continuously nursing her pups for more than 10 min (arbitrarily set time limit). Data were taken from altogether 215 females.

2. Measurements in Situation B

The durations of pup retrieval and of control runs were measured in a slightly different situation. Instead of using loudspeakers for emitting synthesized ultrasound we used pups of the female's own litter as stimulus sources (experimental situation, pre-motivating, and number of tests in a series were the same as before). After pre-motivating, when the female was settled once more in the nest, a pup was gently placed on one or other arm of the running board (random distribution in successive tests) 40 cm from the center of the nest area. Since mouse pups do not continuously emit USC (HAACK et al. 1983), we did not measure the response latency in these tests because it would have depended too much on the sound production rate of the pup, which is a variable we eliminated in situation A. Instead, we measured the durations of pup retrieval and control runs under three different external stimulus conditions:

- a) The females carried the pups back and did control runs in silence (there may have been some occasional ultrasounds and other sounds from the pups in the nest).
- b) During retrieval and control runs the females continuously heard bursts of a preferred USC model (a band of white noise between 40 and 60 kHz, compare EHRET and HAACK 1982), emitted by two ultrasonic speakers mounted 50 cm above the running board so that the sound intensity was equal (75 dB SPL re. 20 μ Pa) in the nest depression and in the next 30 cm of both arms of the running board. In this case, the sound could have only an activating and not a directing component since it was presented from above with no access for the female.
- c) During retrieval and control runs the females continuously heard bursts of a non-preferred sound (pure tone of 20 kHz) presented in the same way as above.

In these tests 18 females were involved.

Results

1. Does Response Latency Depend on the Characteristics of the Ultrasonic Stimulus?

We determined the averaged latencies separately for responses to preferred USC models and non-preferred ultrasounds in tests, a) in which the females had the choice between these two classes of stimuli, b) in which two preferred USC models were the alternatives, and c) in which two non-preferred ultrasounds were presented. Table 1 shows the results. Given the choice, females responded significantly faster to preferred USC models compared with non-preferred sounds. Response latencies were significantly longer in tests with two preferred USC models compared to two non-preferred ultrasounds, and they were significantly longer to non-preferred ultrasounds in tests with a preferred model as alternative, than in tests with two non-preferred ultrasounds. Thus the females hesitated longer with their response to non-preferred ultrasound if a preferred alternative was present.

2. Does Response Latency (Situation A) Depend on the Pre-response Behavior?

We arranged the observed behavioral patterns into 5 classes:

- a) Orientation: sniffing in the nest, sniffing over the nest border, biting the nest border, circling in the nest (but not on the pups), sitting away from the pups and turning the head, listening.

Table 1: Dependence of the response latency on the kind of choice situation. Significant differences (U-test) of the mean latencies occur between I and II ($p < 0.02$), III and IV ($p < 0.05$) and II and IV ($p < 0.01$)

choice	Response latency (s) and number of runs (N)	
	preferred	non-preferred
preferred vs. non-preferred	I 66.7 $\pm$ 21 (N = 724)	II 81.2 $\pm$ 22 (N = 332)
preferred vs. preferred	III 77.7 $\pm$ 22 (N = 583)	-
non-preferred vs. non-preferred	-	IV 67.3 $\pm$ 30 (N = 613)

- b) Self-directed behavior: self-grooming, eating, lying beside the pups and dozing.
- c) "Active" broodcare: grooming, licking, sniffing the pups, digging in the litter, circling on the litter, nest-building.
- d) Warming the pups: lying on at least part of the litter while the pups do not feed.
- e) Nursing: lying on at least part of the litter while the pups are suckling. When a female is nursing, she may at the same time eat, sniff around, listen, or doze.

