Post-Training Vasopressin Injections May Facilitate or Delay Shuttle-Box Avoidance Extinction

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After training to avoid footshock in a two-way shuttle box rats were injected with lysine vasopressin (LVP) and returned to the shuttle box 24 hr later for 10 extinction trials. Experiment 1 shows that when injected 30 min after training subsequent extinction responding varied as an inverted "U"-shaped function of the LVP dose within the range tested (0.036 to 2.97 μg/rat). Responding was increased with 0.11 μg/rat whereas 2.97 μg/rat reduced responding. Experiment 2 shows that these two doses also have opposite effects when injected 30 min after training at a higher shock level (0.45 mA/2 sec). Experiment 3 examined time-dependent effects for these oppositely acting doses and shows that whereas 0.11 μg/rat increased extinction responding when injected 0 or 60 min after training, 2.97 μg/rat was ineffective when injected immediately and increased responding when injected 60 min after training. The data are discussed in relation to the hypothesis that vasopressin facilitates memory consolidation and with respect to anomalous time × dose interactions with various post-training drug treatments.

Neurohypophysial peptide hormones may play important roles in regulating behavior (for review see de Wied and Versteeg 1979) particularly those brain processes related to the consolidation and retrieval of learned responses (Kovacs, Bohus, & Versteeg 1980). Evidence for the involvement of vasopressin in memory mechanisms has accumulated in the years since de Wied (1965) demonstrated that posterior lobectomy facilitated the extinction of a shuttle-box avoidance response independently of disturbances in water metabolism. Normal responding was restored by Pitressin, a crude extract of pituitary lobe tissues, or lysine vasopressin (LVP), when injected during either learning or extinction (de Wied 1969). In intact rats Pitressin increased extinction response levels after repeated

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injections during either training or extinction (de Wied & Bohus 1966). Subsequent experiments showed that a single post-training injection of LVP delayed extinction of pole-jump avoidance responding (de Wied 1971) and enhanced inhibitory (passive) avoidance behavior (Bohus, Ader, & de Wied 1972; Bohus, Urban, van Wimersma-Greidanus & de Wied 1978a; Bohus, Kovacs & de Wied 1978b) (but see Hostetter, Jubb, & Kozlowski 1980). Post-training vasopressin injections were most effective when given within 1 hr of training, potency was reduced with a 3-hr interval and the peptide was ineffective when treatment was delayed for 6 hr (de Wied 1971; Bohus et al., 1978b; King & de Wied 1974). These findings have been interpreted as indicating a role for the peptide in modulating the physiological processes which underlie memory consolidation. Additional evidence from experiments in which the injection precedes retention testing suggests an additional role in retrieval processes (Ader & de Wied 1972; Krejci, Kupkova, Metys, Barth, & Jost 1979).

Further support for the memory hypothesis stems from observations that the des-glycinamide analogs of vasopressin, which lack virtually all antidiuretic and pressor activity, retain their behavioral potency (Lande, Witter, & de Wied 1971; de Wied, Greven, Lande, & Witter 1972) and protect against retrograde amnesias induced by puromycin (Walter, Hoffmann, Flexner, & Flexner 1975; Flexner, Flexner, Hoffmann, & Walter 1977; Flexner, Flexner, Walter, & Hoffmann 1978), anoxia (Rigter, van Reizen, & de Wied 1974), pentylenetetrazole (Bookin & Pfeifer 1977), and electroconvulsive shock (ECS) (Pfeifer and Bookin 1978). That these effects reflect a physiological role for the peptide is suggested by reports that nanogram quantities of arginine vasopressin delayed pole-jump extinction (de Wied 1976) and attenuated passive avoidance extinction (Bohus et al., 1978b; Bohus et al., 1978a) when injected into the lateral ventricles of the brain. Post-training injections of antivasopressin serum into the lateral ventricles of the brain are known to cause deficient passive avoidance retention in otherwise intact rats (van Wimersma-Greidanus, Dogterom, & de Wied 1975). Finally, diabetes insipidus rats with a genetic absence of vasopressin (Brattleboro strain, Valtin & Schroeder 1964) have been shown to have deficient passive avoidance retention (de Wied, Bohus, & van Wimersma Greidanus 1975) despite being able to learn this and other fear motivated tasks such as shuttle-box and pole-jump avoidance (Bohus, van Wimersma-Greidanus, & de Wied, 1975; Celestian, Carey, & Miller 1975) although the interpretation of data from these animals remains controversial (Bailey & Weiss 1979; Celestian et al., 1975; Miller, Barranda, Dean, & Brush 1976).

