Pharmacology Biochemistry and Behavior, Vol. 62, No. 1, pp. 173–178, 1999

0091-3057/99 $–see front matter

ELSEVIER

PII S0091-3057(98)00137-3

# Differential Behavioral Effects of TFF Peptides: Injections of Synthetic TFF3 Into the Rat Amygdala

HELMUT SCHWARZBERG,* HUBERT KALBACHER† AND WERNER HOFFMANN‡

*Institut für Neurophysiologie, Otto-von-Guericke-Universität, D-39120 Magdeburg, Germany,
†Medizinisch-Naturwissenschaftliches Forschungszentrum der Eberhardt-Karls-Universität, D-72074 Tübingen, Germany,
‡Institut für Molekularbiologie und Medizinische Chemie, Otto-von-Guericke-Universität, D-39120 Magdeburg, Germany

Received 7 July 1997; Revised 3 April 1998; Accepted 22 June 1998

SCHWARZBERG, H., H. KALBACHER AND W. HOFFMANN. *Differential behavioral effects of TFF peptides: Injections of synthetic TFF3 into the rat amygdala.* PHARMACOL BIOCHEM BEHAV **62**(1) 173–178, 1999.—TFF peptides (formerly named P-domain peptides or trefoil factors) are also released from the brain as well as being secreted typically by mucin producing cells. The amygdala, besides the hypothalamus, represents a defined neuronal locality of TFF3 synthesis. In a passive avoidance test synthetic TFF3/monomer or 0.9% sodium chloride (control) was injected bilaterally into the basolateral nucleus of the amygdala of male rats either immediately (consolidation test) or 23 h after a footshock (retrieval test). Application of a low TFF3 dose (2 × 6 pg) decreased avoidance latency in a time dependent manner. In contrast, a high dose (2 × 60 pg) increased avoidance latency. Maximal effects of TFF3 were observed about 24 h after the injection. This bidirectional effect was also observed using the elevated plus-maze test. The locomotor activity on the open arms was significantly increased 24 h after a low dose injection of TFF3 into the amygdala. In contrast, a high-dose injection significantly decreased the activity on the open arms. The results of both tests can be explained by an anxiolytic effect at a low dose and an anxiogenic effect at a high dose of synthetic TFF3/monomer. © 1998 Elsevier Science Inc.

TFF domain Fear Amygdala Hypothalamus Anxiolytic Anxiogenic Locomotor activity Passive avoidance response Elevated plus-maze

TFF peptides [formerly called P-domain peptides or trefoil factors; (11,36)] are major secretory products of mucin producing cells and are synthesized in the brain as well. Three TFF peptides have been characterized in a number of mammals including human: TFF1 (formerly pS2), TFF2 (formerly hSP), and TFF3 (formerly hP1.B/hITF). TFF1 is expressed neurally in the hippocampus, the cortex, and the cerebellum of the rat (13), as well as in cultured mouse astrocytes (14) where regulation of expression by various cytokines has been reported (12). In contrast, neural expression of TFF3 has been observed in the hypothalamus (i.e., magnocellular neurons of the supraoptic and paraventricular nuclei) and also the amygdala (28). No data are published concerning a neural expression of TFF2.

The physiological functions of TFF peptides seem to be multiple. They obviously act as motogens influencing cell migratory processes in the gastrointestinal mucosa (10,27) probably via receptors (33). They also interact with mucins, and appear as constituents of viscoelastic mucous gels. The role of TFF peptides in the central nervous system (CNS) has not yet been clarified. They could represent factors influencing the developement of the CNS (13). TFF peptides could also affect behavior similar to the action of oxytocin (3,16). However, transgenic knock out mice where TFF1 or TFF3 have been deleted display no obvious neural abnormalities (19,21). The first direct studies addressing the question on behavioral effects of synthetic TFF3 in rats are presented here. Bilateral application of TFF3 into the amygdala has been chosen for system-

Requests for reprints should be addressed to Prof. Helmut Schwarzberg, Institut für Neurophysiologie, Universitätsklinikum, Leipziger Str. 44, D-39120 Magdeburg, Germany.

atic studies because the amygdala is a defined source for this peptide (28). This brain region is known to be involved in emotional learning and fear (7,18,22,31) and is susceptible to peptidergic stimulation (15,20,29). The use of a classical passive avoidance test as well as the elevated plus-maze allow statements concerning possible effects of TFF3 on processes involving learning, memory, and anxiety (4,5,9,16, 30,35).

## METHOD

Adult male Wistar rats (Harlan GmbH, Borchen, Germany) were kept under a 12 L/12 D schedule (lights on at 0600 h) with free access to standard pelleted food (Altromin GmbH, Lage, Germany) and tap water. The following experimental protocol was approved by an Institutional Review Committee for the use of animal subjects.