Table 2 shows the response latencies for the 5 behavioral classes. Latencies have been calculated from responses to preferred USC models. Shortest latencies occurred when the females were orienting, next longer ones when they did "active" broodcare, again longer ones when they were involved in self-directed behavior; even longer latencies were measured when they were lying on the pups for warming, and longest latencies occurred when they were occupied with nursing. All latencies are significantly different from each other. The response latency to external sound stimuli clearly depends on the behavior before an animal starts responding. The values of the standard deviations in Table 2 correlate significantly ($r = 0.976$, $p < 0.01$) with the mean values. That is, the longer the females, on the average, hesitated, the more variable was their response latency, the less predictable was the beginning of the response.

Table 2: Dependence of response latencies on the pre-response behavior. All mean latencies are significantly different among one another (U-test, $p < 0.01$ at least)

	orientation	self-directed behavior	"active" broodcare	warming	nursing
mean latency (s)	28	65	41	91	246
standard deviation	22	53	29	68	120
number (N) of observations	455	378	234	71	119

3. Does the Response to Different Ultrasounds Depend on the Pre-response Behavior?

Table 3 shows the distributions of responses in favour of preferred USC models and non-preferred ultrasounds (taken from tests in which they had the choice) in dependence on the behavior shown before they responded. In all cases they responded significantly more frequently to the preferred USC model, which could be expected. However, a statistical comparison among the response distributions related to the preceding behaviors points to significant differences, which can already be inferred from the percentage values given in brackets (Table 3). Thus the distribution after self-directed behavior is different from that after "active" broodcare (χ^2 -test, $p < 0.01$) and from that after nursing ($p < 0.03$), and the distribution after "active" broodcare is different from that after orientation ($p < 0.05$). When we add up the values for all three broodcaring activities ("active" broodcare, warming, nursing) and compare these with summed values of non-broodcaring activities (orientation and self-directed behavior) highly significant differences occur ($p < 0.005$). It is obvious that the best situation for starting a run toward a preferred USC model is involvement in a kind of broodcaring activity. After non-broodcaring activities the probability of "mistakes" is higher, although in this case the key-stimulus is also clearly preferred to other sound.

Table 3: Dependence of response distributions to preferred and non-preferred ultrasounds on the pre-response behavior. For each behavior there are significantly more responses to preferred USC models (binomial test; $p < 0.0001$ for orientation, self-directed behavior, "active" broodcare and nursing; $p < 0.008$ for warming

numbers (%) of responses	orientation	self-directed behavior	"active" broodcare	warming	nursing
to preferred USC models	238 (65 %)	169 (62 %)	112 (75 %)	35 (70 %)	88 (73 %)
to non-preferred ultrasound	129 (35 %)	105 (38 %)	38 (25 %)	15 (30 %)	32 (27 %)
total	376	274	150	50	120

4. Does Retrieval Time (Situation A) Depend on the Pre-response Behavior and on the Presence of Sound during Retrieval?

Table 4 shows the average retrieval times after the different pre-response behaviors. Shortest retrieval times occurred after orientation and self-directed behavior, longer durations were measured after the females were warming or nursing their pups, and longest times for pup retrieval were obtained after "active" broodcare. Significant differences exist between retrieval times after self-directed behavior and "active" broodcare and nursing. When the retrieval times are separately averaged for the two classes of non-broodcaring activities ($\bar{x} = 81.2$, s.d. = 39.7) and for the three classes of broodcaring ($\bar{x} = 109.4$, s.d. = 66.5) we obtain highly significant differences in the resulting retrieval

Table 4: Dependence of retrieval times on the pre-response behavior. Significant differences exist (U-test) between retrieval times after self-directed behavior and "active" broodcare ($p < 0.01$), and nursing ($p < 0.02$)

	orientation	self-directed behavior	"active" broodcare	warming	nursing
mean retrieval time (s)	84	80	141	103	105
standard deviation	28	42	74	60	64
number (N) of observations	15	60	12	26	80

times. The females were obviously slower in returning pups to the nest when they were occupied by broodcaring activities before their response.

