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Running Head: DORSAL SUBICULUM AND SHORT-TERM MEMORY

This research is to assess the learning and memory retrieval ability of rats on a delayed non-matching to sample maze learning task. This study will explore the learning processes for short term memory recall and how chemical inhibition of the dorsal subiculum may alter short-term working memory. The majority of neurobiological memory research done in the past several years involves the hippocampal formation. In my assessment of past research I believe that the physiology of memory involves several neurological structures. In the paragraphs below discussion of the interrelation of the hippocampus and subiculum will be discussed. However, including memory and the subicular complex is difficult because subicular complex and memory research is sparse. This research will model similar experiments that deal with memory and learning experiments done with the hippocampal formation. The results of this study should be consistent with the past research done with the hippocampal formation.

Working memory is used when there is a delay in an activity, and the subject has to retrieve the information to use later. D'Amato (1973) expressed the importance of working memory in one of his experiments. D'Amato stated that in order for a subject to perform well at a delayed non-matching to sample task there must be a retention of memory, meaning that the subject must realize which choice has been made in the most recent trial. If this retention does not exist, a trial error in working will occur and no great statistical significance will be found. This will help insure that

the subject has consistently retained the information and the significance will be more valid.

The process of memory being formulated physiologically in the brain is still an immense mystery being researched today. In the attempt to localize the process of short-term memory to a neural circuit, it is important to be familiar with hypothesis of neuronal plasticity. Researchers (Kandel, 1985; Klein and Kandel 1978) have studied this phenomena for many years. Neuronal plasticity is, the adaptation of a neuron due to the presence of a stimuli. Plasticity of neurons may be chemically and/or structurally mediated. The research of neuronal plasticity might show us how a memory is actually formulated.

Bailey and Chen (1983, 1988) presented structural changes of the siphon due to multiple exposure to stimulus. This process is an example of long term potentiation (LTP) which, in turn, is an example of neuronal plasticity. As defined by Pinel (1990):

"After a few seconds of intense high-frequency electrical stimulation to presynaptic fibers, the response of the postsynaptic neurons to low-intensity stimulation of the presynaptic fibers is increased; LTP can last for several Days."

Many researchers (e.g. Teyler and DiScenna, 1984, Kelso and Brown 1986) have studied LTP in the mammalian hippocampal formation. These experiments have supported past hypotheses that the hippocampus has a direct role in memory processes. Examples of

LTP and neuronal plasticity is thought to be the basis of animal memory.

The physiology of memory has mainly been studied for several years with recording of memory trace by mediation of the hippocampal formation. Jarrard (1975) presented an experiment that was designed to test memory retention in hippocampectomized rats and control rats. The rats were preoperantly trained in single alternation task. After this task was well learned the task was given again in a delayed and interpolated delayed (meaning a physical activated in between trials) fashion. Post operative relearning for the single alternation task showed that there was greatly decreased memory retrieval in the hippocampectomized group. The most significant finding in Jarrard's study was the effect of interpolated activity which interfered with performance of the delayed alternation tasks in both control and hippocampectomized subjects.

Jagielo, Nonneman, Isaac, Jackson, and Smith (1990) conducted a study which dealt with delayed matching to sample and simultaneous matching to sample tasks (SMTS). In this study lesions to the hippocampus and fimbria were performed. In the SMTS experiment the post operative group showed decreased signs of relearning. The hippocampal operate group was less likely to relearn the tasks compared to the operate control groups. This indicates to us that reference memory is greatly affected by lesions to the hippocampus. In the delayed matching to sample task the fimbria lesioned rats showed no great difference compared to

the other two groups.

Winocur (1991) studied the differences of learning and memory between the prefrontal cortex and hippocampal areas. In the conditional discrimination learning test the prefrontal cortex group could not rise above a certain performance level. This provides support that several structures may interact for efficient memory retrieval. This is very significant because other groups including the hippocampal lesioned groups out performed the prefrontal group. Rats in this experiment had to use nonspecific memory in order to perform the task successfully. This supports the findings by Winocur Moscovitch (1990) that the prefrontal area is concerned with general task memory and that the hippocampus is concerned with specific memory.

