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Effect of different agonistic experiences on behavioural seizures in fully amygdala kindled rats

Hans J.A. Beldhuis, Jaap M. Koolhaas and Béla Bohus

Department of Animal Physiology, Centre for Behavioural, Cognitive, and Neuro-Sciences, University of Groningen, Haren (Netherlands)

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Fully amygdala kindled rats were exposed to two different inter-male agonistic experiences in order to study the interaction between epilepsy and acute social stress. *Victory* experience did not influence the severity of seizure behaviour, whereas a single acute *defeat* modified both ictal and postictal seizure manifestations. Defeat resulted in less severe and shorter lasting motor seizures, and the accompanied postictal inhibition or behavioural depression was of shorter duration in comparison with pre-stress values. The ability of acute defeat to trigger anticonvulsant activity as implied by the weakened convulsive response is discussed.

Association between stress and convulsions in humans is supported by clinical observations [7, 25]. Several physiological and emotional stressors such as sleep deprivation, somatic overexertion, major life events, and daily hassles are related to the occurrence of convulsions. Kindling is a widely used animal model of human complex partial seizures and epilepsy. Repeated application of an initially subconvulsive electrical tetanus to a limbic brain region induces progressive intensification of evoked behavioural seizures [17]. Electroencephalographic as well as behavioural seizure responses to the kindling tetanus are affected by experimental stressors, such as foot shock [22], immobilisation [20], novelty [11] and handling [2]. However, the results obtained are not uniform in direction and may depend on the type of stressor used. Furthermore, none of these types of stressors are easily comparable with ordinary daily life experiences in humans. An inter-male agonistic design in which a social conflict is provoked by confronting the experimental rat with a conspecific in its semi-natural territory provides a possible solution for this problem [13]. By manipulating the outcome of the conflict it is possible to create winners and losers, which are specified by endocrine and physiological profiles [5]. This approach has proven to be useful in animal studies of stress-related psychosomatic and mental diseases [12], and was used in the present study in order to investigate the effect of acute social stress on the behavioural expression of seizures evoked in fully amygdala kindled rats.

Tryon maze dull S3 rats (TMD-S3; bred in our own laboratory under SPF conditions) were used because of their well-known high levels of intraspecific aggressive behaviour. Adult male rats weighing 242–304 g at begin of the experiment were housed individually and permanently in observation cages (size 80 × 55 × 50 cm), each with a female rendered infertile by ligation of the oviducts. The cages were placed in a temperature-controlled room (22°C) with a 12-h reversed light/dark cycle (lights on 20.00 h). Water and food were available ad libitum. Under pentobarbital anaesthesia (60 mg/kg), trimel-coated bipolar platinum/iridium (90/10%) wires (90 μm diameter) were implanted bilaterally into the medial amygdala (tooth bar at +5 mm; 0.2 mm posterior to bregma; 3.6 mm lateral to median; 8.7 mm ventral to dura [21]). A stainless-steel screw attached to the frontal bone served as a ground wire. After a recovery period of 2–3 weeks, the afterdischarge threshold was established for each rat by stimulating the amygdala at 2-min intervals with an increasing intensity until abnormal electrical activity, the so-called afterdischarge (AD), of 4 s or longer was elicited. In an experimental observation cage (size 80 × 55 × 50 cm), allowing the rat to move freely and undisturbed, recording of bilateral amygdaloid electroencephalographic (EEG) activity and stimulation (1 s, 60 Hz train of 1 ms biphasic rectangular pulses) were made using a computer-controlled set-up. All rats were tetan-

Correspondence: H.J.A. Beldhuis, Department of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands. Fax: (31) (50) 63 52 05.

