Neuroscience Letters, 163 (1993) 109–113

NSL 10011

Extinction of emotional learning: contribution of medial prefrontal cortex

Maria A. Morgan, Lizabeth M. Romanski[b], Joseph E. LeDoux[a,*]

[a]*Center for Neural Science, Department of Psychology, New York University, New York, NY 10003, USA*
[b]*Neurobiology Section, Yale University Medical School, New Haven, CT, USA*

(Received 9 July 1993; Revised version received 27 August 1993; Accepted 27 August 1993)

Key words: Fear; Classical conditioning; Cerebral cortex; Amygdala; Cortex

Stimuli associated with painful or otherwise unpleasant events acquire aversive emotional properties in animals and humans. Subsequent presentation of the stimulus alone (in the absence of the unpleasant event) leads to the eventual extinction of the aversive reaction. Although the neural basis of emotional learning has been studied extensively, considerably less is known about the neural basis of emotional extinction. In the present study, we show that the medial prefrontal cortex plays an important role in the regulation of fear extinction in rats, a finding that may help elucidate the mechanisms and, possibly, the treatment of disorders of uncontrolled fear, such as anxiety, phobic, panic and posttraumatic stress disorders in humans.

The prefrontal cortex has long been suspected of playing some role in the regulation of emotional reactions [4, 10, 22, 24, 25, 28, 32] but the exact role has remained unclear and even controversial [6, 9, 12, 17, 23]. Part of the confusion may be related to the fact that the effects of prefrontal cortex lesions on emotional behavior have not been systematically examined using consistently applied well-defined emotional tasks in a given species. Our goal in the present study was, therefore, to reexamine the contribution of the prefrontal cortex to the acquisition and extinction of emotional reactions in rats using classical fear conditioning, a well-characterized behavioral paradigm that is often used for studying emotional behavior and its neural basis [5, 14, 18, 19]. We focused on the medial prefrontal cortex (mPFC), a region commonly described as being involved in emotional reactivity and autonomic regulation [16, 35, 36].

36 male Sprague–Dawley rats were randomly assigned to three groups: mPFC lesions (n = 11), sham mPFC lesions (n = 13) and unoperated controls (n = 12). Coordinates for mPFC (measured in mm relative to the interaural line and with the surface of the skull level) were 12 anterior, 0.6 medial and 4.8 dorsal. An epoxy-coated stainless steel insect pin (500-μm-exposed tip) was lowered into the brain and anodal direct current (1 mA) was passed for 10–15 s. Sham animals were treated in the same way, except that no current was passed through the electrode. After 14 days of recovery, the animals were trained in a Pavlovian fear-conditioning paradigm involving the presentation of a tone conditioned stimulus (CS; 800-Hz or 10-kHz tone, 80 dB, 20 s), ending with a footshock unconditioned stimulus (US; 0.5 mA, 500 ms delivery of direct current produced by a grid floor shocker). We measured 'freezing' behavior as the conditioned emotional response [2, 3, 8, 21]; the number of seconds spent freezing was ascertained by observing the animal's behavior in the conditioning box and using stop watches to time the total amount of freezing. Animals received two CS–US pairings/day for 2 days after which the CS was presented alone. Freezing was measured during the 20 s CS and during the 20 s before the CS, allowing the assessment of the extent of conditioned emotional reactivity to the CS and to the context in which CS–US pairing took place, respectively [26]. Testing continued daily until extinction criterion was met (2 consecutive days of 5 s spent freezing to the CS and to the pre-CS).

Days 1 and 2 of the experiment were conditioning days. The effects of conditioning on a given day were defined by the amount of freezing during the first trial of the following day [26]. Changes in freezing over days 1–3, thus, reflect fear acquisition. As shown in Fig. 1, animals in control and lesion groups exhibited similar increases in the amount of freezing expressed during the CS and pre-CS periods over days 1–3. Moreover, ani-

*Corresponding author.

mals in all groups appeared to condition to the CS earlier than to the context, as previously reported [26]. A $3 \times 3 \times 2 \times 3$ ANOVA of lesion group by experimental session (initial study and two replications) by stimulus type (pre-CS, CS) by acquisition day (days 1–3) showed no significant difference between the groups in the amount of freezing exhibited during either the pre-CS (context) or the CS over days 1–3 but did show main effects of stimulus type ($F_{1,27} = 28.63$, $P < 0.001$) and day ($F_{2,54} = 179.16$, $P < 0.001$) and an interaction of stimulus type by day ($F_{2,54} = 21.80$, $P < 0.001$). In summary, all groups exhibited conditioning to both the context and the CS and conditioned faster to the CS than to context (interaction of stimulus type by day). Thus, mPFC lesions had no effect on the rate of acquisition to either stimulus type.