The average retrieval time without sound stimulation was 98 ± 51 s ($N = 95$). When the females were stimulated with a preferred USC model (40–60 kHz noise band) retrieval time was 99 ± 72 s ($N = 50$) and with a non-preferred sound (20 kHz tone bursts) it was 93 ± 50 s ($N = 50$). Statistical comparison did not show any significant differences of retrieval times, so that the retrieval speed is not influenced by the presence or absence of different sound stimuli.

5. Does the Duration of Control Runs after Pup Retrieval (Situation B) Depend on Sound Stimulation?

Control runs lasted, on the average, 101 ± 96 s ($N = 94$) when no sound was present, 152 ± 100 s ($N = 49$) when a preferred USC model was presented, and 143 ± 83 s ($N = 48$) in the case of a non-preferred sound. Control runs with sound are statistically different in duration compared with runs without sound (U-test, $p < 0.01$), while the sound quality (preferred against non-preferred) made no difference. The females thus were stimulated by sound to prolong their control runs, i.e. to look for further pups.

6. Is the Behavior after the Control Runs Related to the Pre-response Behavior?

Table 5 shows how frequently the females exhibited the same or a different behavior at the end of a test compared with the beginning. For orientation and warming the females showed significantly more often a different behavior at the end of a test. When they started with self-directed behavior they returned significantly more often to self-directed behavior compared to

Table 5: Dependence of the behavior after the control runs on the pre-response behavior. Significant differences (binomial test) occur for orientation ($p < 0.01$), self-directed behavior ($p < 0.0001$), and warming ($p < 0.04$)

number of cases	orientation	self-directed behavior	"active" broodcare	warming	nursing
behavior the same	2	49	4	7	39
behavior different	13	11	8	19	41

any other behavior after a test. No significant differences could be measured when the initial behavior was "active" broodcare or nursing. It is interesting to note that the motivation for self-directed behavior persisted or was re-activated after a whole sequence of maternal behavior while motivations for other behavioral classes were not.

7. Do Latencies, Durations, and Observed Behaviors Change during the Sequence of 6 Tests?

Fig. 1 shows how the mean response latencies (situation A, values from responses to preferred USC models), the mean retrieval times (situation B), and the mean durations of control runs change in the sequence of 6 tests. A comparison of the means of response latencies did not reveal significant differences among the different tests. The same is true for the durations of control runs. In the case of durations of retrieval, the mean retrieval time of the 1st test was significantly longer than retrieval times of tests 2, 3, and 4 ($p < 0.01$). No other significant differences occurred.

Fig. 1: The dependence of response latency (●), retrieval time (■), and duration of control runs with (▲) and without (▼) sound stimulus on the serial number of the tests. Standard errors of the means are also shown (for clearness partly only in one direction)

These comparisons indicate that the tests are homogeneous among each other so that the time measurements taken to quantify influences on pup searching and retrieving behavior are independent of the temporal course of the experiment. There is one exception: In the 1st test the females took longer to retrieve the pup compared with tests 2, 3, and 4.

Fig. 2 shows the result of a sequential analysis of behavioral classes in the inter-test intervals separately for the situations before each test and after each test in the sequence of 6 tests. The relative frequencies of behaviors before tests 1, 2, ... (situation A) are statistically different only when the 1st test is compared with the 4th, 5th and 6th. In no other case did significant differences occur. Thus the probability of occurrence of the behaviors before a test was rather homogeneous during the sequence of 6 tests. When relative frequencies of behaviors after tests 1, 2, ... are compared among each other

Fig. 2: Relative frequencies of occurrence of behaviors before (striped bars) and after (black bars) the 1st, 2nd, ... test

(situation B), no statistically significant differences could be measured. Evidently, the probability of occurrence of the behaviors after a test (after the end of the control runs) did not change significantly during the sequence of 6 tests.

From these results of the sequential analysis we are confident that the measures taken before, during and after the 6 tests of a female in a sequence are minimally or not at all influenced by the serial number of the test.