The animals were anesthesized with 50 mg/kg sodium pentobarbital (Sigma-Aldrich GmbH, Deisenhofen, Germany) and then placed in a stereotactic instrument. A pair of stainless steel guide cannulae of 1.0 mm o.d. and 13 mm length were introduced through small burr holes on the skull and were placed 1.0 mm above the basolateral amygdala of the left and right hemispheres. All coordinates were provided by the atlas of Paxinos and Watson (25). The cannulae were fixed chronically to the skull with dental cement (Harvard GmbH, Berlin, Germany) and acrylate (Spezialchemie, Leipzig, Germany). Cannulae were implanted in a total of 312 rats. The animals were sacrificed at the end of each experiment to verify the position of the injection; only results with the cannulae in the correct position entered the statistical evaluation. The rats were killed by an overdose of pentobarbital. Their brains were fixed in 10% formaldehyde for at least 1 week, and the placement of the cannulae was visualized on 30-μm frozen sections after injection of methylene blue into the cannulae. Experiments were started 7 days after operation. During the recovery period, the animals were handled each day to decrease stress during the experiment.

Synthetic TFF3 based on the deduced rat sequence (28) was synthesized on a MilliGen9050 peptide synthesizer similarly as described (17,24). Cys-57 was blocked by an acetamidomethyl group to avoid dimerisation via this residue. TFF3 was purified by reverse-phase HPLC on a C2/C18 column and the sequence confirmed by electrospray mass spectrometry. Oxidation of the six deblocked cysteine residues was for 48 h, and the monomer was purified by gel filtration followed by HPLC.

TFF3/monomer was diluted in 0.9% NaCl and injected bilaterally into the basolateral nucleus of the amygdala by protrusion of the injection needle (0.45 mm o.d.) 1.0 mm beyond the tip of the guide cannula. A dose of 0.5 microliters was injected in each hemisphere. The infusion rate was 0.5 μl/30 s. Control rats were given equal volumes of 0.9% saline solution.

All behavioral measurements were made between 0900 and 1200 h. The passive avoidance behavior was studied by using a one-trial learning paradigm in a step-through situation as described (1,2). The experimental arrangement consisted of an illuminated runway attached to the front center of a dark chamber with a grid floor. The rats were placed on the platform and allowed to enter the dark compartment. Because rats prefer dark to light, they normally entered within 10 s (exploration trial). After an additional trial on the following day (preshock trial), a single 3-s unavoidable foot shock (current 2 mA, pulse duration 2 ms, interval 10 ms) was delivered through the grid floor immediately after rats entered the dark compartment. The animals were treated with TFF3 or 0.9% NaCl (controls) either immediately (consolidation test) or 23 h after the foot shock (retrieval test). The rentention of the passive avoidance behavior was tested for each rat 24, 48, and 72 h after the foot shock (retention trials 1–3) by placing the rats on the runway and measuring the latency necessary to enter the dark chamber. Cutoff time was 180 s.

Motor coordination of the rats was tested 15 min after injection of TFF3. This was done on a platform of glass (78 $cm^2$) by measuring the time spent on the smooth surface up to the cutoff time of 20 s.

The elevated plus-maze [(26); purchased from TSE, Bad Homburg, Germany] consisted of two "closed" (25 cm high walls) and two "open" arms (40 × 15 cm each) interconnected by a 15-cm square central area. The maze was elevated 70 cm above the floor. Light conditions were similar (30 lx) in the open and closed arms. Separate series of rats, naive to the apparatus, were placed onto the square central area facing a closed arm 1 or 24 h after the bilateral injection into the amygdala and allowed to explore the maze for 3 min. The animals showed their main locomotor activity within these 3 min. The following parameters were scored with the help of an automatic video tracking system: (a) the time spent in the different compartments of the maze, (b) the number of entries into the different compartments, and (c) the distance walked within the different compartments (measuring exactly the locomotor activity).

Analgesic effects were measured in separate series 15 and 60 min after injection of TFF3 (two series with doses of 2 × 6 or 2 × 60 pg) by means of a tail-flick analgesiometer (6). Briefly, a focused light beam is directed to the tail of the rat. The rat stops light and measurement of time by a short sudden movement of the tail, if pain is perceptible. Control rats treated with 0.9% NaCl switched off the light between 7 and 10 s.

Statistical significance was evaluated by using Levene's test for equality of variances and the two-tailed *t*-test (SPSS software 7.5, SPSS Inc. 1996). Differences between the TFF3-injected animals and their respective controls were considered significant if $p < 0.05$.

## RESULTS

### *Passive Avoidance Tests*

Passive avoidance behavior was tested after low-dose (2 × 6 pg; Fig. 1) or high-dose injections (2 × 60 pg; Fig. 2) of TFF3 into the amygdala.

