The Emergence of Non-Match-to-Sample Behavior in the Developing Rat Pup

Alan G. Gittis
Psychology Department, Westminster College, New Wilmington, PA 16172

Between 15 and 18 days of age, rat pups develop an alternation strategy in a spatial non-match-to-sample task. Adapting the rat pups to the apparatus by housing them in it overnight, the difference in behavioral patterns can readily be observed in a single laboratory session. The opportunity of the pups to "nipple attach" to an anesthetized dam serves as the reinforcer. Discussion questions are formulated that encourage students to analyze the behaviors structurally, functionally and developmentally.

Key words: non-match-to-sample, alternation, working memory, hippocampus

Behavioral neuroscience texts incorporate several types of analyses when describing behavior. For example, Rosenzweig et al. (2002) discuss five research perspectives. In their text, the neural basis of behavior is 1) described functionally and structurally, 2) placed in comparative and evolutionary perspective, 3) analyzed developmentally, 4) reduced to underlying mechanisms, and 5) from these analyses applications are considered. The exercise described here can be related to each of these perspectives. It is a study of the emergence of spatial non-match-to-sample behavior (NMTS) in the developing rat. Rat pups are observed as they select a path that takes them to a nursing mother. The task requires the pups to alternate between two paths and the ability to do this structurally depends upon the maturation of neural circuitry and, functionally, illustrates the emergence of adult foraging patterns. The questions presented in the *Issues for Discussion* section revisits these five perspectives.

NMTS has been used to study higher level cognitive processes including those that depend upon both cortical and subcortical structures, particularly the hippocampus, a structure centrally involved both in memory and spatial processing (see Squire et al., 1993). When the task is used with more highly evolved animals such as monkeys, the animal is shown an object under which is hidden a food reward. The object serves as a *sample* and after a time delay the monkey is shown the sample and a novel object. To receive a reward, the animal must select the novel object (i.e., non-match). The task requires the animal to store a representation of the sample and, on the basis of that representation, select an object that fails to match that representation.

The NMTS task used for the monkey is beyond the capabilities of most sub-primate animals, however, a spatial variation of the task is readily learned by the rat, illustrating evolutionary continuity and the rat's particular spatial adaptations. The development of this adaptation will be studied in 15 and 18 day old rat pups as they learn an alternation pattern in a Y-maze. In order to access a dam (a lactating female) a pup must remember the arm it previously entered and then select the alternative one.

Besides NMTS, this procedure has also been called *conditioned alternation* and *win-shift.*

The exercise is based on a study by Gittis et al. (1988) who found that the ability to alternate between arms emerges between 15 and 18 days of age. This time frame correlates nicely with the structural development of the hippocampus (Altman and Das, 1965). The integrity of the hippocampus as well as portions of the frontal lobe are necessary for successful performance of spatial tasks similar to the one used in this project (Kolb, Sutherland and Whishaw, 1983). Functionally, the time frame of 15 to 18 days of age correlates with changes in food consumption patterns. Although rat pups wean naturally some two weeks later, 18 day pups can consume solid food providing them with the ability to survive if separated from their litter.

MATERIALS AND METHODS

Apparatus *(Note: if the project needs to be completed in a single test day, two mazes will be needed)*. A photograph of the Y-maze apparatus is seen Figure 1. The floor is constructed from plywood and walls from 1.5 cm (3/4 in) shelving lumber cut to a 9 cm width. To permit visibility, the lids covering the various maze areas should be fashioned from Plexiglas®. The apparatus is composed of a start area, the arms of the Y and a goal area. The start area is 9 x 8 cm, the arms are 5 x 19 cm and the goal area is 12 x 21 cm. All compartments of the maze need to be separated with manually operated guillotine doors that can be constructed of Plexiglas. The two doors separating the arms from the goal area should have holes drilled in them such that odors from the goal area can reach the pups. A wood block (7 x 3.5 x 8.5 cm) is used as a barrier between the start box and entry to an arm.

Both arms of the Y permit access to the goal area. A divider bisects the goal area. A 7 cm notch is removed from the center divider permitting placement of an anesthetized reclining dam. With the goal area so divided, when pups enter from one of the arms, their access is restricted to either the rostral or caudal nipples of the lactating female. Modeling clay placed around the reclining dam further restricts pups movements in the goal area. It is important that the pups, after entering the goal area,

have essentially nowhere else to explore and nothing to distract them from nipple attaching.

Figure 1. **Photograph of Y-maze**. Note hinged Plexiglas lids for each maze section. These can be taped down during adaptation. Holes are drilled in lid over goal area (raised in this picture) for air circulation.