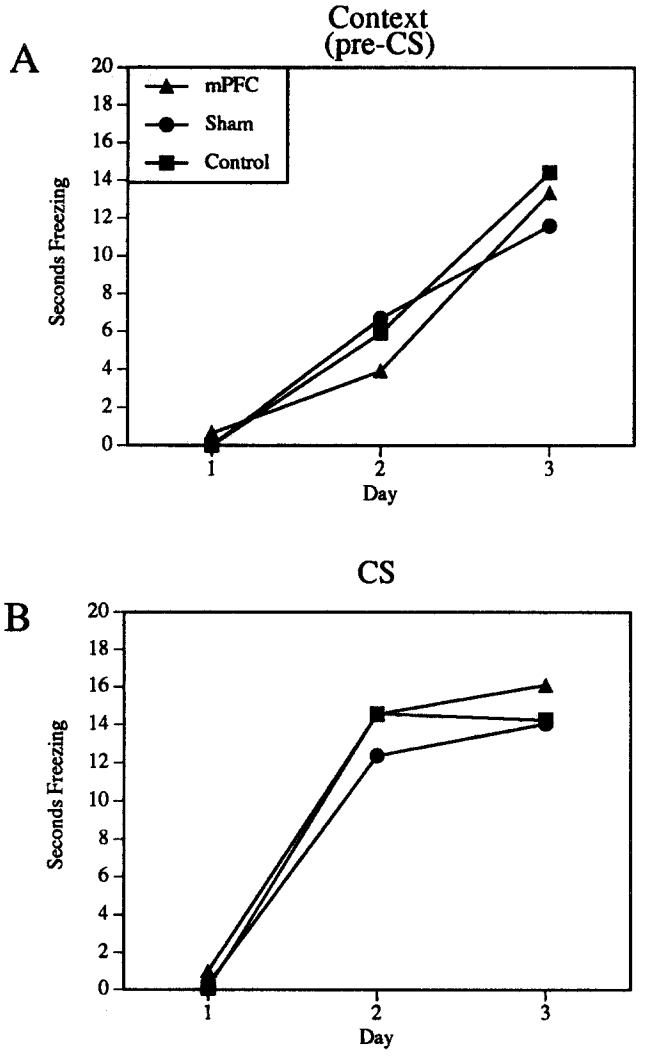

Fig. 1. Acquisition of fear. Mean number of seconds spent freezing during an exposure to the conditioning context (A) and to the conditioned stimulus (B) is shown. Freezing was measured during the 20 s before the onset of the conditioned stimulus (context test) and during the 20-s conditioned stimulus (conditioned stimulus test) on each day. Freezing responses during the first trial of days 2 and 3 reflect the effects of conditioning trials on days 1 and 2 (see text for details).

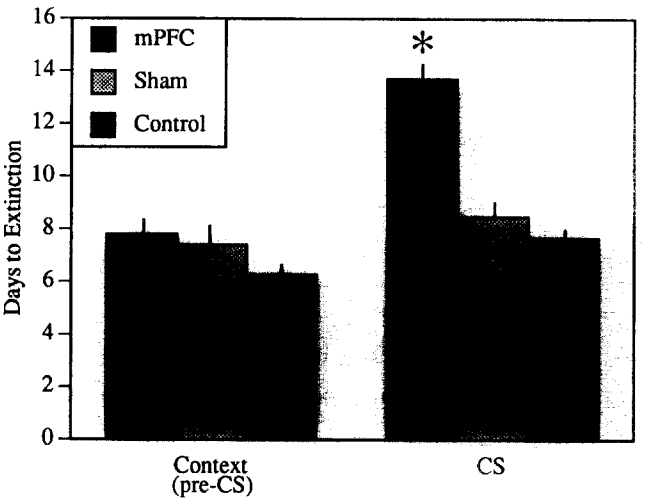

Fig. 2. Extinction of fear. Mean number of days to reach criterion is shown. Extinction criterion was defined as < 5 s of freezing during the conditioned stimulus and, independently, during the context test period on 2 consecutive days (see Fig. 1 and text for more details).