McNaughton and Morris (1982) have reformulated the possible storage of an associated memory. In this "neuronal model", the proposal of memory formulation is very much like the trisynaptic circuit of the hippocampal formation from the perforant pathway to the CA3 field and then projections to the CA1 field of the hippocampal formation. McNaughton and Morris found that through electrical saturation and chemical blockade tests in rats supported the LTP hypothesis.

In the present research, the dorsal subiculum (much like past research done with the hippocampal formation) will be chemically controlled and retrieval of short-term memory ability will be observed. The chemical control should directly effect the relay of information that involves the efferent and afferent projections of

the dorsal subiculum causing a decrease in memory retrieval.

The research in this study will be concerned with the dorsal subiculum. It is important to be familiar with the afferent and efferent connections. Finch and Babb (1980, 1981) have detailed the afferent connections of the subiculum. Their research has indicated that the primary afferents originate from the CA1 hippocampal sub-fields. Supporting Finch and Babb, Tamamaki and Nojyo (1990) have extensively mapped out the afferents from the CA1a, CA1b, and CA1c fields to the subiculum, using the injections of horseradish peroxidase. With this distinct linkage it is important to emphasize the possibility of short-term memory transference in the subiculum through these neuronal pathways.

The neural pathways continue out of the subiculum into other areas that may influence memory. Swanson and Cowan (1977) have extensively shown the efferent subicular complex projections. The dorsal subiculum projects to the lateral subiculum via the fornix, and then into the mammillary bodies. The rostral projections of the ventral subiculum pass through the septofimbrial nucleus and on to the lateral septo complex. Some fibers go further ventrally into the medial part of the nucleus accumbens and into the infralimbic cortex. If the present research show a significant deficit in memory retrieval the structures above may need to be tested for their importance in memory formulation with respects to transference and retrieval. Swanson and Cowan discuss other important connections to various parts of the subiculum but the emphasis in this research is on the dorsal subiculum to determine

its role in the memory retrieval process. In the present research both afferents and efferents will be chemically controlled by stopping the action potential in the dorsal subiculum and it is my proposal that this will directly affect short-term memory.

METHODS

The subjects will be ten male Long Evans hooded rats about two months old and weighing two hundred to three hundred grams. They will be housed separately in hanging metal cages. The subjects will be supplied with rat chow and will be free to eat at all times. The room will be set at a twelve hour light and dark cycle.

All rats will be handled daily to facilitate placement of injection needles and to ensure that they will be as calm as possible during behavioral testing.

SURGERY and HISTOLOGY

All subjects will be assigned to one dorsal subiculum surgical group. The rats will be anaesthetized with sodium pentobarbital (50 mg/kg ip injection). Once they reach a surgical plane of anesthesia (which means no limb retraction in response to paw pinches) their scalps will be shaved. The animals will be positioned in the Kopf model 1204 small stereotaxic instrument with the tooth bar adjusted at a height of -3.3 mm above the interaural plane for dorsal subiculum cannula implantation 4 mm below cortex level. Cannula implantation will be interaurally produced by drilling bilaterally into the skull -/+ 1.5 mm to the midline. The dura mater will be split with a 26 gauge hypodermic needle to ensure correct cannula placement. The cannulas will be secured in place by the mounding of dental acrylic. To prevent material from

blocking the cannulas, stainless steel stylettes will be inserted into the cannula and fixed in place with the addition of further dental acrylic. The wound will then sutured shut and the animal allowed to recover.

Lidocaine will be delivered bilaterally through two 30 ga hypodermic needles inserted into the cannulae and protruding 1 mm past the cannula tips. The distal ends of the hypodermic needles were hydraulically continuous with polyethylene (PE) tubing with .38 mm inside diameter and 1.09 mm outside diameter (Clay Adams PE-90). The PE tubing had marks running its length spaced such that 1 ul of solution was contained in the volume of tubing between successive marks. The PE was connected to a 1 cc Hamilton syringe mounted on a Razel Model A99 infusion pump. When lidocaine will be infused. the injection needle and the first two or three feet of the PE tubing will be loaded with lidocaine. The rest of the PE tubing and Hamilton syringe will be filled with aniline blue dye in solution with distilled water. A small air bubble separated the drug from the dye. When animals are infused, they will have two separate injection assemblies attached to their cannulas. The infusion pumps will be turned on simultaneously to start injections. Infusion speed will be set to allow a flow rate of approximately 1 ul/min. The amount of drug infused will be verified by visual inspection of the distance travelled by the dye in each injection line.