Subjects The rats pups you will use need to be 15 and 18 days of age at testing. At 15 days, the eyes of the pups are just about ready to open. Although pups as young as 12 days of age have been tested successfully in this procedure, the activity and mobility of the 15 day old pups make them ideal. If possible, pups from the same litter should be tested at each age, however, if it is necessary to test animals at both ages on one day, cross-litter comparisons should yield comparable results.

Procedure

Design You will be comparing the non-match-to-sample performance of 15 and 18 day old pups. If you have two mazes and two litters of rats, you can complete the procedure in about two hours. If you have only one maze and one litter, you should not use the same pups for the two tests. You can ear punch the pups you used or paint their tails with a permanent marker pen to identify them.

Deprivation and Adaptation Groups of four to six pups are placed in the Y-maze 14 to 16 hours prior to testing. All guillotine doors are removed permitting the animals to explore and adapt to the apparatus. The plastic lids over the various compartments must be secured firmly –if not, you may find yourself hunting high and low for your subjects at the time of testing. The apparatus should be housed in a room that is maintained at normal room temperature.

Just prior to testing remove the pups and place them in a holding cage. You should number the pups on their backs with a permanent marker pen.   Anesthetize a nursing dam and position her in the goal area. Sodium pentobarbital (45 mg/kg) can be used as the anesthetic but any injected anesthetic that will keep the dam deeply anesthetized for approximately 90 minutes will suffice. Supplementary injections may have to be administered. Although it may be more convenient, it is not necessary to use the mother of the litter being tested. Figure 1 shows the dam lying on her side with all her nipples easily accessible.   Shape modeling clay around the dorsal surface of the dam and between the two goal compartments to keep pups from climbing behind her. Insert the two guillotine doors that separate the goal area from the arms and place each pup in each goal compartment until it attaches to a nipple for 15 sec.

Testing A test trial is composed of two components, a forced run and a choice run. On the forced run, only one arm is accessible, access to the other is blocked with the barrier. The forced run serves as the *sample*. The pup is placed in the start box for a few seconds with the guillotine door down. The door is lifted and the rat allowed to find its way down the available arm until it reaches the goal door. When the animal contacts the goal door, lift it and allow the pup to attach to a nipple for 15 sec. (Be patient here, particularly on the first couple of trials). Detach the pup from the nipple and then immediately return it to the start box with the guillotine door in place. Remove the arm barrier. Open the door and allow the pup to choose an arm. To *non-match-to-sample* the pup must select the arm that was previously blocked. If it chooses that arm allow it to reach and touch the goal door, lift it, and allow the pup to attach to a nipple. If the pup selects the same arm as on the forced run, allow it to touch the goal door, but do not lift it. After the pup completes the two components of the trial, return it to the holding cage and select a new pup and repeat the procedure.

Each pup is tested for 25 trials. Test each pup in the same sequence to maintain a comparable inter-trial interval. If the pup made the correct choice on a previous trial, use the other arm as the sample on the next trial. If the animal incorrectly chooses, maintain the same arm from the previous trial as the sample.   This procedure prevents the animal from developing a position preference and equalizes the reward given for each arm choice.

PREDICTIONS AND REPORTING DATA

At 15 days of age, rat pups should non-match-to-sample close to chance (50% of the time). At 18 days of age, the pups should non-match-to-sample more frequently than the 15-day-old pups. A t-test can be used to compare frequency of alternation in the two groups. A bar graph can be used to display this data.

Another way to describe the data is to track the acquisition of the alternation habit by calculating *trial to successive criteria level*. In learning studies, researchers often define a criterion level to be an indicator of learning, for example eight correct responses in ten trials. You can track an animals' progress in acquiring NMTS by computing the criterion level achieved at each trial within groupings of ten trials (e.g., trials 1-10, 2-11, 3-12, etc.) and recording the trial at which each successive criterion level has been achieved (1/10 at trial 1, 2/10 at trial 3, ….etc).   The older pups are likely to reach the 8/10 criterion level by the end of testing while the younger pups hover around 5/10. You can use a t-test to compare the criterion level achieved by the pups in each age group.