*Retrieval tests.* The injection of TFF3 took place about 23 h after the initial foot shock, a point at which memory consolidation is not affected. Neither of the two doses significantly affected the retention trial 1 compared with the control animals treated with 0.9% NaCl. Injection of a low TFF3 dose reduced the avoidance latency significantly only in retention trials 2 and 3 (Fig. 1A). In contrast, injection of a high TFF3 dose increased the latency significantly in retention trial 2 (Fig. 2A).

*Consolidation tests.* Passive avoidance latency was also tested after an injection scheme typical of a consolidation test, as shown in Figs. 1B and 2B. Both TFF3 doses were injected immediately after the foot shock when memory consolidation takes place. Injection of the lower dose decreased the avoidance latency significantly in all retention trials (Fig. 1B), whereas an increase of the avoidance latencies was observed

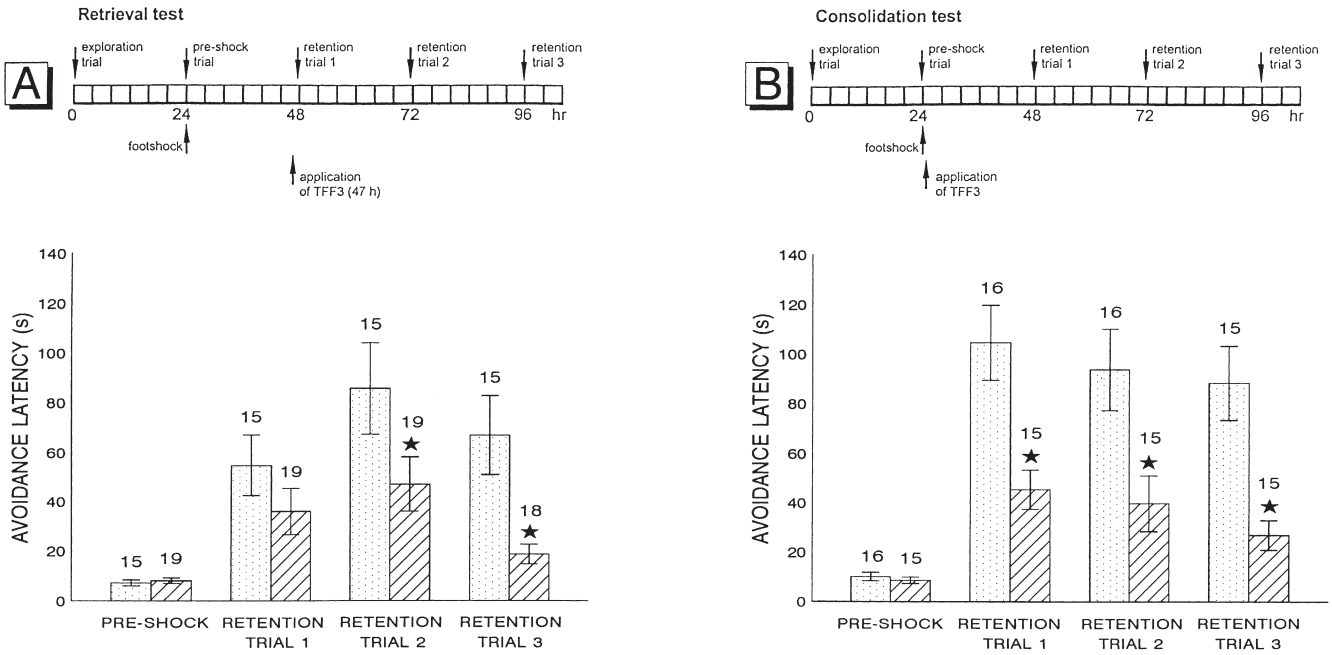


FIG. 1. Passive avoidance latencies after bilateral injection of 2 × 6 pg TFF3 into the rat amygdala (hatched). The results from a typical retrieval test (A) or a consolidation test (B) are shown. As a control, 0.9% NaCl was injected (dotted). The number of animals tested in each group is given above the bars. Mean values ± SE; stars indicate statistically significant $p$-values: A/trial 2: $p < 0.042$ ($t = 2.144$), A/trial 3: $p < 0.01$ ($t = 2.944$), B/trial 1: $p < 0.002$ ($t = 3.476$), B/trial 2: $p < 0.012$ ($t = 2.700$), B/trial 3: $p < 0.001$ ($t = 3.828$).

