6-OHDA bilateral lesions to the central amygdala do not affect vasopressin improvement of recall in rats

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Abstract. The influence of vasopressin (AVP) on recall of information in a passive avoidance situation after bilateral 6-OHDA lesions to the central amygdala was tested. AVP given 15 min before the retention testing at the icv dose of 1 μg significantly prolonged avoidance latencies both in lesioned and in sham-operated rats in comparison with the respective icv saline injected animals. Insignificant increase of spontaneous locomotor activity in rats lesioned to the central amygdala was unlikely to interfere with the cognitive effect of AVP. These results suggest that dopaminergic projection to the central amygdala is not responsible for the facilitatory effect of AVP on retrieval process in a passive avoidance situation.

Key words: vasopressin, central amygdala, passive avoidance, locomotor activity, rats
Earlier studies performed in our laboratory have shown that angiotensin II (AII) and its fragments stimulate learning and memory in rats (Baranowska et al. 1983, Braszko et al. 1987, 1991). Mouw et al. (1971) found that AII given intracerebroventricularly (icv) causes release of vasopressin (AVP), a peptide which plays an important role in memory consolidation and retrieval (Bohus et al. 1978, De Wied and Versteeg 1979, Kovacs et al. 1979, De Wied and Jolles 1982). Although AII stimulates mostly acquisition, while AVP consolidation processes, both peptides have been found to facilitate recall of information in a passive avoidance situation (Braszko et al. 1991, Care et al. 1993). The evidence obtained in our laboratory suggests that AII improves cognition in rats through an action on central dopamine systems (Wiśniewski and Braszko 1984). It has also been shown that bilateral destruction of dopaminergic endings with 6-OHDA in the central amygdala abolished facilitatory effect of AII and its 3-7 fragment on memory retrieval in a passive avoidance situation (Winnicka et al. 1988, 1996) and also on object recognition (Winnicka 1995). The relationship between mesolimbic dopamine system and AVP has also been found. Parvicellular vasopressinergic neurones projecting to the lateral septum (De Vries and Buijs 1983) have been found in medial amygdaloid nucleus of rats (Caffé and Leeuwen 1983). Ishizawa et al. (1990) suggest that a portion of AVP binding sites in the lateral septum may be localized presynaptically on the terminals of noradrenergic and possibly dopaminergic neurones. Recently the existence of V2 receptors mRNA in rat hippocampus, another structure of mesolimbic system, which receives dopaminergic input, has been presented (Hirasava et al. 1994).

In order to investigate whether in facilitating effect of AVP on memory motivated affectively is engaged dopaminergic projection to the central amygdala, bilateral 6-OHDA induced lesions were made before testing of the influence of AVP on the retrieval process in a passive avoidance situation.

Male Wistar rats weighing 150-155 g on the day of surgery and 170-175 g at the time of behavioural testing were used. The animals were housed in plastic cages with a 12 h light/12 h dark cycle beginning at 7.00 h with food and water freely accessible.

The rats were anaesthetized with chloral hydrate and were placed in a Kopf stereotaxic apparatus with tooth bar 5 mm above the interaural line. The skull was exposed, and burr holes 1.5 mm in diameter were drilled above the appropriate coordinate targets. The coordinates for the central amygdala, anterior (A) from the bregma, lateral (L) to the midline and below (V) the skull surface were selected with the aid of the atlas of König and Klippel (1963) at A: -0.8, L: 3.7, V: 8.0. Each site was infused with 1 µl of 0.9% NaCl containing 10 µg of 6-OHDA (free base, Sigma). The neurotoxin was dissolved in a vehicle solution containing 5 mg/ml of ascorbic acid and injected over 10 min through a stainless steel cannula (O.D. = 0.3 mm) at the rate of 0.1 µl/min. The cannula was left in place for an additional 5 min after the end of the infusion. Eighteen randomly selected rats received 6-OHDA to the central amygdala, the remaining 18 sham-operated control rats underwent the same procedure except that they only received the vehicle solution. Thirty minutes before the surgery all rats were pre-treated with an intraperitoneal injection of 25 mg/kg desmethylimipramine (Sigma), an inhibitor of norepinephrine (NE) uptake, which has been shown to protect NE neurones from destruction by 6-OHDA (Breese and Traylor 1971). After 6-OHDA infusions, an additional burr hole 0.5 mm in diameter, was drilled in the rats skull 2.5 mm laterally and 1 mm caudally from the point of intersection of the bregma and the superior sagittal suture on the right side of the head for the icv injection. Behavioural testing started after ten days of the recovery period. AVP (Calbiochem) dissolved in 5 µl of 0.9% NaCl was given icv in the amount of 1 µg per rat 15 min before behavioural testing. The icv injection was made freehand into the right cerebral ventricle with a Hamilton syringe, using a removable KF 730 needle cut 4.5 mm from its base, according to the technique described earlier (Braszko et al. 1991). This procedure allowed lowering the tip of the needle about 0.5 mm below the ceiling of the lateral
cerebral ventricle. It was relatively nontraumatic as the animal, gently fixed in the left hand of the experimenter, was usually quiet and no vocalization occurred. Half of lesioned and sham-operated rats received at the same time 0.9% NaCl.

Locomotor and exploratory activity was measured in an open field which was square, 100 x 100 cm, a white floor divided by 8 lines into 25 equal squares and surrounded by 47 cm high wall. Four plastic bars, 20 cm high, were located in four lines crossing in the central area of the floor. Following 1 min of adaptation crossings, rearings, and bar approaches were counted manually for 10 min.

