Chapter IV
Design and Methodology

The following section deals with the details of the design and methodology used by the experimenter to investigate the problem formulated in Chapter III.

Sample:
A sample of 60 male albino rats (six of which were rejected since they did not attain the training criterion and four died during the experimentation), approximately 90 days old, weighing 120 ± 10 gms. were randomly selected from the rat population of the animal house of the Psychology Department, M.D.University, Rohtak. Identification numbers were assigned to each animal and they were housed in wire cages (5 animals/cage).

Design:
A multigroup design, with four groups was employed to study the antagonistic effect of VP on morphine induced amnesia. The dosage of morphine and VP were chosen on the basis of earlier investigations (Sunita, 1983; Monisha, 1985; Yadava, 1985) and some initial pilot work.

After training in a single trial passive avoidance task, the animals were given two successive ip injections of either morphine (3.0 mg/kg), VP (3.0 I.U./Kg) or Saline (0.25 ml/animal).
<table>
<thead>
<tr>
<th>Group</th>
<th>Number of subjects</th>
<th>Administration of Drug Immediately after training</th>
<th>Administration of Drug After five minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13</td>
<td>Saline</td>
<td>Saline</td>
</tr>
<tr>
<td>II</td>
<td>13</td>
<td>Morphine</td>
<td>Saline</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>Saline</td>
<td>Vasopressin</td>
</tr>
<tr>
<td>IV</td>
<td>12</td>
<td>Morphine</td>
<td>Vasopressin</td>
</tr>
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Retention tests were taken after 1, 2 and 7 days.

Instrumentation:

Active Passive avoidance apparatus:

The avoidance apparatus used in the present investigation has been shown on the photographic plate on the opposite page. It was a rectangular alley with the following dimensions:

- Length: 91 Cm
- Width: 20 Cm at the top and 6 Cm at the bottom
- Height: 51 Cm

It was divided into two compartments by a wooden partition. The larger compartment was 60 Cm in length while the smaller one was 30 Cm long. The partition had a circular opening at the base (6 Cm), fitted with a sliding door, through which the animal could cross over from one compartment to the other. The outer surface of the
entire apparatus was covered with white sunmica, and the inner surface was covered with an aluminium sheet, separated by a gap of 1 cm at the base. Both the compartments were covered with transparent glass covers so that the activity of the animal could be observed. The smaller compartment was illuminated by a table lamp while the larger compartment was connected to an automatic shock generator adjusted to deliver a shock of 750 mA for 0.5 second.

Electric shock generator:

The shock generator was SG-C type, purchased from Techno Electronics, Lucknow. It had the following specifications:

Input - 220 volts AC ± 5%

Output - 0 to 4 mA (Calibrated in short circuit current from 0.05 to 4 mA in 20 steps).

Time range - 0.1 to 10 seconds (continuously adjustable).

This shock generator was connected through a servo controlled voltage stabilizer (Nelco Type No. Tan 2010) with the following specifications:

Input : 180-250 Volts AC

Output : 230 Volts ± 1%

The intensity and duration of the foot shock was controlled by the shock generator. In order to ensure
that the intensity of the shock did not vary due to fluctuations in the input voltage the stabilizer was put into the circuit.

Since another variable that could influence the amount of current actually passing through the animal is the body weight of the animal, a digital multimeter, Type 445 was put into the circuit to record the actual amount of current passing through the body of the animals. But the weight of these animals varied only by ± 10 gms. and no drastic difference was observed in the amount of shock actually passing through the body of the animal.

White Noise:

The screening technique was employed to control an extraneous variable—external sounds—which could have had a profound influence on the results. Since the comparative laboratory at M.D. University is not sound proof, the extraneous sounds had a distracting effect on the animals. These external sounds were masked by playing taped white noise whenever the animals were being trained or tested.

Analogous to white light which is a combination of light waves of seven different frequencies, white noise is also a combination of a number of sounds of different frequencies mixed up in such a way that the resultant sound has a fixed amount of energy. The noise produced from
FIG. 2. CHEMICAL STRUCTURE OF VASSOPRESSIN.
the exhaust of an airjet is a very good example of white noise flat spectrum (Chapanis, 1949). Although this noise can be recorded from the airfield, variations might occur due to differences in engine acceleration, distance of the recording equipment from the exhaust, environmental noises etc. In view of these difficulties, it is safer to use an electronic white noise generator, which gives pure white noise of uniform intensity.

