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Mineralocorticoid Receptor Blockade During a Rat's First Violent Encounter Inhibits Its Subsequent Propensity for Violence

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In individuals naïve to serious conflict in an unfamiliar environment, violence has long-lasting effects on subsequent aggressive behavior. This effect of the stressful experience of a first violent conflict occurs in victims as well as offenders. The authors study in the male rat as offender the role of a rapid corticosterone signal mediated by brain mineralocorticoid receptors (MR) in adjusting the threshold of aggressive responses. For this purpose, the authors have applied electrical stimulation of the brain's aggression circuit via the hypothalamic attack area or HAA. Using this paradigm, they found that in inexperienced rats, retesting of the animals on subsequent days facilitated aggression. Hypothalamic attack thresholds decreased to about 50% of their initial level. However, blocking the MR once with the mineralocorticoid antagonist spironolactone, during the very first evoked attacks, permanently prevented attack facilitation in subsequent conflicts in that same environment. The MR-mediated effect blocked by the antagonist occurred within an hour following the start of the first aggression tests only. A later MR blockade was not effective. These findings suggest that the corticosterone stress response during a very first serious conflict initializes an enhanced propensity for violent aggression through the brain MR.

Keywords: facilitation hypothalamic aggression, first offender, rat, corticosterone, spironolactone

Starting a fight in an unfamiliar environment is a risky venture for an individual with no experience in handling violent conflicts. The individual may lose, or may incur serious injury, even when winning. Inexperienced individuals mostly fight if there is an immediate serious threat to survival or reproductive potential. However, to refrain from starting a fight has its downside too, because the outcome of a first fight in a new environment often determines the direction of subsequent aggression and future social relationships (Anderson, Buckley, & Carnagey, 2008; Chase, Tovey, Spangler-Martin, & Manfredonia, 2002; Drews, 1993; Hemelrijk, Wantia, & Isler, 2008; Hsu, Earley, & Wolf, 2006; Mazur, 1973, 1985; Savin-Williams, 1977). Therefore, "to fight or not to fight," is an important decision. Winning often increases the propensity for future aggression (Bondar, Boyarskikh, Kovalenko, Filipenko, & Kudryavtseva, 2009; de Boer, Caramaschi, Natarajan, & Koolhaas, 2009; Fish, DeBold, & Miczek, 2005), suggesting that aggression-control has been changed. But what precisely mediates such a change is only partly understood. Moreover, the balance between cost and benefit of the behavioral response is not the only important factor to consider in starting a fight. The condition of the internal resources required to cope with the strains of fighting is an equally important factor. The individual should be fit to fight.

The secretion of the adrenocortical hormones rapidly rises during exposure to the threat of a conflict or fight in an unfamiliar environment. These hormones are crucial in mobilizing the physiological resources and in coordinating the brain functions necessary to cope with the demands of fighting. They rapidly enter the brain, where they can modulate the aggression-control neuronal network that is already activated by the social factors signaling the threat of an impending fight. They do so by rapid nongenomic effects within the time frame of one single conflict. These effects are followed by slower genomic effects, which probably also act in the aftermath of conflicts to produce lasting changes in the setting of the aggression control network (Fish et al., 2005; Kruk, Halász, Meelis, & Haller, 2004; Mikics, Kruk, & Haller, 2004). Accord-

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ingly, corticosteroids seem crucial to the integration and coordination of the physiological and behavioral requirements of fighting (Summers et al., 2005).

In *experienced* animals, there is ample evidence for the important role of corticosteroid signaling in aggression. Both territorial and hypothalamic aggression are accompanied by a fast surge of corticosterone, which rapidly facilitates aggression (Kruk et al., 2004; Mikics et al., 2004). Corticosterone levels that rise in anticipation of aggression also are critical for escalated aggression in mice (Fish et al., 2005). Territorial aggression is more likely when circulating corticosterone levels are rising than when they are falling (Fish et al., 2005; Haller, Halász, Mikics, Kruk, & Makara, 2000; Haller, Millar, van de Schraaf, De Kloet, & Kruk, 2000). Accordingly, blocking corticosterone synthesis with metyrapone (Fish et al., 2005; Mikics et al., 2004) or blocking the mineralocorticoid receptors (MR) with spironolactone strongly inhibits territorial aggression (Haller, Millar, & Kruk, 1998; Haller, Millar, et al., 2000).