It appears that the physiological presence of the peptide is required for normal expression of conditioned avoidance responding, that post-training injections delay subsequent response extinction, that the effectiveness of post-training vasopressin injections diminishes as a function

of the interval between training and injection, and that post-training vasopressin injections alter some aspect of memory consolidation. Experiments on the effects of delaying post-training vasopressin injections have relied on training–injection intervals ranging from 0 or 60 min up to 6 hr; however, when injections of LVP (1 μg) were given 30 min after rats had been trained to avoid shock in an automated shuttle box Hagan (1980) found subsequent extinction to be reduced compared to saline control performance. As this finding may pose difficulties for a straightforward explanation of the behavioral effects of post-training vasopressin injections in terms of effects on consolidation processes we report here the results of a number of experiments designed to replicate and extend the original finding.

GENERAL METHODS

Subjects

Adult male Wistar rats of an inbred strain (CPB TNO, Zeist, The Netherlands) were housed five to a cage with ad libitum access to food and water at a constant room temperature (22°C) with regulated illumination. (The animal house was in darkness between 1900 and 0500 hr.) Rats weighed 200–220 g and were brought to the laboratory 1 hr before experimental sessions which were run between 1300 and 1700 hr.

Apparatus

A two-way shuttle box (internal dimensions, 48 × 25 × 17 cm) with a central hurdle (height, 4 cm) was housed in a sound and light attenuating chamber. The shuttle box was lit by a single overhead houselight and a constant level of background noise was maintained by a footshock scrambler. A loud buzzer placed behind the shuttle box was used as the conditioned stimulus (CS). Ten seconds of the CS alone was followed by ten seconds during which the CS was accompanied by a scrambled footshock (the unconditioned stimulus, UCS, 0.15 mA, except where stated).

If the rat crossed the hurdle after the onset of the CS but before the UCS then shock was cancelled (AVOIDANCE RESPONSE), if the crossing occurred during shock (ESCAPE RESPONSE) the remaining shock was cancelled. In both cases responding also terminated the CS. In order to avoid excessive shock exposure no trial was allowed to exceed 20 sec. Each trial began with the onset of the CS every 60 sec. If the rat crossed the hurdle in the absence of the CS this was counted as an intertrial response (ITR).

Procedure

After 5 min of adaptation to the shuttle box training began and continued until each rat had made 10 correct consecutive avoidance responses.

Having reached the criterion rats were removed from the shuttle box
and returned to the home cage for treatment as described in the individual
experiments. Approximately 24 hr later they were returned to the shuttle
box and after 2 min of adaptation were tested with 10 extinction trials
during which shock was omitted but other aspects of the schedule were
identical to the training phase. A response within 10 sec of the CS onset
was counted as an avoidance and responses in the absence of the CS
were counted as intertrial responses. Use of the two-way shuttle box
reduces handling of the animals to a minimum and eliminates handling
between extinction trials thereby reducing the influence of experimenter
bias in this respect. In addition the rapidity of extinction responding
virtually eliminated experimenter bias from discrimination of avoidances
from either intertrial responses or failures to respond. This was confirmed
by the low incidence of reponses with latencies greater than 10 sec but
less than 20 sec as revealed by preliminary inspection.

Peptides

Peptides were stored at 16°C and solutions were freshly prepared before
each session by adding a single drop of HCl (0.01 N) plus saline (0.9%)
to yield the required dose in a constant injection volume of 0.5 ml. Lysine
vasopressin (LVP, pressor activity 325 IU/mg) was supplied by Organon,
Oss, The Netherlands. All injections were made subcutaneously (sc).