In contrast, examination of the number of days taken to reach extinction criterion (2 consecutive days of 5 s spent freezing during CS and pre-CS) indicates that mPFC lesions do have an effect on extinction (Fig. 2). Days to extinction criterion was examined between groups and experimental session and across stimulus type (pre-CS, CS) using a $3 \times 3 \times 2$ ANOVA. There was a main effect of lesion group ($F_{2,27} = 10.07$, $P < 0.001$) and stimulus type ($F_{1,27} = 60.42$, $P < 0.001$) and an interaction between lesion group and stimulus type ($F_{2,27} = 16.29$, $P < 0.001$). Follow-up simple-effects tests showed that there was a significant difference between lesion groups in the number of days to extinction of the freezing response to the CS ($F_{2,27} = 19.22$, $P < 0.01$) but not to the context ($F_{2,27} = 2.32$, $P > 0.1$). Posthoc t tests further showed that for the CS the mPFC group was significantly different from the sham control group ($t_{27} = 11.15$, $P < 0.001$) and from the unoperated control group ($t_{27} = 12.59$, $P < 0.001$) but these two control groups did not differ from each other ($t_{27} = 1.73$, $P > 0.09$). Thus, lesioned rats extinguished to the pre-CS (context) at the same rate as controls but took significantly longer than controls to extinguish to the CS.

Histological analysis of the mPFC lesions showed that all lesions included damage to the infralimbic cortex, prelimbic cortex and caudal portions of medial orbital cortex (Fig. 3). Damage to the anterior cingulate cortex, which lies just dorsal to the prelimbic region, and to the tenia tecta, which is ventral to infralimbic cortex, was slight and was variable from case to case.