The goal of this study is therefore to examine the role of the brain MR in the effect of corticosterone during the first conflict. We focus on the MR because previous studies have demonstrated its role in appraisal processes and the onset of the stress response (de Kloet, Joëls, & Holsboer, 2005) likely through its nongenomic mode of action (Joëls, Karst, de Rijk, & de Kloet, 2008). For this purpose, hypothalamic-evoked aggression seems a useful paradigm to test the possible role of corticosteroids during a first conflict in an initially naive attacker, in a novel, unfamiliar environment. Hypothalamic aggressive responses are evoked from an area within the mediobasal or tuberal hypothalamus, depending on the species studied. Its anatomical distribution and connections have been extensively studied in rats and cats (Siegel, Roeling, Gregg, & Kruk, 1999; Toth, Fuzesi, Halász, Tulogdi, & Haller, 2010). These species-specific responses seem to be evolutionary well preserved since they have been demonstrated in, for example, fishes (Demski, 1973), lizards (Sugerman & Demski, 1978), domestic fowl (Putkonen, 1966), mice (Lin et al., 2011), rats (Kruk, van der Poel, & de Vos-Frerichs, 1979; Panksepp, 1971; Vergnes & Karli, 1970), guinea pigs (Martin, 1976), mini pigs (Ettrup, Sorensen, Rodell, Alstrup, & Bjarkam, 2012), cats (Hess, 1928; Romaniuk, 1965), opossums (Roberts, Steinberg, & Means, 1967), marmosets (Lipp & Hunsperger, 1978) rhesus monkeys (Herndon, Perachio, & McCoy, 1979), and even in humans (Bejjani et al., 2002). A comparison of the two most extensively studied species the cat and the rat can be found in (Siegel et al., 1999). Hypothalamic behavioral responses have been accepted as direct evidence for the existence of behaviorally specific mechanisms in the brain (von Holst & von Saint Paul, 1963), which may have been somewhat premature. Others have dismissed these responses as meaningless artifacts of the experimental conditions (Valenstein, Cox, & Kakolewski, 1969). However, even if the latter extreme viewpoint would be true, these responses could still provide a useful perspective on aggressive behavior in pathological conditions. It is interesting that hypothalamic attack in the rat is selectively inhibited by drugs frequently used to treat human aggression in clinical settings (Kruk, 1991). Hypothalamic functions in abnormal aggression have recently been reviewed (Haller, 2013).

In rats, hypothalamic attacks can be evoked in an initially unfamiliar environment where such violent aggression is normally absent (Kruk et al., 1979). Attacks are similar to the type of attacks in the "escalated" aggression version of resident-intruder conflict (de Boer et al., 2009) as they lack the introductory social interactions and threats normally observed in territorial aggression in experienced rats. Even though these responses are uncommon in the experimental settings in which they are usually studied, they constitute a regular element of the normal behavioral repertoire observed in territorial conflict. In territorial conflict, these short-lasting but violent actions could very well be decisive in determining future dominance relationships. The latter consideration suggests that it may be worthwhile to study how such responses become part of the agonistic repertoire in the first place.

Hypothalamic attacks are evoked within seconds after stimulation onset. Such unrestrained aggression represents a first experience with a novel type of conflict, in an unfamiliar environment. The stimulation paradigm is organized in such a way that the stimulated rat always wins. Thresholds for hypothalamic attack decrease by about 50% upon repeated testing on subsequent days. In experienced fighters, such decreased and ultimately stable hypothalamic attack thresholds have been extensively used to determine effects of drugs, brain lesions, and hormones (Kruk, 1991; Kruk et al., 2004, 1998) and to study underlying brain mechanisms (Halász et al., 2009; Halász, Liposits, Kruk, & Haller, 2002; Halász, Liposits, Meelis, Kruk, & Haller, 2002; Kruk et al., 1983; Lammers, Kruk, Meelis, & van der Poel, 1988; Roeling, Kruk, Schuurmans, & Veening, 1993; Toth et al., 2010).