Data Analysis

Training performance was recorded using four measures: the number
of trials, avoidances, escapes, and intertrial responses taken to reach
criterion. Independent t tests and one-way analysis of variance (Winer
1962) were used to determine significant differences between groups in
training. For the analysis of extinction data avoidances and intertrial
responses were summed across subjects within each group to obtain the
total number of each response type on every trial. Trial totals, or means
in cases of unequal N, were analyzed using two-way analysis of variance
(treatment × trials) (Winer 1962). The Neuman–Keuls test (Winer 1962)
was then used to determine significant differences between peptide and
saline groups, between peptide doses, and between trials. For all tests
$p < .05$ was accepted as significant. Extinction data summarized in Tables
1–3 is expressed as the percentage of avoidance responses per trial for
each group.

EXPERIMENT 1

The first experiment examined the effects of five LVP doses on sub-
sequent extinction responding when injections were delayed for 30 min
after training. Rats were returned to the home cage immediately after
training and were randomly allocated for injection with either saline or

LVP. In the first of two independent phases three doses (0.11, 0.33, 0.99 μg) were compared with saline and in the second phase the dose range was extended to 0.036 and 2.97 μg.

Results

The results from Experiment 1 are summarized in Table 1. During training there were no significant differences between groups in either phase.

Analysis of phase 1 extinction data revealed significant treatment effects on avoidance ($F(3/27)$ = 16.4, $p < .01$) and intertrial responding ($F(3/27)$ = 11.6, $p < .01$). In addition the trials had significant effects on avoidance ($F(9/27)$ = 7.12, $p < .01$) and intertrial responding ($F(9/27$ = 15.3, $p < .01$). Neuman–Keuls comparisons showed that 0.11, 0.33, and 0.99 μg significantly increased avoidance responding compared to saline (p's $< .01$). Furthermore, rats injected with 0.11 μg made significantly more avoidances than those injected with either 0.33 or 0.99 μg (p's $< .01$).

Analysis of the trial's effects showed that avoidance responding was higher on Trial 1 than on any subsequent trial (p's $< .05$) and higher on Trial 2 than subsequent trials (p's $< .05$). Analysis of the trial's effects in the intertrial response data showed that intertrial responding on Trials 2 and 3 was significantly lower than on Trials 5–10 (p's $< .05$), intertrial responding on Trial 1 was lower than on both Trials 6 and 8 ($p < .05$), and responding was greater on Trial 8 than all other trials (p's $< .05$).

Analysis of phase 2 extinction data showed significant effects of treatment ($F(2/18)$ = 5.51, $p < .05$) and trials ($F(9/18)$ = 5.23, $p < .05$) on avoidance responding but no effects on intertrial responding. Avoidance responding was significantly reduced by both 0.036 μg ($p < .05$) and 2.97 μg ($p < .01$) compared to saline controls. Furthermore avoidance responding after 2.97 μg was significantly lower than after 0.036 μg ($p < .01$). Neuman–Keuls analysis of the trial's effects showed that avoidance responding on Trial 1 was significantly higher than on subsequent trials (p's $< .05$) and responding on Trials 2 and 10 was significantly higher than on Trial 4 ($p < .05$).

Discussion

All five vasopressin doses altered avoidance extinction responding but the direction of effects depended on the dose. This can be seen clearly from Fig. 1.

Avoidance responding was reduced by the lowest (0.036 μg) and highest (2.97 μg) dose and increased by intermediate doses (0.11, 0.33, 0.99 μg). The most potent dose in this respect was 0.11 μg which yielded higher avoidance response levels than either 0.33 or 0.99 μg and was the only dose to significantly increase intertrial responding. The results with 0.11,

TABLE 1

Acquisition and Extinction of Shuttle-Box Avoidance Responding Using a 0.15-mA Footshock in Training and a 30-Min Interval between the End of Training and Injection of Various Doses of Lysine Vasopressin