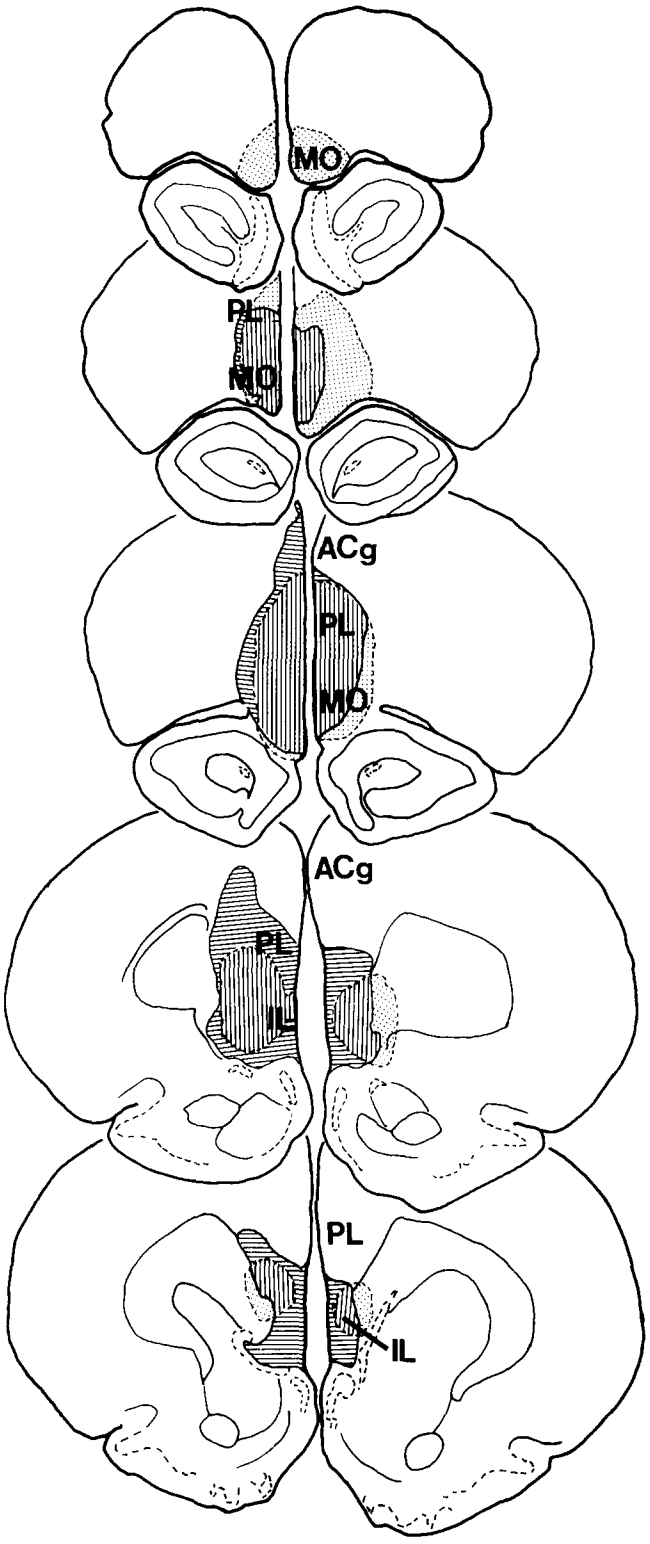

Fig. 3. Medial prefrontal cortex lesions. mPFC as defined here includes the various cortical ares lying along the medial wall of the anterior frontal lobe, specifically the infralimbic (IL), prelimbic (PL), anterior cingulate (ACg) and medial orbito-frontal (MO) cortical regions [33]. Areas damaged consistently included the prelimbic, infralimbic and medial orbital cortex. The anterior cingulate region was damaged in some but not all cases. The most rostral extending lesion (stippled), the most caudal extending lesion (horizontal lines) and the overlap of the two (vertical lines) is shown.

Some studies have suggested that mPFC lesions alter fear levels in rats, with both increases and decreases having been reported [6, 9, 12, 17, 23]. mPFC-lesioned animals did not freeze any more or less than controls to the CS or context during acquisition and the early trials of extinction, ruling out the possibility that the prolonged responding of mPFC-lesioned rats over days is due to an increase in fear per se. Also, a change in general levels of fear would most likely be reflected in freezing to contextual stimuli as well as to the CS during extinction. Differences with past studies may be due to differences in the strains of rats studied (wild vs. laboratory-bred rats), the extent to which the various areas of mPFC and surrounding cortical areas are included in the lesion, variations in the procedures used to measure fear (fear conditioning vs. passive avoidance vs. open-field behavior) and/or the extent to which performance on a given task is controlled by continuously present background contextual stimuli vs. discrete phasic-conditioned stimuli.

Previous studies have shown that lesions of sensory areas of cortex interfere with extinction of conditioned fear responses elicited by conditioned stimuli presented in the sensory modality corresponding to the damaged cortical area [20, 29]. On the basis of these findings, it was suggested that damage to sensory cortex interferes with the flow of sensory information about the conditioned stimulus to higher-order cortical association regions, such as the hippocampus or frontal cortical areas [20]. The present findings suggest that the mPFC might be the critical recipient of this information.

The amygdala is an essential component of the neural system underlying the acquisition and expression of fear conditioning; it is believed to be the site where the 'emotional significance' of threatening stimuli is determined [5, 14, 18, 19]. The mPFC has extensive projections to the amygdala and several amygdaloid projection targets in the brainstem that are also involved in the expression of conditioned fear [1, 13, 30, 31, 34]. These projections may allow the organism to adapt the expression of learned fear reactions to changing stimulus relations by modulating neuronal activity in the amygdala and/or its projections during exposure to the CS. A similar conclusion was reached by Rolls [27] on the basis of neurophysiological studies of primate orbito-frontal cortex and the amygdala. The finding that extinction of fear responses elicited by a phasic CS is interfered with by blockade of NMDA receptors in the amygdala [7] suggests a possible mechanism through which fronto-amygdala connections might exert their influence.