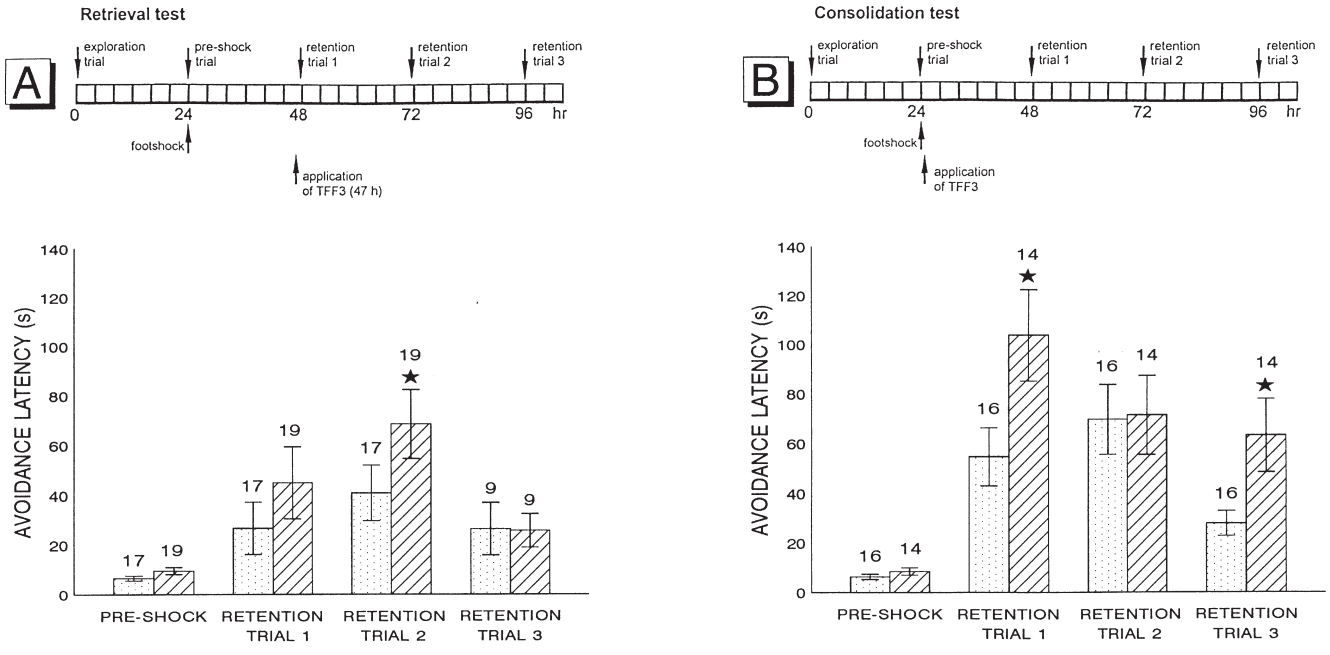


FIG. 2. Passive avoidance latencies after bilateral injection of 2 × 60 pg TFF3 into the rat amygdala (hatched). The results from a typical retrieval test (A) or a consolidation test (B) are shown. As a control, 0.9% NaCl was injected (dotted). The number of animals tested in each group is given above the bars. Mean values ± SE; stars indicate statistically significant $p$-values: A/trial 2: $p < 0.048$ ($t = -2.068$), B/trial 1: $p < 0.036$ ($t = -2.231$), B/trial 3: $p < 0.038$ ($t = -2.257$).

in most retention trials after injection of the higher dose (Fig. 2B).

*Elevated Plus-Maze Tests*

The following significant behavioral effects were observed after low-dose (2 × 6 pg; Fig. 3) or high-dose injections (2 × 60 pg; Fig. 4) of TFF3. The number of open-arm entries was not affected at both doses (data not illustrated).

Animals injected with a low dose of TFF3 showed a decreased total locomotor activity 1 h after injection when compared with control animals (Fig. 3A). However, no effect was observed concerning the activity or the time on the open arms. TFF3 at this time point apparently did not trigger mechanisms of fear (Fig. 3A). In contrast, the activity on open arms significantly increased 24 h after injection, but not the total activity (Fig. 3B). The time on open arms showed a tendency towards an increase at this time point.

Animals injected with a high dose of TFF3 could easily be distinguished by their different behavior when compared to the controls even 1 h after injection (Fig. 4A). The animals entered all arms in a manner similar to the controls but left the open arms immediately. This behavior resulted in a significantly decreased locomotor activity on open arms and a tendency towards a decrease in the time spent on open arms ($p < 0.075$) 1 h after injection. The total activity did not change in comparison to the controls at this time point (Fig. 4A). Furthermore, the open-arm activity together with the time on open arms was still reduced 24 h after injection (Fig. 4B). However, also the total activity decreased significantly at this time point.

*Platform Tests, Tail-Flick Tests*

None of the two doses applied (2 × 6 pg, 2 × 60 pg) had effects in the platform and tail-flick tests showing that motor coordination and pain sensitivity of the TFF3-treated animals were within the same range as the controls.