	No. of subjects	Acquisition[a]			Extinction[b]	
		Trials	Avoidances	Escapes	Avoidances	ITRs
Saline	8	18.63 ± 1.58	12.62 ± 0.42	5.87 ± 1.24	50.0 ± 4.5	36.2 ± 7.1
LVP 0.11 µg	8	20.87 ± 2.11	13.87 ± 1.53	6.37 ± 0.9	64.3 ± 6.0[d,e]	111.2 ± 10.4[d]
LVP 0.33 µg	7	21.28 ± 1.99	14.43 ± 1.04	6.0 ± 1.23	59.9 ± 6.3[d]	38.5 ± 7.1
LVP 0.99 µg	8	23.25 ± 2.87	13.25 ± 0.97	8.87 ± 2.31	61.2 ± 3.9[d]	30.0 ± 7.5
Saline	8	23.75 ± 2.51	15.0 ± 1.08	8.62 ± 1.74	68.7 ± 3.7	65.0 ± 8.1
LVP 0.036 µg	8	22.62 ± 2.25	13.75 ± 0.99	8.25 ± 1.37	63.7 ± 4.7[c]	36.2 ± 11.4
LVP 2.97 µg	8	23.25 ± 2.53	15.75 ± 1.38	7.12 ± 1.39	56.2 ± 3.8[d]	37.5 ± 8.7

[a] Mean ± SEM.
[b] Percentage responses (mean ± SEM per trial, see text).
[c] $p < .05$ (compared to saline controls).
[d] $p < .01$.
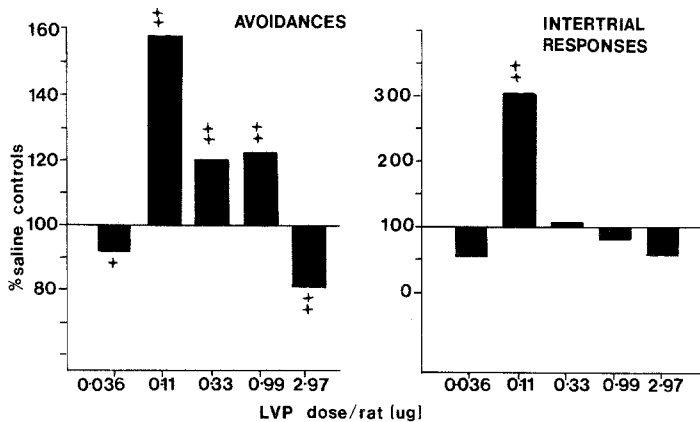[e] Two-way ANOVA followed by Neuman–Keuls test.

FIG. 1.   The effects of five LVP doses injected (sc) 30 min after training on extinction responding 24 hr later. Mean response levels for peptide-treated rats are expressed as a percentage of their saline controls. +, p .05; + +, p .01; ANOVA followed by Neuman–Keuls test.

0.33, and 0.99 μg confirm previous reports that post-training vasopressin injections increase resistance to extinction in intact rats (de Wied & Bohus 1966; de Wied 1971; Bohus et al., 1972; King & de Wied 1974; Bohus et al., 1978a, 1978b; Krejci et al., 1979). In addition however, the reduced responding seen after 0.036 and 2.97 μg support previous findings by Hagan (1980) who found that LVP injected 30 min after avoidance training reduced extinction responding 24 hr later. Furthermore despite a number of methodological differences the results from the present experiments agree with earlier findings (Hagan 1980) that when injected 30 min after training doses in excess of 1 μg give a negative dose–response curve during subsequent extinction testing. The direction and magnitude of LVP's effects on avoidance extinction vary as an inverted "U"-shaped function of the dose when injected 30 min after training. Hoglund and Meyerson (1980) have also reported a biphasic dose–response curve for vasopressin injected prior to inhibitory (passive) avoidance retention testing.

Avoidance response levels were high during early extinction trials then declined rapidly. In contrast, intertrial responding in phase 1 was low and increased up to Trial 8; within-test reductions in responding after intermediate doses may therefore reflect reductions in stimulus control as opposed to general mobility. Absence of within-test trial differences in phase 2 intertrial data suggests that although avoidance responding declines rapidly over trials with response reducing doses this is not paralleled by increased intertrial responding, suggesting reduced mobility. Avoidance and intertrial response levels showed some variability between phases 1 and 2 despite the fact that in both phases rats were trained to

the same learning criterion. A number of factors may contribute to this difference; avoidance responding in extinction is by nature variable particularly when responding is weakly established and extinction performance criteria are not used to select good responders (see for example de Wied 1971; Le Moal, Koob, Koda, Bloom, Manning, Sawyer, & Rivier 1981). Furthermore, although equated according to the learning criterion slight differences in rate of response acquisition may have contributed to extinction differences. In order to confirm that the behavioral effects of post-training vasopressin injections are robust despite baseline variations the following experiment was designed to test the peptide's effects after manipulating training conditions to substantially alter extinction response levels.