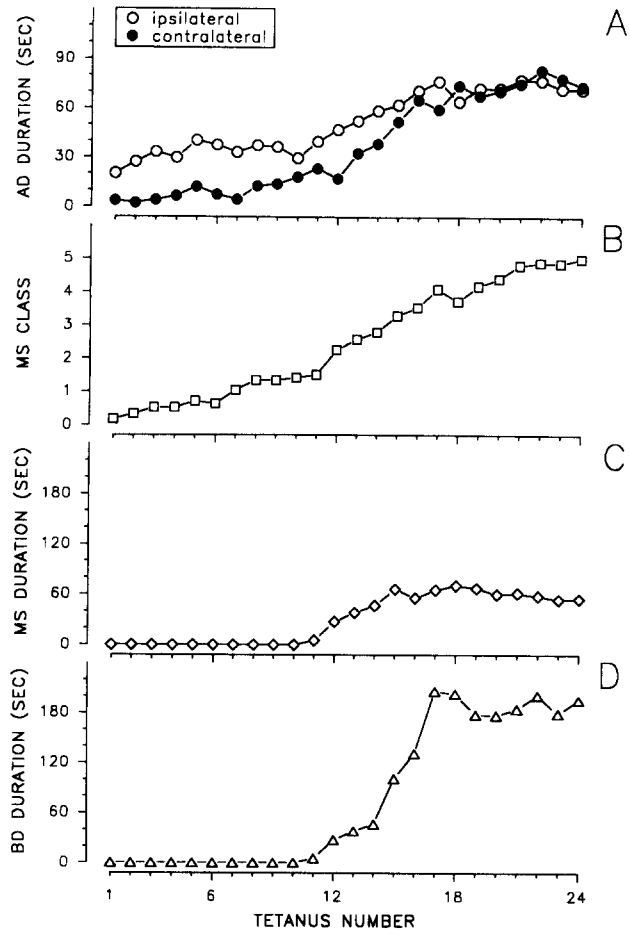

Fig. 1. Development of seizure parameters during daily tetanization of the medial amygdala. A: mean afterdischarge duration in ipsi- and contralateral amygdala. B: mean motor seizure class. C: mean motor seizure duration. D: mean behavioural depression duration.

ized once daily, 7 days a week, with a fixed intensity set at two times the mean AD threshold of all rats in order to evoke seizure behaviour. AD duration was measured by on-line EEG recording, while the severity of the motor seizure (MS) behaviour was assessed using Racine's 5-point classification [23]. Duration of the ictus and the postictal behavioural depression phase (BD), in which the rat is temporarily torpid or immobile, were measured relative to the end of stimulation. Rats were considered fully kindled when they had at least five MS class 5 (generalized clonic) seizures on consecutive tests.

Subsequently, the resident/intruder paradigm was used to induce either a victory ($n = 7$) or a defeat ($n = 9$) experience in fully kindled rats [13]. In the victory test a socially naive male TMD-S3 rat (younger and lighter in weight), whereas in the defeat test an experienced fighter TMD-S3 rat (older and heavier in weight), was introduced into the experimental observation cage in which the experimental rat was already present for 1–2 min. The differences in age, weight, and fighting experience between experimental kindled rats and intruders assures clear dominant-subdominant relations in both the victory and the defeat test. After 15 min, the intruder rat was removed and, shortly afterwards (approximately 1 min), the experimental rat was tetanized in order to assess the direct effect of the preceding agonistic experience. On following days rats were tetanized with similar stimulation parameters in order to assess a possible long-term effect. The kindling stimulation and agonistic experiences took place under dim light, from 09.00 to 17.00 h during the dark phase. At the end of the experiment, all rats were sacrificed with an overdose of anaesthetic and their brains were processed with immunocytochemistry to examine the electrode locations. Only those animals with electrodes in the medial amygdala complex were included in the final analysis. Unfortunately, due to a technical problem, the AD duration in relation to stress could not be analyzed. All data are given as means ± standard error. Significance of differences was calculated by the Wilcoxon signed-rank test for paired replicates. All procedures in this study were approved by the Committee on Animal Bio-ethics of the University of Groningen.

The mean threshold for eliciting an AD was 80.3 (± 4.7) μA. Rats were therefore kindled with a stimulus intensity of 160 μA (peak-to-peak). As is shown in Fig. 1, daily stimulation of the medial amygdala led to a gradual development of bilaterally generalized motor convulsions. On average, an AD lasting 71.2 (± 6.8) s ipsilateral and 73.2 (± 5.9) s contralateral was accompanied after 24 tetanizations by a class 5 MS of 54.6 (± 2.6) s and a BD of 195.2 (± 26.7) s.

The effect of the two agonistic experiences, victory and defeat, on seizure behaviour is shown in Fig. 2. A single acute victory experience did not affect either MS severity, MS duration or BD duration. In contrast, a single acute defeat reduced the duration both of the MS (29.0 ± 9.6 s; $P < 0.01$), and of the BD (117.8 ± 40.1 s; $P < 0.05$). On the following day BD was still decreased by 51.0 ± 18.7 s ($P < 0.05$), whereas the duration of the MS had already returned to the baseline level. A reduction of MS severity as a result of defeat was observed in 3 rats ranging from 4 to 5 MS classes, whereas in the remaining 6 rats no change was detected. Subsequent tetanizations produced full-blown MS class 5 seizures comparable to pretreatment values regarding the duration of MS and BD.