## DISCUSSION

Passive avoidance tests showed maximal effects about 1 day after the application of synthetic TFF3 into the amygdala, re-

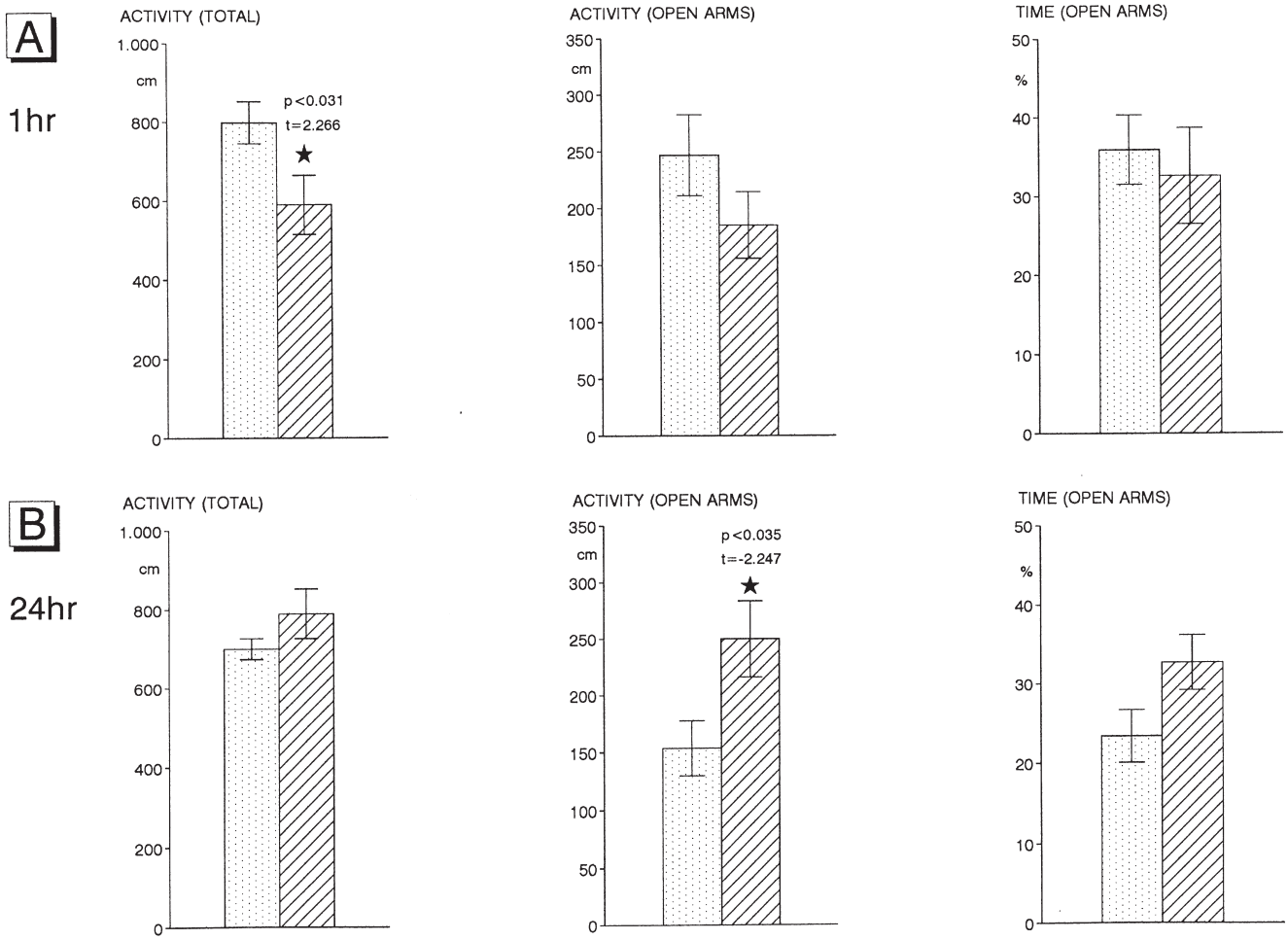


FIG. 3. Results from the elevated plus-maze tests 1 h (A) or 24 h (B) after bilateral injection of 2 × 6 pg TFF3 into the rat amygdala (hatched). As a control, 0.9% NaCl was injected (dotted). The number of animals was: (A) TFF3 $n = 15$, controls $n = 15$; (B) TFF3 $n = 13$, controls $n = 11$. Mean values ± SE; stars indicate statistically significant $p$-values.