EXPERIMENT 2

It has previously been shown using inhibitory (passive) avoidance training that manipulation of training parameters, such as small increases in footshock intensity and the use of overtraining procedures, reduces the amnesic effects of protein synthesis inhibitors (Flood, Bennett, Rosenzweig, & Orme 1973, 1974). Similarly, the duration of amnesic treatment must be prolonged in order to be effective in mice which have been overtrained in active avoidance responding (Flood, Bennett, Orme, & Rosenzweig 1975). Results from Experiment 1 show that extinction avoidance responding varied as an inverted "U"-shaped function of the LVP dose when injected 30 min after training. Similar dose–response curves have been reported for ACTH injected after passive or Y-maze avoidance training (Gold & van Buskirk 1976; Sands & Wright 1979). Gold and van Buskirk (1976) found a strong interaction between dose and training shock level, a high dose facilitated retention after low shock but disrupted retention after intermediate or high shock. Moreover a low dose facilitated retention after both low and intermediate training shock levels but disrupted retention after high shock. The authors argued that ACTH injections modulated the normal hormonal responses to shock thereby mimicking the effects of higher footshock in training (Gold & van Buskirk 1976; Gold and McGaugh 1977). Although Gold and McCarty (1981) have recently demonstrated that footshock during inhibitory (passive) avoidance training acts as a powerful stimulator of peripheral catecholamine release it also seems clear from their results that, though large, this effect is relatively short lived. Plasma catecholamine levels of rats exposed to footshock were well within the range of controls within 40 min after training. Assuming that similar changes follow shuttle-box training a direct interaction between vasopressin injections and peripheral catecholamines seems unlikely, nevertheless one possible explanation of the inverted "U"-shaped dose–response curve for post-training vasopressin injections may be that the peptide injections alter the hormonal consequences of shock experienced

in training. In this case the inverted "U"-shaped dose–response curve for post-training LVP injections delayed by 30 min should be altered by increasing the shock level in training. Two oppositely acting doses, 0.11 and 2.97 μg, were therefore selected and injected 30 min after training in the shuttle box using 0.45-mA footshock. The effects of increased shock level in training are well documented and are known to depend on the particular behavioral requirements of the avoidance training procedure. In the two-way shuttle box shock increases cause disruption of avoidance responding (Moyer & Korn 1964; Levine 1966; McAllister, McAllister, & Douglas, 1971). Varying extinction response levels in this manner aim to test the peptide's effects over a wider range of response baselines.

Methods

The procedures were identical to Experiment 1 with the exception that the training shock level was increased to 0.45 mA and only two LVP doses (0.11 and 2.97 μg) were tested.

Results

Data from Experiment 2 are summarized in Table 2. During training there were no significant differences between groups in the number of avoidances, escapes, intertrial responses, or trials taken to reach criterion.

Analysis of extinction data revealed significant effects of treatment ($F(2/18) = 16.1, p < .01$) and trials ($F(9/18) = 14.5, p < .01$) on avoidance responding. A dose of 0.11 μg significantly increased avoidance responding compared to saline ($p < .01$) and 2.97 μg ($p < .01$). Furthermore, 2.97 μg reduced avoidances compared to saline ($p < .01$). Avoidance responding on Trial 1 was significantly higher than on all subsequent trials (p's < .05). Intertrial responding was almost totally suppressed during extinction and there were no significant effects of either trials or doses.