A commonly observed effect of extensive damage to the frontal cortex is response perseveration [10, 11], a failure or inability to inhibit responses which are no longer appropriate. Our findings suggest that persevera-

tion extends into the emotional domain. Further, our findings localize a specific region of the prefrontal cortex that is involved in emotional perseveration. When mPFC is damaged, emotional associations mediated by the amygdala may not be inhibited during nonreinforcement and, thus, conditioned responding may be prolonged over time. Emotional perseveration may represent a failure to inhibit fear to the CS after the threatening properties of the stimulus should have been diminished by nonreinforcement. To the extent that such a mechanism exists, it is extraordinarily specific to the CS itself as prolonged extinction to contextual stimuli did not occur. However, contextual conditioning is mediated by the hippocampal formation, as well as the amygdala [15, 26], and mPFC does not have extensive projections to the hippocampal region [1, 13]. Thus, the effect of mPFC lesions on the extinction of fear to the CS, but not to context, may be related to the fact that somewhat different mechanisms underlie the acquisition and/or expression of fear to a CS and to contextual stimuli. Further elucidation of these mechanisms may provide insights into the neurobiology of fear extinction; this may, in turn, shed light on the mechanisms and possibly the treatment of disorders of uncontrolled fear, such as anxiety, phobic, panic and posttraumatic stress disorders.

Supported by USPHS Grants R37MH28774, R01MH46516 and K02MH00956 and NSF Grant IBN9209646. Data in this paper were presented at the Cold Spring Harbor Laboratory Conference, Learning and Memory, held from 9 September to 4 October 1992.

1 Berendse, H.W., Galis-de Graaf, Y. and Groenewegen, H.J., Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat, J. Comp. Neurol., 316 (1992) 314–347.

2 Blanchard, R.J. and Blanchard, D.C., Crouching as an index of fear, J. Comp. Physiol. Psychol., 67 (1969) 370–375.

3 Bouton, M.E. and Bolles, R.C., Conditioned fear assessed by freezing and by the suppression of three different baselines, Anim. Learn. Behav., 8 (1980) 429–434.

4 Damasio, A.R., Tranel, D. and Damasio, H., Individuals with sociopathic behavior caused by frontal damage fail to respond autonomically to social stimuli, Behav. Brain Res., 41 (1990) 91–94.

5 Davis, M., The role of the amygdala in conditioned fear. In J.P. Aggleton (Ed.), The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction, Wiley-Liss, New York, NY, 1992, pp. 255–306.

6 Divac, I., Mogenson, J., Blanchard, R.J. and Blanchard, D.C., Mesial cortical lesions and fear behavior in the wild rat, Physiol. Psychol., 12 (1984) 271–274.

7 Falls, W.A., Miserendino, M.J.D. and Davis, M., Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala, J. Neurosci., 12 (1992) 854–863.

8 Fanselow, M.S., Conditional and unconditional components of postshock freezing, Pavlov. J. Biol. Sci., 15 (1980) 177–182.

9 Frysztak, R.J. and Neafsey, E.J., The effect of medial frontal cortex lesions on respiration, 'freezing', and ultrasonic vocalizations during conditioned emotional responses in rats, Cereb. Cortex, 1 (1991) 418–425.

10 Fuster, J.M., The Prefrontal Cortex. Anatomy, Physiology, and Neuropsychology of the Frontal Lobe, 2nd edn., Raven, New York, NY, 1989, 255 pp.

11 Goldman-Rakic, P., Circuitry of primate prefrontal cortex and regulation of behavior by representational memory. In F. Plum (Ed.), Handbook of Physiology. Section 1: The Nervous System. Vol. V, Higher Functions of the Brain, American Physiological Society, Bethesda, MD, 1987, pp. 373–418.

12 Holson, R.R., Mesial prefrontal cortical lesions and timidity in rats. I. Reactivity to aversive stimuli, Physiol. Behav., 37 (1986) 221–230.

13 Hurley, K.M., Herbert, H., Moga, M.M. and Saper, C.B., Efferent projections of the infralimbic cortex of the rat, J. Comp. Neurol., 308 (1991) 249–276.

14 Kapp, B.S., Wilson, A., Pascoe, J., Supple, W. and Whalen, P.J., A neuroanatomical systems analysis of conditioned bradycardia in the rabbit. In M. Gabriel and J. Moore (Eds.), Learning and Computational Neuroscience: Foundations of Adaptive Networks, MIT Press, Cambridge, MA, 1990, pp. 53–90.