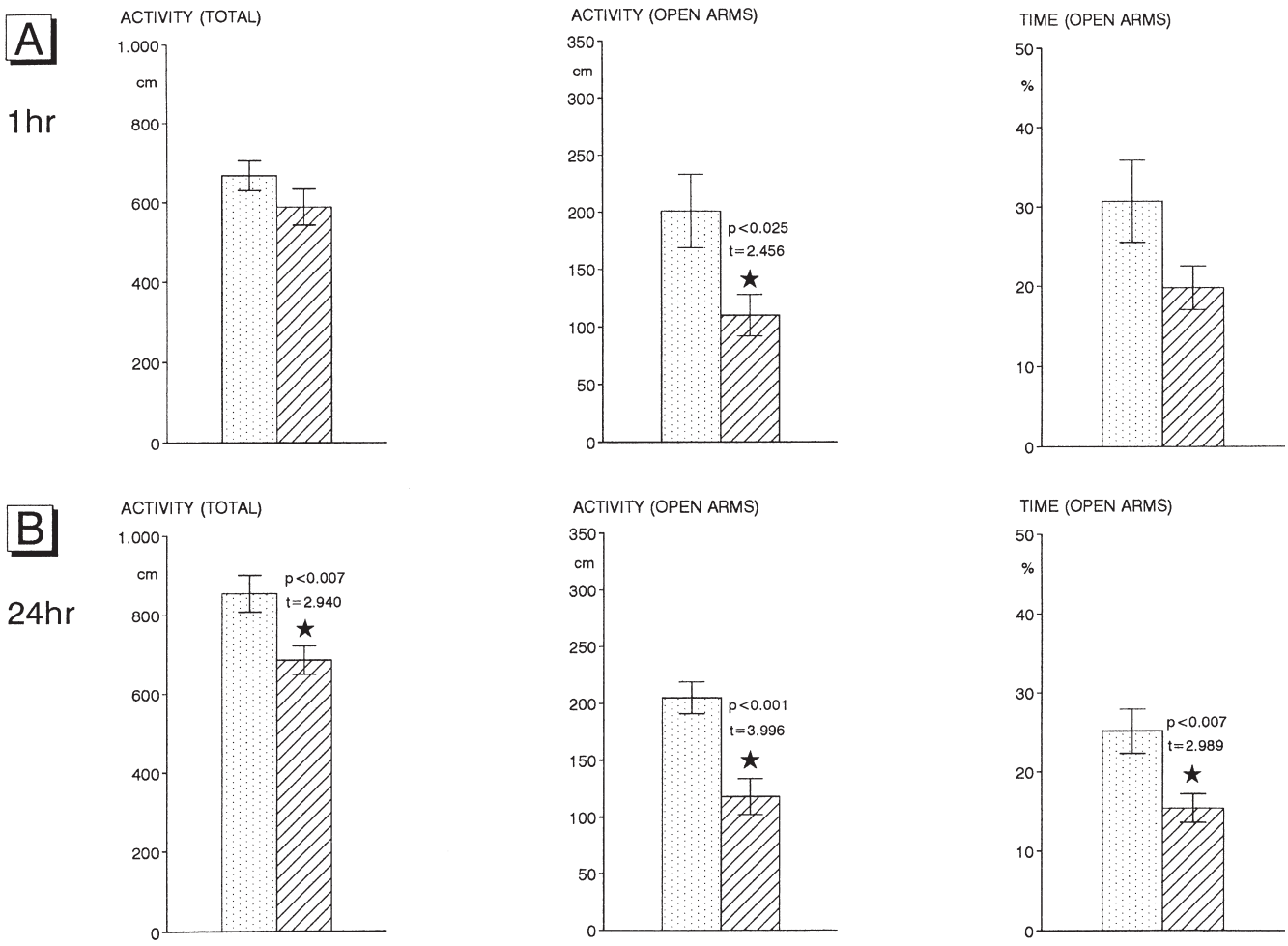


FIG. 4. Results from the elevated plus-maze tests 1 h (A) or 24 h (B) after bilateral injection of 2 × 60 pg TFF3 into the rat amygdala (hatched). As a control, 0.9% NaCl was injected (dotted). The number of animals was: (A) TFF3 $n = 12$, controls $n = 12$; (B) TFF3 $n = 13$, controls $n = 11$. Mean values ± SE; stars indicate statistically significant $p$-values.

gardless if a retrieval test or a consolidation test had been performed. The maximal effect was consequently at retention trial 2 in retrieval tests, whereas TFF3 was most active already at retention trial 1 in the consolidation test. One hour after application (i.e., retention trial 1 in the retrieval tests) was obviously too early to observe a significant effect. However, a tendency was visible at this time. The reason for this delay is currently not known; it is unlikely that the lag phase is due to generation of biologically active proteolytic degradation products of TFF3 because TFF peptides are remarkably stable even in the aggressive lumen of the gastrointestinal tract (11).

The different TFF3 doses injected had opposite effects in the passive avoidance tests. This was observed for both the retrieval and the consolidation test. A direct comparison of the maximal effects in the retrieval tests (i.e., retention trial 2) and the consolidation tests (i.e., retention trial 1) clearly accentuates the increased avoidance latency at the low dose (2 × 6 pg), whereas the high dose (2 × 60 pg) decreased the latency. A similar biphasic dose dependent effect has been described for CRH (34).

The avoidance latency reduction at the low dose of TFF3 could be due to an anxiolytic effect. This would be in line with the results from the elevated plus-maze test 24 h after injection. However, mechanisms of fear do not seem to be affected, as judged by the elevated plus-maze test results 1 h after injection of TFF3. The decrease in the total locomotor activity at this time is in agreement with a report that the amygdala generally plays an important role in the modulation of alerting mechanisms and arousal states (32).