Discussion

Experiment 2 extended the findings of Experiment 1, 0.11 μg increased whereas 2.97 μg decreased subsequent avoidance responding in extinction when injected 30 min after avoidance training. Increased training shock levels in the present experiment did not markedly disrupt the learning rate compared to rats trained with the lower shock level of 0.15 mA in other experiments although intertrial response levels in training were generally lower for those rats trained at the higher shock level. Much more pronounced was the almost total suppression of intertrial responding in extinction and the reduction of avoidance response rates by almost 50% in rats trained at 0.45 mA. The replication of opposite effects for different vasopressin doses injected 30 min after training argues against any major confounding influence due to changes in control group baseline

TABLE 2

Acquisition and Extinction of a Shuttle-Box Avoidance Response Using 0.45 mA of Footshock in Training Followed by 0.11 or 2.97 μg of LVP Injected 30 Min after Training

| | No. of subjects | Acquisition[a] | | | | Extinction[b] | |
		Trials	Avoidances	Escapes	ITRs	Avoidances	ITRs
Saline	8	23.87 ± 3.32	14.87 ± 1.23	8.5 ± 2.44	2.0 ± 0.84	33.7 ± 5.3	3.7 ± 2.6
LVP 0.11 μg	8	22.12 ± 2.28	14.12 ± 1.12	8.25 ± 1.56	0.62 ± 0.37	40.0 ± 4.1[d]	2.2 ± 1.6
LVP 2.97 μg	8	22.5 ± 1.28	12.87 ± 0.76	9.5 ± 1.44	1.87 ± 1.06	25.0 ± 11.7[d]	3.7 ± 2.6

Note. See Table 1 for key.

rates which were seen in the previous experiment. The level of responding with 0.45 mA was such as to preclude further study of the interaction between shock level and vasopressin dose using this particular behavioral procedure. Despite this limitation it seems clear that, unlike the effects of post-training ACTH reported by Gold and van Buskirk (1976), the effectiveness of low and high LVP doses remained essentially the same after training at the higher level of footshock. Thus although considerable evidence implicates peripheral catecholamines in modulating memory storage (Walsh & Palfai 1979; Gold & McCarty 1981) and despite evidence for peripheral catecholamine release following avoidance training (Gold & McCarty 1981) it seems unlikely that vasopressin injected up to 1 hr after training mediates its subsequent effects on extinction by altering the hormonal consequences of shock, particularly in view of the relatively rapid decline in peripheral catecholamine levels after training (Gold & McCarty 1981). Clearly, however, in the absence of direct measurement of the effects of vasopressin on peripheral catecholamine levels after training this conclusion remains speculative.

EXPERIMENT 3

Time-dependent changes in the effectiveness of post-training vasopressin injections have been a central aspect in the evidence relating the peptide's effects on the maintenance of avoidance responding to processes involved in memory consolidation (de Wied 1971; Bohus et al., 1972; King and de Wied 1974; van Wimersma-Greidanus et al., 1975; Bohus et al., 1978b, 1978b). As has been shown in the two previous experiments, that 0.11 and 2.97 μg have opposite effects when injected 30 min after training, it was of interest to examine the pattern of time-dependent changes for these doses. In the present experiment either 0.11 or 2.97 μg were injected immediately or 60 min after training.

Methods

The procedures were identical to those described for Experiment 1 with the exception that rats were injected with saline or LVP (0.11 or 2.97 μg) 0 or 60 min after training. Rats in the 60-min groups were retained in the home cage for the intervening period.

Results

During training there were no significant differences between groups in the number of avoidances, escapes, intertrial responses, or trials to criterion. The data from acquisition and extinction phases are summarized in Table 3.

Analysis of extinction data revealed significant effects of treatment ($F(2/18) = 9.9, p < .01$) and trials ($F(9/18) = 2.95, p < .05$) on avoidance responding but no significant effects on intertrial responding when rats

TABLE 3

Acquisition and Extinction of a Shuttle-Box Avoidance Response Using 0.15 mA Footshock in Training followed by 0.11 μg or 2.97 μg LVP Injected Either Immediately or 60 Min after Training