Discussion

The sequential analysis of latencies, durations and behaviors observed and quantified in the present study showed (Figs. 1 and 2) that the measures taken during the 6 tests in a row were homogeneous to a great extent. This shows that pre-motivating the females had one of the desired effects, namely adjusting them to a rather homogeneous and stable level of arousal and alertness, which can serve as a baseline from which one can measure modulations induced by external stimuli and by short-term motivational tendencies. In the following, data from tests 1 to 6 of a female will be treated as representing statistically independent samples with regard to the measures taken. Thereby we accept a minor error introduced in some cases by inclusion of the first test.

Summarizing the results, we can define the conditions for optimal retrieval of a lost pup: The female should already be aroused or alerted, e.g. by previous retrievals, and should show a kind of orienting behavior when a pup starts calling. The slowest retrieval can be expected when the arousal and alertness levels are low and when the female is nursing her pups in the nest.

Influences on the Decision to Start Responding

The data on the response latency suggest that the decision process is not bound on judging positive and negative alternatives but rather on making only a positive decision for the preferred USC model. The females responded with short latencies (67.3 s) when two non-preferred signals were presented. This means that 67.3 s is a general measure for the females' average activation time by sound on the background of a controlled increased arousal and alertness level through pre-motivating. In a choice situation with a preferred alternative almost the same short latency (66.7 s) to the preferred alternative was measured, indicating that making a decision (to a USC model) takes no extra time beyond the general activation time. The significantly longer latencies in this situation for the non-preferred sound can be interpreted as a delay effect of the preferred USC model on moving away from it. When the females had to decide between two preferred USC models the latencies were significantly longer (77.7 s) compared with the situation with two non-preferred sounds, and they were longer compared with those to preferred ultrasound in the true choice situation. The reason may be a mutual delay effect of both preferred USC models because in each case the female had to move away from a key-stimulus.

The response latency from stimulus onset to response onset also clearly depends on the behavioral tendency present when the animals are activated by the sound stimulus. The females showed the shortest latencies while orienting, i.e. when their behavior was directed to perceive stimuli from the surroundings. They also quickly started responding when they were involved in what we called "active" broodcare. In this case they had to switch from one broodcaring activity (in the nest) to another (outside the nest). Latencies became longer when the females were occupied with self-directed behavior. This can be expected since they had to give up a behavior which has nothing to do with broodcare and far-field external stimuli, before a maternal response to ultrasound could be started. Long latencies were measured when the females were lying on the pups, either warming and, especially long, when nursing them. Obviously strong stimuli from the nest make the females hesitate to care for a single lost pup outside the nest. A single lost pup has to "fight for priority" against the rest of the litter which has close contact to the mother. According to BELL's (1974) hypothesis that ultrasounds of pups produce arousal, quite a number of USC from the lost pup (or other sounds, as we have shown) are necessary to increase the unspecific arousal in the females to such a level that they become ready to respond in the presence of other stimuli from the nest. In fact, if we had used females without pre-motivating, response latencies of nursing mothers could have been much longer (HAACK 1978, and unpubl.).

If we accept the arousal hypothesis, the latencies in Table 2 could be used as indicators of the relative arousal level during certain activities. All these data show that the internal states of the females, expressed either by the behavioral program just produced or by the arousal level, exert strong in-

fluences on the readiness to respond to pup ultrasound from outside the nest. Table 2 indicates that the more the females hesitated the less predictable were the response latencies. This, in addition to the latencies themselves, points to a strong influence of the momentary internal state on the response latencies.

The next question (whether or not different behavioral states influence the decision about what kind of stimulus to approach) is answered by the data presented in Table 3. Self-directed behavior and orientation led to significantly lower rates of "correct" responses (toward preferred USC models) compared with behavior related to broodcare. This is an important result, since it shows that recognition of the key-stimulus configuration for pup USC can be influenced by ongoing behavior. Being already involved in broodcaring activity in the nest increases the probability for recognition of a broodcare-demanding stimulus from outside the nest.