15 Kim, J.J. and Fanselow, M.S., Modality-specific retrograde amnesia of fear, Science, 256 (1992) 675–677.

16 Kolb, B.J., Prefrontal cortex. In B.J. Kolb and R.C. Tees (Eds.), The Cerebral Cortex of the Rat, MIT Press, Cambridge, MA, 1990, pp. 437–458.

17 Kolb, B.J., Social behavior of rats with chronic prefrontal lesions, Comp. Physiol. Psychol., 87 (1974) 466–474.

18 LeDoux, J.E., Emotion. In F. Plum (Ed.), Handbook of Physiology. 1: The Nervous System. Vol. V, Higher Functions of the Brain, American Physiological Society, Bethesda, MD, 1987, pp. 419–460.

19 LeDoux, J.E., Brain mechanisms of emotion and emotional learning, Curr. Opin. Neurobiol., 2 (1992) 191–198.

20 LeDoux, J.E., Romanski, L.M. and Xagoraris, A., Indelibility of subcortical emotional memories, J. Cog. Neurosci., 1 (1989) 238–243.

21 LeDoux, J.E., Sakaguchi, A. and Reis, D.J., Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned by acoustic stimuli, J. Neurosci., 4 (1984) 683–698.

22 Mark, V.H. and Ervin, F.R., Violence and the Brain, Harper and Row, New York, NY, 1970, 170 pp.

23 Markowska, A. and Lukaszewska, I., Emotional reactivity after fronto-medial cortical, neostriatal or hippocampal lesions in rats, Acta Neurobiol. Exp., 40 (1980) 881–893.

24 Myers, R.E., Role of prefrontal and anterior temporal cortex in social behavior and affect in monkeys, Acta Neurobiol. Exp., 32 (1972) 567–579.

25 Nauta, W.J.H., The problem of the frontal lobe: a reinterpretation, J. Psychiatr. Res., 8 (1971) 167–187.

26 Phillips, R.G. and LeDoux, J.E., Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning, Behav. Neurosci., 106 (1992) 274–285.

27 Rolls, E.T., Neurophysiology and functions of the primate amygdala. In J.P. Aggleton (Ed.), The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction, Wiley-Liss, New York, NY, 1992, pp. 43–165.

28 Stuss, D.T. and Benson, D.F., The Frontal Lobes, Raven, New York, NY, 1986, 303 pp.

29 Teich, A.H., McCabe, P.M., Gentile, C.C., Schneiderman, L.S., Winters, R.W., Liskowsky, D.R. and Schneiderman, N., Auditory

cortex lesions prevent the extinction of Pavlovian differential heart rate conditioning to tonal stimuli in rabbits, Brain Res., 480 (1989) 210–218.

30 Terreberry, R.R. and Neafsey, E.J., Rat medial frontal cortex: a visceral motor region with a direct projection to the solitary nucleus, Brain Res., 278 (1983) 245–249.

31 Terreberry, R.R. and Neafsey, E.J., The rat medial frontal cortex projects directly to autonomic regions of the brainstem, Brain Res. Bull., 19 (1987) 639–649.

32 Teuber, H.-L., The riddle of frontal lobe function in man. In J.M. Warren and K. Akert (Eds.), The Frontal Granular Cortex and Behavior, McGraw-Hill, New York, NY, 1964, pp. 410.

33 Uylings, H.B.M. and van Eden, C.G., Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans, Prog. Brain Res., 85 (1990) 31–62.

34 van der Kooy, D., Koda, L.Y., McGinty, J.F., Gerfen, C.R. and Bloom, F.E., The organization of projections from the cortex, amygdala, and hypothalamus to the nucleus of the solitary tract in rat, J. Comp. Neurol., 224 (1984) 1–24.

35 Vogt, B.A., Finch, D.M. and Olson, C.R., Functional heterogeneity in cingulate cortex: the anterior executive and posterior evaluative regions, Cereb. Cortex, 2 (1992) 435–443.

36 Zbrozyna, A.W. and Westwood, D.M., Stimulation in prefrontal cortex inhibits conditioned increase in blood pressure and avoidance bar pressing in rats, Phys. Behav., 49 (1991) 705–708.