Figure 2 shows how this data can be graphed.   To construct this graph, compute the mean trial at which each successive criteria level was achieved for each group. Graph with criterion level on the ordinate and trial number on the abscissa to track the different acquisition rate of the pups at 15 and 18 days of age.
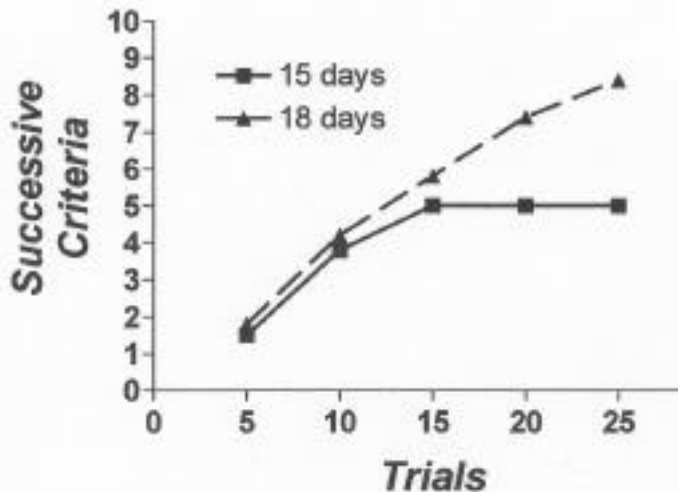
Figure 2. A graph contrasting the acquisition of an alternation habit by 15- and 18-day-old rat pups.

ISSUES FOR DISCUSSION

1) To structurally describe what you observed, you should analyze what the animal needs to remember in order to perform accurately. Does accurate performance necessitate habitual repetition of a movement, generally termed reference memory, or does it seem to require a more transient memory of preceding events, i.e., working memory? You may want to read Olton (1977) for a discussion of the relation between working and spatial memory. A functional explanation might make reference to foraging theory. According to foraging theory, when an animal visits one location, presumably depleting the resources at that location, it is adaptive to visit an alternate, non-depleted location (Olton et al., 1981). Consider foraging behavior as an explanation for the behavior of the older pups and then critique it.

2) The alternation behavior you observed has been analyzed as motivated by the detection of novelty (Gaffan and Davies, 1982). It is speculated that animals are attracted to a stimulus that is novel relative to previous stimuli experienced. How does this type of explanation help establish a linkage between the behavior seen in rats and the NMTS performance of monkeys? What evolutionary factors might account for the similarities and differences in the task used for rats and the task used for monkeys?

3) In just three days of development, the behavior of the rat pups has changed qualitatively. In that the older pups could successfully live independently from the dam, can you think of reasons why NMTS behavior appears in the 18 day old pups? Describe ways to connect the pattern of behaviors you observed to the weaning process itself. Finally, a lactating dam is not a "patchy" resource. Can that account for the absence of alternation in the 15-day-old pups? Design a variation of this experiment to evaluate whether the behavior of the younger pups is more adapted to a non-patchy resource.

4) Try to generate several explanatory mechanisms to account for behaviors you observed. How do the older pups do it? What are they remembering? Generate several hypotheses and see if you can design an experiment to test these hypotheses. What neural systems are needed to mediate the mechanisms that you hypothesized? How might an analysis of the developing anatomy and neurophysiology complement the behavioral analysis? A classic analysis of the linkage of working memory to the hippocampus is Olton (1977). Recent developments linking working memory to the frontal lobes in humans and primates can be found in Beardsley (1997).

5) These results suggest there is a stage-like transition between the behavior of the younger and older pups. Have you encountered stage-like developmental theories in human development? Draw parallels between the behaviors you observed in this project and some models of human cognitive development. How might you be able to apply the insights obtained from animal behavior to principles of neural and cognitive development in people? (See Bauer, 2002).

REFERENCES

Altman J, Das GP (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 124:319-36.

Bauer PJ (2002) Long-term recall memory: Behavioral and neuro-developmental changes in the first 2 years of life. Curr Direct in Psych Sci 11:137-141.

Beardsley T (1997) The machinery of thought. Sci Amer 277(8): 78-83.

Gaffan EA, Davies J (1982) Reward, novelty and spontaneous alternation. Q Jour Exp Psych 34B:31-47.

Gittis AG, Stark H, Arnold C, Geter B, Frazier D, Olton DS (1988) Emergence of choice strategies in the rat: Shift-stay differentiation precedes win-lose differentiation. Ani. Learn. Behav 16: 15-18.

Kolb B, Sutherland RJ, Whishaw IQ (1983) A comparison of the contributions of the frontal and parietal association cortex in spatial localization in rats. Behav Neurosci 97:13-27.

Olton DS (1977) Spatial Memory. Sci Amer 236(6):82-98.

Olton DS, Handelman GA, Walker JA (1981) Spatial memory and food searching strategies. In *Foraging Behavior : Ecological, Ethological and Psychological Approaches* Kamil AC, Sargent TD (eds) pp 333-54). New York: Garland STPM Press.

Rosenzweig MR, Breedlove SM, Leiman AL (2002) *Biological Psychology, 3rd edition.* Sunderland, MA, Sinauer.

Squire LR, Knowlton B, Musen G (1993) The structure and organization of memory. Annu Rev Psych 44:453-95.