The present results thus show that a single agonistic confrontation with a conspecific male suppressed the duration both of the motor seizure and of the subsequent behavioural depression in male rats, provided that the experience was one of defeat rather than victory. Such

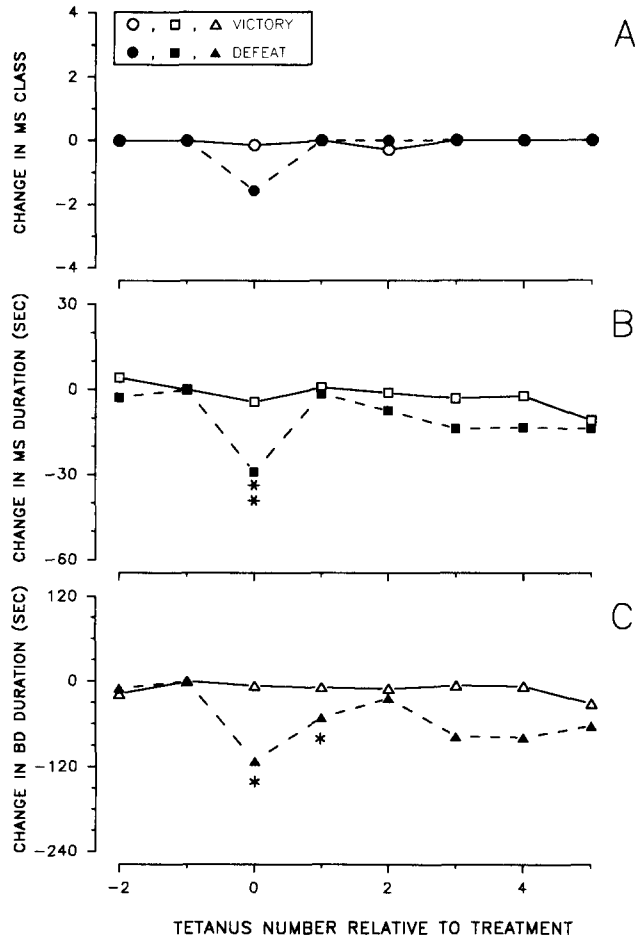

Fig. 2. Effect of agonistic experiences victory and defeat on behavioural seizure activity in fully amygdala kindled rats. Motor seizure class, motor seizure duration and behavioural depression duration are expressed as absolute difference of individual control values observed during the preceding tetanization. Tetanus number 0 is applied directly after the agonistic treatment. Significance of differences between pre- and post-experience values is indicated by asterisks (*$P < 0.05$, **$P < 0.01$).

social stress failed to affect these behavioural seizure variables in rats which won the social encounter. These data suggest that a stressful condition triggers an anticonvulsant mechanism in fully kindled rats. Previous data in animals as well in humans have also shown that stress is associated with an anticonvulsant activity [22, 27].

Several possibilities for the underlying mechanism can be considered. Firstly, it is known that stress caused by an acute defeat in an aggressive encounter in mice increases the brain levels of the opioid peptides, β-endorphin and met-enkephalin [18]. Defeated mice become analgesic, an effect which can be suppressed by an opiate μ-receptor antagonist. Anticonvulsant activity in fully amygdala kindled rats could be mediated by opiate μ-receptor agonists [1], but pro- as well as anti-convulsant actions of opioids are on record [6]. Adrenocorticotropin hormone and related neuropeptides, released during stress, may be considered as partial agonist/antagonists of opiate receptors [26], and their anticonvulsant properties have indeed been demonstrated in hippocampal kindled rats [4]. Therefore, activation of opiate receptors by means of opioids released by stress could result in an anticonvulsant action. Secondly, also the peripheral catecholaminergic systems are affected by agonistic confrontations, such that defeat (but not victory) results in marked elevations of plasma noradrenaline and adrenaline [5]. As far as the central catecholamines are concerned, immobilisation stress increases noradrenaline release in the amygdala [24]. Participation of the central catecholaminergic mechanisms in the development of amygdala kindling is wellfounded. For example, destruction of the noradrenergic and dopaminergic neurons facilitates amygdala kindling [3]. These results were related to a disinhibition of the spread of epileptiform activity from the stimulated amygdala. Recently, it was reported that stress by restraint suppresses electrophysiological activity measured by evoked potentials in the entorhinal-dentate pathway [9]. However, in fully amygdala kindled rats depletion of noradrenaline does not affect the seizure expression [28], by which a contribution of catecholamines to the anticonvulsive effect observed in the present study is not likely. Third of all, acute defeat results in a prolonged elevation of plasma corticosteroid in rats, whereas victory results in no change or only a very short increase [5]. Steroids and their naturally occurring metabolites, the so-called neurosteroids, enhance the affinity of GABA receptors [15, 16]. Interestingly, defeated mice show an increased binding of the GABA-benzodiazepine receptor complex [19]. GABAergic mechanisms play a significant role in the development and maintenance of amygdala kindling [8, 10]. For example, injection of a GABA-elevating substance (γ-vinyl-GABA) in the substantia nigra strongly reduces the AD duration of the seizures in amygdala kindled rats, whereas a similar trend is observed in the MS severity [14]. Consequently, release of steroids in the periphery by stress could result in an activation of a centrally acting anticonvulsive system.

In conclusion, the agonistic experience defeat results in a weakened convulsive response in fully amygdala kindled rats. This effect may be attributed to the evoked release of opioid peptides or steroids or both. A more definite conclusion can be obtained by studies using selective antagonists, such as steroid antagonists which are currently under investigation in our laboratory.

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