The enhanced avoidance latency after injection of a high TFF3 dose into the amygdala might be caused by an anxiogenic effect, which is also in agreement with the elevated plus-maze test results presented. However, the effect at 24 h is correlated to a change in the total locomotor activity which can give rise to false positive results in the elevated plus-maze test (8,9,23).

In summary, injection of synthetic TFF3/monomer into the rat amygdala seems to affect anxiety bidirectionally in a dose-dependent manner. A low dose (2 × 6 pg) is anxiolytic, whereas a high dose (2 × 60 pg) has an anxiogenic effect, and both doses are capable of modulating total locomotor activity. Both behavioral parameters are mediated by the serotonergic transmission system in the amygdala (5,32). The basolateral nucleus, which received the TFF3 injections in the present investigations, showed the most pronounced serotonergic innervation (32).

The passive avoidance test by itself represents a paradigm typically used for investigations of learning and memory. However, the results with the elevated plus-maze presented here do not allow a clear statement concerning the effect of TFF3 on learning and memory. Further studies necessary to clear this point will be of special interest, as the amygdala seems to represent a brain region where fear and processes of learning and memory converge (22,31).

ACKNOWLEDGEMENTS

We thank C. Obst, K. Tauber (Magdeburg) and T. Bauer (Tübingen) for their excellent technical assistance and Dr. M. Oertel for his comments on the manuscript. This work has been supported by the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (BMBF, Neuroverbund 01 ZZ 9510/project B8 to W.H. and H.S.) and the Deutsche Forschungsgemeinschaft (SFB 120 to H.K.).