After the experiment the rats will be deeply anaesthetized and euthanasia will be performed. The rat head will be stored in a

solution of formyl saline solution. The brain will be harvested from the subject and frozen. Every fifth coronal section will be kept and microscopy will be performed to insure the correct implantation of the cannulas. The sections will be sliced at a thickness of forty microns. All sections will be stained and compared with the Paxinos and Watson (1986) atlas.

APPARATUS

A T-Maze will be used for the maze learning test. The maze was painted a flat grey and placed in a well lit room. The arms of the T-Maze are 127 cm in length. The start box of the maze is 7 cm in width and 23 cm in length. From the start box to the entrance of the T-maze arms is 26 cm in length. The entire T-Maze area for the rat to work with is 7.5 cm in width and the height is 7.

PROCEDURE

The rats will be monitored on their daily intake of water for three days prior to preoperative maze-training. Following the recording of the rats daily intake they will be deprived of water for a day and on the next day they will begin preoperative training on the T-Maze. The rats will be allowed to drink only during testing and for five minutes directly following their test runs in their housing cages. The rats will be weighed daily and monitored closely to ensure their health.

During the testing procedures the rat will be placed in the start box. When the sliding door is opened the rat will be forced (by the blockade of a randomly predetermined arm) to pick an arm to go down. After the rat runs down the open arm it will be allowed

to drink for ten seconds then the rat will be taken out and placed in a holding cage. After the set delay period (of 1, 2, 5, minutes depending upon the rats criterion level) the rat will be placed in the maze and allowed to choose which arm to go down. If the rat proceeds down the arm it had previously gone down it will be recorded as no mistake. Once the maze learning is effectively performed three days in a row the rat will be ready to go through surgery.

The rat will be given four days to recover with water and food supplied, and then water monitored for three days on their daily intake. One day of water deprivation was again put into effect. After a day of water deprivation the rat will be considered ready to undergo the maze task exercises for the experiment. The delay period starting off was one minute. The rats delay period will increase when the rat has successfully met the criterion. Criterion will be determined by the correct responses of the rat having two or less mistakes three days in a row. On the fourth day an injection will be performed. The delays will be set at 1, 2, 5 minute intervals depending on the performance level (criterion) of the subject. After the time period has elapsed the rat will be placed back in the start box and the process of choosing an arm will again be performed. The rats are to choose the opposite arm in which it had not previously gone down. If the rats performance of this task is correct for three trials in a row the rats have met criterion. During the delay period on the fourth day the rat will be given an injection of lidocaine bilaterally into the dorsal

subiculum through the implanted cannula.

The results of this research should be consistent of past research which deals with the hippocampal formation and memory retrieval. It is the belief of this researcher that the dorsal subiculum plays an important role in memory retrieval. Upon analysis of the results, there should be significant statistical evidence which supports the researchers belief that the dorsal subiculum is necessary for working memory retrieval.

Results

The histologically-determined locations of lidocaine injection sites in animals tested revealed that the injection sites were placed too far above the dorsal subiculum. Sufficient lidocaine profusion could not reach the desired discrete brain area for proper inactivation.

A total of six animals were used in t-maze running for this experiment. Following the histological procedures all six were found to have misplaced cannulae which resulted in the inability for lidocaine to sufficiently control the subiculum.

It is regretful for me to state that all data in this experiment was insufficient, and therefore unable to be used. There were no significant findings.

Discussion

When an experiment doesn't work it is important to find out what went wrong in order to have future success. This experiment showed consistent placement of the cannulae about 4mm above the desired location. At first it was my belief that my newness to this type of procedure had caused a great confound. But due to the consistent placement of the cannulae it seemed more likely that something else had gone astray.

I checked all possible confounds and could find nothing that would cause such a result. It wasn't until a colleague of mine in the lab found similar problems while working on his thesis. It was brought to my attention that the coordinates of the two stereotaxic

instruments I had used were contradicting. I had lined the cannulae on the intraoral line of one instrument and when shifting to the kopf stereotaxic table the alignment was drastically changed. Thus my cannulae placement might have been right, but the equipment was not compatible.

My colleagues and I have since made the proper adjustments and plan to run this project again. It is my hypothesis that the Dorsal subiculum is an important pathway for memory and my proposal that future research will support this claim.