A C-90 cassette recorded from an electronic white noise generator was obtained from the Comparative Psychology Laboratory of the University of Sheffield, U.K., and was played through a Philips tape recorder whenever the rats were being trained/tested. Any possible effect of white noise on the performance of the animals was controlled since it was played in the case of all the groups.

Vasopressin:

VP is probably the most widely known biologically active peptide. It is a vasoactive and antidiuretic substance that is formed from a precursor molecule in the paraventricular (PVN), the supraoptic (SON) and the suprachiasmatic nucleus (SCN). It travels along the nerve fiber tract to the posterior pituitary for storage or release. Chemically, it is a cyclic polypeptide consisting of a cyclic pentapeptide and tripeptide side chain (Figure 2).
The level of VP increases with a rise in plasma osmolarity mediated by receptors in the thoracic veins, a fall in plasma volume mediated by receptors in the thoracic veins, emotional stress and various drugs like morphine and nicotin. In the present investigation VP (manufactured by Ferring GMBH Kiel/W-Germany, Trade name-Postaction) available in injectable form (10 I.U./½ ml) was used.

Morphine:

Morphine is an alkaloid obtained from opium, which is the dried juice of the poppy plant Papaver Somniferum. The different alkaloids found in opium fall into two categories; the phenanthrene alkaloids and the benzylisquinoline compounds. Of the latter group only papaverine has achieved any medical importance as an antispasmodic and vasodilator. The main constituent (10% by weight) of opium is morphine which was initially extracted in 1803 by a German Pharmacist-Sertturner. By the middle of the 19th century, this substance was being used for inducing euphoria and relaxation. The euphoria produced by morphine and other opiate substances like herion and codein is generally accompanied by a sense of tranquility and detachment.

Morphine is available in injectable, crystalline, capsule, tablet and powder form. In the present investigation it was used in injectable form (15 mg/ml).
Method

The selected male albino rats were placed in wire cages, 5 animal\textsuperscript{c} cage and the respective identification numbers were marked on their tails with a felt pen. Each rat was handled for three days, five minutes/day so that rapport could be established between the animal and the experimenter. All the animals were weighed for three consecutive days before the actual experimentation in order to obtain the mean body weight for calculation of the exact amount of drug to be injected to each rat.

One day before the training session, the animals were given orientation trials to familiarize them with the apparatus. The overhead lights were switched on and the rat was allowed to move about in the apparatus for five minutes. The apparatus was swabbed with 20\% alcohol solution and dried with the help of a Wolf NWB air blower before placing any animal in the apparatus so that the odour traces of the earlier run rat could be removed. It has been reported that rats leave different odours depending on their experience which in turn influence the behaviour of the subsequent run rats, thereby contaminating the results of psychopharmacological experiments (Walia and Muhar, 1981).

The actual training was given 24 hours after the orientation trial. The overhead lights were switched off.
The entire room was dark except for the smaller compartment of the apparatus which was illuminated with the help of a lamp. Taped white noise was played whenever the animals were being trained or tested to mask the effect of external noises which could distract the animals being run.

After making all the required arrangement, a rat was placed in the smaller compartment facing the rear wall and a stop watch was switched on. As soon as the rat turned around/after 10 second, the stop watch was switched off, the second stop watch was switched on and the door between the two compartment was lifted. When the rat entered the larger dark compartment, the second stop watch was switched off, the door was replaced and a shock of 750 μA for 0.5 seconds was delivered. The rat was retained in the dark compartment for 30 seconds. From the first stop watch, turning time and from the second latency period was recorded. Any animal which failed to enter the larger compartment within 90 seconds was eliminated. Six rats were eliminated because they did not achieve this criterion.

After removing the rats from the dark compartment, the rats were given an immediate ip injection of saline (.25 ml/animal) or morphine (3.0 mg/Kg) followed by an ip injection of saline (.25 ml/animal) or vasopressin (3.0 I.U./Kg) after an interval of 5 minutes depending
upon the group to which they belonged. The respective concentrations of the drugs were prepared by diluting the available solutions with saline half an hour before the experimentation.

The entire experiment was conducted double blind. The 'E' running the animals was not aware as to which group the animal belonged. This was helpful in avoiding contamination of the results due to experimenter bias.

Retention tests were given to each animal after 1, 2 and 7 days after training. The procedure for retention testing was the same as that for the training paradigm except that no shock was given. If a rat failed to cross over to the second compartment within 300 seconds, the retention trial was terminated and a latency of 300" was recorded.

The obtained results have been presented and discussed in the next chapter.