## REFERENCES

1. Ader, R.; Weijnen, J. A. W. M.; Moleman, P.: Retention of a passive avoidance response as a function of the intensity and duration of electric shock. Psychon. Sci. 26:125–128; 1972.
2. Bernstein, H.-G.; Schwarzberg, H.; Poeggel, G.; Reiser, M.: Intracerebroventricular but not intraperitoneal administration of aluminum attenuates vasopressin-enhanced retrieval of a passive avoidance task in rats. Pharmacol. Biochem. Behav. 47:587–590; 1994.
3. Bohus, B.; Kovács, G.L.; De Wied, D.: Oxytocin, vasopressin and memory: Opposite effects on consolidation and retrieval processes. Brain Res. 157:414–417; 1978.
4. Bucherelli, C.; Tassoni, G.; Bures B.: Time-dependent disruption of passive avoidance acquisition by post-training intra-amygdala injection of tetrodotoxin in rats. Neurosci. Lett. 140:231–234; 1992.
5. Chopin, P.; Briley, M.: Animal models of anxiety: The effect of compounds that modify 5-HT neurotransmission. Trends Pharmacol. Sci. 8:383–388; 1987
6. D'Amour, F. E.; Smith, D. L.: A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72:74–79; 1941.
7. Davis, M.: The role of the amygdala in emotional learning. Int. Rev. Neurobiol. 36:225–266; 1994.
8. Dawson, G. R.; Crawford, S. P.; Collinson, N.; Iversen, S. D.; Tricklebank, M. D.: Evidence that the anxiolytic-like effects of chlordiazepoxide on the elevated plus maze are confounded by increases in locomotor activity. Psychopharmacology (Berlin) 118: 316–323; 1995.
9. Dawson, G. R.; Tricklebank, M. D.: Use of the elevated plus maze in the search for novel anxiolytic agents. Trends Pharmacol. Sci. 16:33–37; 1995.
10. Dignass, A.; Lynch-Denaney, K.; Kindon, H.; Thim, L.; Podolsky, D. K.: Trefoil peptides promote epithelial migration through a transforming growth factor β-independent pathway. J. Clin. Invest. 94:376–383; 1994.
11. Hoffmann, W.; Hauser, F.: The P-domain or trefoil motif: a role in renewal and pathology of mucous epithelia? Trends Biochem. Sci. 18:239–243; 1993.
12. Hirota, M.; Awatsuji, H.; Furukawa, Y.; Hayashi, K.: Cytokine regulation of pS2 gene expression in mouse astrocytes. Biochem. Mol. Biol. Int. 33:515–520; 1994.
13. Hirota, M.; Awatsuji, H.; Sugihara, Y.; Miyashita, S.; Furukawa, Y.; Hayashi, K.: Expression of pS2 gene in rat brain. Biochem. Mol. Biol. Int. 35:1079–1084; 1995.
14. Hirota, M.; Miyashita, S.; Hayashi, H.; Furukawa, Y.; Hayashi, K.: pS2 gene especially expressed in the late G1/S phase of mouse astrocytes. Neurosci. Lett. 171:49–51; 1994.
15. Introini-Collison, I. B.; Ford, L.; McGaugh, J. L.: Memory impairment induced by intraamygdala β-endorphin is mediated by noradrenergic influences. Neurobiol. Learn. Mem. 63:200–205; 1995.
16. Kovács, G. L.; De Wied, D.: Peptidergic modulation of learning and memory processes. Pharmacol. Rev. 46:269–291; 1994.
17. Kropshofer, H.; Max, H.; Müller, C.A.; Hesse, F.; Stevanovic, S.; Jung, G.; Kalbacher, H.: Self-peptide released from class II HLA-DR1 exhibits a hydrophobic two-residue contact motif. J. Exp. Med. 175:1799–1803; 1992.
18. LeDoux, J. E.: Emotion: Clues from the brain. Annu. Rev. Psychol. 46:209–235; 1995.
19. Lefebvre, O.; Chenard, M.-P.; Masson, R.; Linares, J.; Dierich, A.; LeMeur, M.; Wendling, C.; Tomasetto, C.; Chambon, P.; Rio, M.-C.: Gastric mucosa abnormalities and tumorigenesis in mice lacking the pS2 trefoil protein. Science 274:259–262; 1996.
20. Lu, Y.-F.; Moriwaki, A.; Hayashi, Y.; Tomizawa, K.; Itano, T.; Matsui, H.: Effects of neurotensin on neurons in the rat central amygdaloid nucleus in vitro. Brain Res. Bull. 40:135–141; 1996.
21. Mashimo, H.; Wu, D.-C.; Podolsky, D. K.; Fishman, M. C.: Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. Science 274:262–265; 1996.
22. McKernan, M. G.; Shinnik-Gallagher, P.: Fear conditioning induces a lasting potentiation of synaptic currents in vitro. Nature 390:607–611; 1997.
23. Moser, P. C.: An evaluation of the elevated plus-maze test using the novel anxiolytic buspirone. Psychopharmacology (Berlin) 99: 48–53; 1989.
24. Pawelec, G.; Max, H.; Halder, T.; Bruserud, O.; Merl, A.; da Silva, P.; Kalbacher, H.: BCR/ABL leukemia oncogene fusion peptides selectively bind to certain HLA-DR alleles and can be recognized by T cells found at low frequency in the repertoire of normal donors. Blood 88:2118–2124; 1996.
25. Paxinos, G.; Watson, C.: The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1986.
26. Pellow, S.; Chopin, P.; File, S. E.; Briley, M.: Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14:149–167; 1985.
27. Playford, R. J.; Marchbank, T.; Chinery, R.; Evison, R.; Pignatelli, M.; Boulton, R. A.; Thim, L.; Hanby, A. M.: Human spasmolytic polypeptide is a cytoprotective agent that stimulates cell migration. Gastroenterology 108:108–116; 1995.
28. Probst, J. C.; Skutella, T.; Müller-Schmid, A.; Jirikowski, G. F.; Hoffmann, W.: Molecular and cellular analysis of rP1.B in the rat hypothalamus: In situ hybridization and immunohistochemistry of a new P-domain neuropeptide. Mol. Brain Res. 33:269–276; 1995.
29. Roberts, G. W.: Neuropeptides: Cellular morphology, major pathways, and functional considerations. In: Aggleton, J. P., ed. The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction. New York: Wiley-Liss; 1992:115–142.
30. Rodgers, R. J.; Dalvi, A.: Anxiety, defence and the elevated plus-maze. Neurosci. Biobehav. Rev. 21:801–810; 1997.
31. Rogan, M. Z.; Stäubli, U. V.; LeDoux, J. E.: Fear conditioning induces associative long-term potentiation in the amygdala. Nature 390:604–607; 1997.
32. Sanford, L. D.; Tejani-Butt, S. M.; Ross, R. J.; Morrison, A. R.: Amygdaloid control of alerting and behavioral arousal in rats: Involvement of serotonergic mechanisms. Arch. Ital. Biol. 134: 81–99; 1995.
33. Tan, X.-D.; Hsueh, W.; Chang, H.; Wei, K.-R.; Gonzalez-Crussi, F.: Characterization of a putative receptor for intestinal trefoil factor in rat intestine: Identification by in situ binding and ligand blotting. Biochem. Biophys. Res. Commun. 237:673–677; 1997.
34. Veldhuis, H. D.; De Wied, D.: Differential behavioral actions of corticotrophin-releasing factor (CRF). Pharmacol. Biochem. Behav. 21:707–713; 1984.
35. Weyers, P.; Fritze, J.: Tiermodelle der Angst. In: Kasper, S.; Möller, H.-J., eds. Angst- und Panikerkrankungen. Jena, Stuttgart: Gustav Fischer Verlag; 1995:233–269.
36. Wright, N. A.; Hoffmann, W.; Otto, W. R.; Rio, M.-C.; Thim, L.: Rolling in the clover: The trefoil factor family (TFF)-domain peptides, cell migration and cancer. FEBS Lett. 408:121–123; 1997.