Influence during the Time of Responding

Retrieval times did not differ if the females heard key-stimuli or non-preferred or no sound. Thus the females were, while retrieving a pup, irresponsive to sound stimulation by e.g. other pups also displaced from the nest. This kind of refractoriness is well adaptive, since a female can carry only one pup at a time and certainly can retrieve most effectively when she does not react to further calling. In this respect, retrieving a pup may be called a consummatory act.

The retrieving time, however, depends on the behavior shown before a female left the nest. In general, non-broodcaring behavior before retrieval had a smaller delay effect than a kind of broodcare. Thus it seems that switching from one behavioral context (non-broodcare) to another (broodcare) increases the arousal level in the females which leads to a faster retrieval.

After retrieval the females normally conduct control runs, thus "making sure" that they did not forget a pup. The duration of the control runs depended, contrary to the duration of the retrieval, on the presence of sound stimuli. Sound stimuli prolonged the control runs. This result can be expected on the basis of the arousal hypothesis (BELL 1974). Sound stimuli, especially when presented from an unusual position, namely from above the nest and running board, will keep the arousal and alertness and so the tendency for seeking further pups on a high level. This results in longer control runs. It is evident that the females did not discriminate between preferred and non-preferred ultrasounds, confirming the hypothesis of increase of non-specific arousal (alertness).

Influences on the Behavior after the Response

Here we tested the "persistence" of behavioral tendencies from before responding through the retrieving tests and control runs to after the tests. We found that the tendency for self-directed behavior either persisted during the retrieving tests or was preferentially re-activated thereafter. On the other hand, orientation and warming the pups was less frequently shown after the tests. It seems that stimulus-induced maternal pup searching and retrieving

behavior represented an interruption of self-directed behavior, which is continued as soon as possible after the sequence of maternal behavior is finished. It is also conceivable that orientation behavior was less frequently found after the tests because it was extensively shown just before and during the control runs.

A Flow-diagram of the Whole Behavioral Sequence

Fig. 3 combines all the measured influences on pup searching and retrieving behavior. The processing of sound stimuli up to the recognition level has already been described (EHRET 1983). Once a stimulus has been recognized as a key-stimulus, the females can show a discriminative behavior to the stimulus. At the same time, general arousal or alertness, which is enhanced by sound stimuli and thus by the key-stimulus, has a strong positive effect on starting the response. The response threshold depends on the general arousal and alertness level and on stimulus characteristics like the acoustic energy transmitted (NOIROT and PYE 1969) and on call specificity (present paper). If the decision is "no" (caused e.g. by low arousal, low stimulus repetition rate, no key-stimulus configuration, which was not the case in the present study), the females stay in the nest and can perceive further stimuli. If the decision is "yes", then retrieving behavior will be initiated. Since we have seen that there is always a certain response latency, which depends on the kind of ongoing behavior before the response is started, we can introduce delays (specified by the ongoing behaviors), relative strength being indicated by the arrow thickness. After

Fig. 3: A flow diagram of ultrasound-elicited pup retrieving behavior of lactating female mice. Negative signs indicate a delay effect or negative feedback, positive signs an enhancing effect or positive feedback. Widths of arrows are proportional to relative strength of the influence or, in case of behaviors shown at the end of a test, to the probability that post-test is the same as pre-test behavior (for further explanations see text)

the behavior-characteristic delay time the retrieving response will start. The duration of retrieving again is influenced by the behavioral tendency before retrieval. These tendencies prolong the retrieval by characteristic amounts. When retrieval is finished, the duration of the control runs is influenced by the presence or absence of sound. Sound stimuli prolong the control runs. Finally the females get settled in the nest again and start with a behavior from the 5 classes mentioned. Positive feedback was seen for self-directed behavior, negative feedback occurred with orientation and warming. No special effect was measured with "active" broodcare and nursing.