	No. of subjects	Training-injection interval (min)	Acquisition[a]				Extinction[b]	
			Trials	Avoidances	Escapes	ITRs	Avoidances	ITRs
Saline	8	0	19.5 ± 2.28	13.5 ± 1.24	5.75 ± 1.22	2.0 ± 0.65	68.7 ± 6.2	41.7 ± 8.5
LVP 0.11 μg	8	0	25.0 ± 2.85	15.62 ± 1.74	6.75 ± 1.13	4.75 ± 1.2	83.7 ± 1.9[d]	71.2 ± 9.7
LVP 2.97 μg	8	0	21.12 ± 1.74	13.87 ± 1.27	7.12 ± 1.26	5.75 ± 2.15	63.8 ± 2.4	58.7 ± 7.2
Saline	8	60	26.87 ± 3.82	16.37 ± 2.21	8.75 ± 1.21	5.0 ± 1.37	53.7 ± 3.2	35.0 ± 6.1
LVP 0.11 μg	8	60	24.5 ± 3.54	15.62 ± 2.32	8.0 ± 1.72	6.87 ± 2.11	67.5 ± 3.8[d]	61.2 ± 9.0
LVP 2.97 μg	8	60	24.12 ± 2.96	16.87 ± 1.87	6.5 ± 1.21	2.75 ± 0.92	66.2 ± 5.3[d]	48.7 ± 7.1

Note. See Table 1 for key.

were injected immediately after training. A dose of 0.11 μg significantly increased avoidance responding relative to saline and 2.97 μg (p's < .01), whereas 2.97 μg did not affect avoidance responding relative to saline. Analysis of the trial's effects showed that avoidance responding on Trial 1 was higher than on Trial 5 (p < .05).

When injections were witheld for 60 min there were significant effects of treatment on avoidance responding ($F(2/18) = 4.7$, p < .025) but no effects on trials. Both 0.11 and 2.97 μg significantly increased avoidance responding relative to saline (p's < .01). There were no significant effects on intertrial response data. The extinction data, together with that from Experiment 1, for these two doses are shown in Fig. 2.

GENERAL DISCUSSION

The results from the final experiment show that 0.11 μg increased avoidance responding when injected 0, 30, or 60 min after training, confirming earlier observations on the efficacy of vasopressin injections within 1 hr of training (de Wied 1971; Bohus et al., 1972; King and de Wied 1974). As shown by Experiment 1 this dose was more effective when injected 30 min after training than after either 0 or 60 min. In contrast the effects of the high dose (2.97 μg) varied in direction as a function of the intervening interval. When injected immediately after training it had no effect, when injected 30 min after training subsequent extinction responding was reduced, and when injected after 60-min extinction responding was increased. Thus the interaction between doses and injection intervals appears to be more complex than a simple model of facilitation or inhibition of memory consolidation processes would predict.
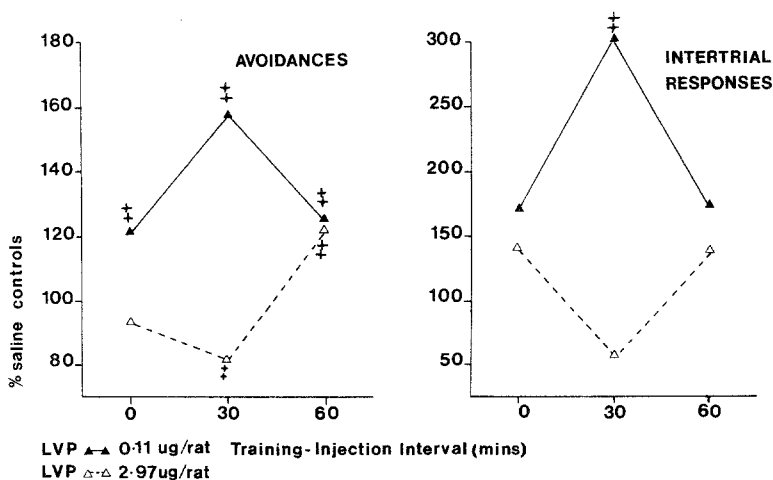

LVP ▲–▲ 0·11 ug/rat   Training-Injection Interval (mins)
LVP △·–△ 2·97ug/rat

FIG. 2.   The effects of 0.11 and 2.97 μg LVP injected (sc) 0, 30, or 60 min after training on extinction responding 24 hr later (See Fig. 1 for explanation).