Passive avoidance performance was studied in a one trial step-through passive avoidance situation (Adler et al. 1972). The apparatus consisted of an illuminated platform attached to a large dark compartment. The subjects were placed on the platform and were allowed to enter the naturally preferred dark compartment. Two more trials were given on the following day. At the end of the second trial an inescapable, scrambled electric footshock (0.5 mA for 3 s) was delivered through the grid floor of the dark compartment. Retention of passive avoidance behaviour was tested 24 h after the single learning trial by measuring the latency to re-enter the dark compartment up to a maximum of 300 s.

Placement of the cannula was examined histologically. At the end of the behavioural testing the rats were sacrificed and their brains were removed and fixed in 10% formaldehyde for 7 days. Subsequently, coronal sections (20 μm thick) of the cannula tract were cut using frozen sectioning method, saving every fifth section through the lesion, mounted on slides, and stained with cresyl violet. Rats in which the cannula tip was located outside the centre of target structure, and with incorrect icv injections were excluded from the experimental data. According to Agid et al. (1973) the location of the tip of the cannula is particularly critical for reproducibility of the decrease in dopamine levels in lesioned structures. After infusion of 1 μl of 6-OHDA distribution of neurotoxin has a spherical shape with the diameter 3.0 mm. Typical sites of 6-OHDA injections into the central amygdala are shown in Fig. 1. In the final analysis 16 lesioned and 16 sham-operated animals were included. Two sham-operated and two lesioned to the central amygdala groups of rats injected icv with AVP or saline consisted of 8 rats.

Statistical comparisons were made with ANOVA followed by Newman-Keuls test. In all comparisons between particular groups a probability of 0.05 or less was considered significant. The facilitating effect of AVP was still observable in rats which sustained 6-OHDA lesions (Fig. 2). ANOVA of 2 lesioned and 2 sham-operated groups, injected icv with AVP or saline, yielded F_{3,28}=13.09, P<0.001. Post hoc comparisons between these groups made with Newman-Keuls test revealed significant increase of the mean step-through latency in the sham-operated and lesioned to the central amygdala groups of rats injected with AVP as compared with respective saline injected groups. Sham-operated rats, injected icv with saline, stayed on illuminated platform about 40 s, while the lesioned subjects almost immediately entered the dark compartment on retention test, the mean step-through latencies being less than 20 s, but the difference was insignificant. Lesioned animals injected icv with AVP stayed on the platform shorter than sham-operated controls injected with the peptide but this difference was also insignifi-
The motility of rats lesioned to the central amygdala (estimated by crossings of squares, rearings and bar approaches) was slightly higher in comparison with motility of sham-operated animals (Table I). However this difference was insignificant and it could not account for the results obtained in a passive avoidance situation. Also Simon et al. (1988) did not observe significant changes in spontaneous locomotor activity in rats after bilateral 6-OHDA lesions to the central amygdala.

The results obtained in this study indicate, that bilateral lesions to the dopaminergic innervation of the central amygdala do not affect the improvement of recall of the passive avoidance behaviour after icv injection of AVP.

Our earlier study with AII and its 3-7 fragment (Winnicka et al. 1988, 1996) indicated that bilateral destruction of dopaminergic endings in the central amygdala abolished improving effect of both peptides on recall in the same behavioural test.

The involvement of amygdala in affectively motivated memory was supported by numerous experiments. In the majority of studies amygdalatomized rats were impaired in acquiring or relearning a two-way avoidance problem (Yeudall and Walley 1977, Schütz and Izquierdo 1979, Eclancher and Karli 1980). Also amygdalatomized rats were found to be impaired in passive avoidance tests (Nagel and

<table>
<thead>
<tr>
<th>Group</th>
<th>Crossings</th>
<th>Rearings</th>
<th>Bar approaches</th>
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<tbody>
<tr>
<td>Sham-operated</td>
<td>38.21</td>
<td>10.96</td>
<td>3.35</td>
</tr>
<tr>
<td>(4.92)</td>
<td>(1.53)</td>
<td>(0.61)</td>
<td></td>
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<tr>
<td>Lesioned to the central amygdala</td>
<td>43.86</td>
<td>12.62</td>
<td>4.43</td>
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<tr>
<td>(5.74)</td>
<td>(0.90)</td>
<td>(0.92)</td>
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Values are means from 16 subjects and SEM in parentheses.
Kemble 1976, Russo et al. 1976, Liang et al. 1982). The involvement of dopaminergic projection to the central amygdala in the positive effect of angiotensins but not AVP on retrieval in a passive avoidance situation could be explained by different relationship of these peptides with structures of mesolimbic system. While extensive AII immunoreactive terminal field was found in the central amygdala (Lind et al. 1985), a structure which, according to Kesner et al. (1993), plays a crucial role in memory motivated affectively, parvicellular vasopressinergic neurons were found in medial amygdaloid nucleus. Lesions to the basolateral or central amygdaloid nuclei consistently produced an impaired passive avoidance behaviour (Pellegrino 1968, Grossman et al. 1975), whereas corticomedial amygdaloid lesions induced only a minor deficit or had no effects (Kemble and Tapp 1968, Pellegrino 1968). The results obtained in this study indicate, that dopaminergic input to the central amygdala is not responsible for the facilitating retrieval effect of AVP in a passive avoidance situation in rats.

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