REFERENCES

Bailey, C. H., & Chen, M. C. (1983). Morphological basis of long-term habituation and sensitization in Aplysia. Science, 220, 91-93.

Bailey, C.H., & Chen, M.C. (1988). Long-term memory in Aplysia modulates the total number of varicosities of single identified sensory neurons. Proceedings of the National Academy of Sciences (USA), 85, 2373-2377.

D'Amato, M.R. Delayed matching and short-term memory in monkeys. In G.H. Bower (Ed.),The psychology of learning and motivation: Advances in research and theory (Vol. 17). New Yew York: Academic Press, 1973.

Finch, D.M., and Babb T.L. (1980) Neurophysiology of the caudally directed hippocampal efferent system in the rat: projections to the subicular complex. Brain Res. 197: 11-26.

Finch, D.M., and Babb T.L. (1981) Demonstration of caudally directed hippocampal efferents in the rat by intracellular injection of horseradish peroxidase. Brain Res. 214: 405-410.

Gazzaniga, M. S., (1988). Perspectives in Memory Research. Cambridge: Massachusetts Institute of Technology.

Jagielo, J. A., Nonneman, A.J., Isaac, W. & Jackson-Smith, P. (1990). Hippocampal lesions impair rats performance of non-spacial matching to sample task. Psychobiology, 18(1), 55-62.

Jarrard, L. (1975). Role of Interference in Retention By Rats with Hippocampal Lesions. Journal of comparative and Physiological

Psychology, 89, 400-408.

Kelso, S.R.,& Brown, T.H. (1986). Differential conditioning of associative synaptic enhancements in hippocampal brain slices. Science, 232, 85-87.

McNaughton, B.M. & Morris, R.G.M. (1987). Hippocampal synaptic enhancement and information storage within a distributed memory system. Trends in Neural Science, 10 (10), 408-415.

Moore, J. (1979). Information processing in space-time by the hippocampus. Physiological Psychology, 7, 224-232.

Olton, D., Becker, J. & Handelmann, G. (1979). Hippocampus, Space and Memory. Behavioral & Brain Sciences, 2, 313-365.

Paxinos, G., (1985). The Rat Nervous System (Vol.,1). Academic Press, Inc.

Pellegrino, L. & Cushman, A. (1967). A Stereotaxic atlas of the rat brain. New York: Appleton-Century-crafts.

Pinel, J. (1990). Biopsychology. Boston: University of British Columbia.

Rawlins, F., Aggleton, J. & Blindt, H. (1989). Effect of Amygdaloid and Amygdaloid-Hippocampal Lesions on Object Recognition and Spatial wording Memory in Rats. Behavioral Neuroscience, 103 (5), 962-974.

Solomon, P. R. (1980). A time and a Place for Everything? Temporal processing views of hippocampal function with special reference to attention. Physiological Psychology, 8, 254-261.

Squire, L. R., (1987). Memory And Brain. New York: Oxford University Press.

Swanson, L.W. & Cowan, W.M. (1977). An Autoradiographic Study of the Organization of the Efferent Connections of the Hippocampal Formation in the Rat. Journal of Comparative Neurology, 172, 49-84.

Tamamaki N., & Nojyo Y., Disposition of the Slab-Like Modules Formed by Axon Branches Originating From Single CA1 Pyramidal Neurons in the Rat Hippocampus. Journal of Comparative Neurology, 291, 509-519.

Teyler, T.J., & DiScenna, P. (1984). Long-term potentiation as a candidate mnemonic device. Brain Research Reviews, 7, 15-28.

Van Groen, T. & Wyss J. M. (1990). Connections of the Retrosplenial Granular A Cortex in the Rat. Journal of Comparative Neurology, 300, 593-606.

Winocur, G., & Hilbert, M. (1984). The hippocampus, Context, and information processing. Behavioral & Neural Biology, 40, 27-43.

Winocur, G., & Moscovitch, M. (1990). Hippocampal and Prefrontal cortex contributions to learning and memory: Analysis of lesion and aging effects on maze-learning in rats. Behavioral Neuroscience,104, 544-551.

Winocur, G. (1991). Junctional dissociation of the hippocampus and prefrontal cortex in learning and memory. Psychobiology, 19 (1), 11-20.