The mechanism by which LVP injections alter subsequent extinction performance is unknown. Central mediation has been assumed on the basis that nanogram quantities of vasopressin and its behaviorally active fragments have similar effects when injected directly into the ventricular system (de Wied 1976; Bohus et al., 1978a, 1978b) and on recent evidence showing alterations in catecholamines from discrete brain nuclei following ventricular vasopressin injections (Tanaka, de Kloet, de Wied, & Versteeg 1977). Pressor and antidiuretic effects of the peptide were not thought to contribute significantly to its behavioral effects (Lande et al., 1971; de Wied et al., 1972). Recent evidence has shown that doses of AVP which prolong extinction responding also induce an early pressor response; conversely, injections of the antagonist peptide (dPTyr-(Me)AVP) block both the pressor and behavioral effects of vasopressin (Le Moal et al., 1981). Although the receptors which mediate the behavioral effects of vasopressin appear to be similar to those involved in the mediation of pressor effects the role of the short-term pressor response per se in mediating the behavioral effects of the peptide is controversial but seems unlikely in view of findings that des-glycinamide vasopressin analogs lack pressor potency despite their behavioral activity (Lande et al., 1971; de Wied et al., 1972). Furthermore, post-training peripheral injections of antivasopressin serum which abolish the endogenous peripheral peptide, as shown by diuresis, did not alter inhibitory (passive) avoidance behavior whereas much smaller quantities of ventricularly injected antivasopressin serum altered behavior while not affecting peripheral vasopressin levels (van Wimersma-Greidanus et al., 1975).

The present experiments with 0- and 60-min post-training injections confirm previous reports of post-training injections of vasopressin leading to increased extinction responding (de Wied & Bohus 1966; de Wied 1971; Bohus et al., 1972; King & de Wied 1974; Bohus et al., 1978a, 1978b). Whereas responding also increased following 30-min post-training injections of 0.11 μg it was further found that injections of 2.97 μg at this interval decreased subsequent extinction response levels, as shown previously (Hagan 1980). The mechanism by which increasing LVP doses results in an inverted "U"-shaped dose response curve during subsequent extinction is not clear. Although a biphasic dose response curve has also been reported for LVP injected 60 min before passive avoidance retention testing (Hoglund & Meyerson 1980) the underlying mechanism may not be the same in each case. Results from Experiment 2 suggest that the opposite effects of 0.11 and 2.97 μg are not due to modulating the hormonal consequences of shock as may be the case for ACTH (Gold & van Buskirk 1976).

Changes in the direction of high-dose effects and sensitivity to low-dose effects as a function of training–injection intervals may suggest time-dependent changes in the substrate which mediates vasopressin's

behavioral effects, possibly related to anomalous effects in other exper-iments. Gold, van Buskirk, and Haycock (1977) reported a similar dose–response relationship between post-training epinephrine injections and subsequent passive avoidance retention: a low dose (50 μg/kg) increased subsequent retention when injected immediately, but not 10, or 30 min after training, whereas 500 μg/kg was ineffective when injected immediately and 30 min after retention but improved retention when injected after 10 min. More recently Messing, Jensen, Martinez, Spiehler, Vasquez, Soumireu-Mourat, Liang, and McGaugh (1979) reported that intermediate naloxone doses (1 mg/kg) increased subsequent retention when injected immediately and 30 min after passive avoidance training whereas 0.1 and 10 mg/kg were ineffective. Furthermore 0.5 mg/kg naloxone was inactive when injected immediately after passive avoidance training but significantly reduced retention when injection was delayed for 30 min. Similar dose–response curves and time × dose interactions with different drugs may suggest a common mode of action. A-adrenergic receptors appear to play an important role in mediating various experimental amnesic procedures (Gold & Sternberg 1978; Sternberg & Gold 1981). Coupled with extensive evidence for vasopressin interacting with monoaminergic transmission in the brain (for review see Kovacs et al., 1980) these findings may be accounted for in terms of post-training, time-dependent changes in central monoamines. However, in view of evidence suggesting that vasopressin may act as a corticotropin releasing factor (Gillies & Lowry 1979), al-ternative explanations either in terms of an interaction between vasopressin and other neuropeptides of brain and/or pituitary origin or of peptide influences on labile memory (Gibbs & NG, 1978) cannot be ruled out yet.

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