Predictions from the Flow-diagram

1. The positive feedback for self-directed behavior predicts that during multiple retrieving tests in a sequence, self-directed behavior should become more and more frequent. We can test this prediction by comparing the relative rate of self-directed behavior before the first, second ... sixth test and similarly after the tests (Fig. 2). In fact, the relative occurrence of self-directed behavior increases from 21% before the first test to 38 % before the sixth test and from 32 % after the first test to 52 % after the fifth test (here the number in the sixth test is too low to expect meaningful results). Negative feedback for orientation predicts a decrease of this class of behavior in the sequence of the tests. A trend in this direction is evident inasmuch as orientation decreases from 40 % before test 1 to 31 % before test 6. Regression analysis shows significant correlations between the increase of the serial number of tests and the increase of % self-directed behavior before ($r = 0.951$, $p < 0.01$) and after ($r = 0.927$, $p < 0.05$) the tests.

2. The diagram (Fig. 3) can be used to predict the fine structure of the behavioral sequence lactating females show, when they hear sound stimuli from outside the nest, which may be ultrasounds from a lost pup. It does not consider other cues like olfactory stimuli which have been shown to influence response speed for pup retrieval in rats (SMOTHERMAN et al. 1978) and differential choice in rats and mice (SMOTHERMAN et al. 1974). Olfactory stimuli thus contribute to the arousal, which shortens the response latency, and contribute to the specificity for recognition of a lost pup. Olfactory stimuli, in cases when they can convey *specific* information about a lost pup, could therefore be introduced in the flow-diagram at the appropriate places and could add to the influences shown. In this connection we can predict that specific olfactory stimuli from a lost pup prolong the duration of control runs¹).

3. Influences of hormonal states (NOIROT 1964 a, 1972 b), of priming and experience (NOIROT 1964 a, 1965), which certainly can affect arousal or alertness produced by the ultrasounds, and the readiness to respond, have not been considered in the diagram (Fig. 3). Such influences may be integrated when their effects on the decision to start a response, on response latencies, duration

¹) In our study olfactory stimuli of the whole litter and nest were constantly present before the tests started and during the whole test session. Thus no *differential* olfactory stimuli were available.

times for retrieval and control runs, and behaviors after the response are more quantitatively known. We can expect that experience with stimuli from lost pups will shorten the time for recognition and will increase the alertness to perceive further stimuli, so that the response latency will become shorter. Experience with handling a pup during retrieval may shorten retrieval times by increased effectiveness. Such influences of experience could be tested by comparing females caring for their first, second, . . . litter.

Zusammenfassung

1. Wir haben Einflüsse von Schallsignalen und Motivations- und Erregungs- (Aufmerksamkeits-)zuständen auf schallinduziertes Jungeeintrageverhalten von Mäuseweibchen in Wahltests untersucht.

2. Die Weibchen reagierten signifikant schneller auf Schlüsselreize (bevorzugte Ultraschall-Lautmodelle) als auf nicht bevorzugten Ultraschall, wenn sie die Wahl zwischen diesen Alternativen hatten. Die Reaktionslatenz auf Ultraschall hängt weiterhin von dem Verhalten vor den Tests ab und ist am kürzesten, wenn die Weibchen sich orientieren, und am längsten, wenn sie die Jungen säugen (dazwischenliegende Latenzen treten bei „aktiver“ Brutpflege, Eigenverhalten und Wärmen der Jungen auf).

3. Nach einer Brutpflegeaktivität wählen die Weibchen signifikant häufiger Schlüsselreize als nach anderen Verhaltensweisen.

4. Die Eintragezeit ist nach Brutpflegeverhalten im allgemeinen länger als nach anderen Aktivitäten. Sie bleibt von Schallsignalen während des Eintragens unbeeinflusst.

5. Die Präsenz von Schallsignalen (Schlüsselreize oder nicht bevorzugter Schall) verlängert die Kontrollgänge im Vergleich zur Situation ohne Schall.

6. Das nach den Kontrollgängen gezeigte Verhalten hängt vom Verhalten vor dem Eintragen ab.

7. Die Ergebnisse werden in einem Flußdiagramm zusammengefaßt und interpretiert, das halb-quantitativ die Auslösung und den Verlauf der Verhaltenssequenz Jungesuch- und Eintrageverhalten von Hausmäusen beschreibt.

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