In this article, we present results of four experiments on the early role of the MR in the initialization of a circuit underlying aggression facilitation following repeated exposure to violent conflict in an initially unfamiliar environment. The MR antagonist spironolactone, given just once, prior to the very first aggression test, permanently prevents the facilitation of hypothalamic attack upon repeated testing. These results suggest that the brain MR mediates a corticosterone signal enabling a lasting facilitation of subsequent aggression that apparently happens within the short-time domain of the very first conflict of inexperienced animals in an unfamiliar environment.

Method

General Experimental Approach

In the first experiment, the time course of the gradual but long-lasting facilitation of hypothalamic aggression caused by repeated testing was studied. In the second experiment, the early onset of that facilitation was assessed. In the third and fourth experiments, the effect of blocking the mineralocorticoid receptor (MR) with spironolactone on different time points during the facilitation of aggression was determined. Spironolactone was chosen because it inhibits resident–intruder aggression in a time-dependent way (Haller, Halász, et al., 2000; Haller, Millar, et al., 1998). Moreover, spironolactone also blocks the rapid, nongenomic effects in other paradigms for example, (Karst et al., 2005; Zhou et al., 2010).

Subjects

Male Wistar rats, weighing 350–400 g, were obtained from Charles River Laboratories (via Broekman, Veldhoven, the Netherlands). Rats recovered from transportation for 2 weeks. They

were fed standard laboratory food and given free access to tap water. Temperature was maintained at 22 ± 1 °C, and humidity was 60 ± 10%. Rats were housed in Macrolon Type IV cages in groups of 5 before electrode implantation and individually in Macrolon Type III cages thereafter. Opponents were male rats of 250–300 g from the same supplier and maintained under the same conditions. Opponents received an intraperitoneal injection of morphine (10 mg/kg) 20 min before encounters to produce profound sedation and analgesia during attacks. Opponents were used only once and killed immediately afterward with an overdose of Nembutal. The experiments were all performed in the active (dark) phase of both stimulated rats and their opponents. A 12:12-hr inverted day–night schedule was imposed on the rats, with lights on at 2000. All experiments were carried out in accordance with the European Council Directive of 24 November 1986 (86/609/EEC) and reviewed and approved by the Ethical Committee on Animal Experimentation of Leiden University, in accordance with Dutch laws on animal experimentation DEC # 04128.

Electrode Implantation

Electrode implantation and stimulation were performed as described in Kruk et al. (1979). In brief, male adult Wistar rats were deeply anesthetized by an intraperitoneal injection of a mixture of 0.5 mg/kg midazolam (Hoffman-la Roche), 1.0 mg/kg atropine, and 1 ml/kg Hypnorm (Janssen Pharmaceuticals). Teflon-isolated bipolar Pt-Ir electrodes (MedWire PtIr3T) were aimed at the hypothalamic attack area (HAA) at a point corresponding with the rostrocaudal 1.9, mediolateral 1.0, and dorsoventral 8.2 point with respect to bregma in Paxinos and Watson's stereotaxic atlas (1998). Electrodes and connectors were kept in place by dental carboxylate cement covered by acrylate dental cement and anchored to the skull by stainless steel screws. Rats recovered during 1 week from surgery. Because of individual variation not all electrodes reach the core of the HAA, resulting in position dependent variation in attack thresholds. In previous series of implantations, between 50% and 70% of all electrodes evoked aggression. Accordingly, the number of animals in which both left and right hypothalamic electrodes evoked aggression ranged between 25% and 50% (Kruk, 1991; Kruk et al., 1979, 1983).

Spironolactone Dosage

We dissolved 30 mg Spironolactone in 3 ml of 0.9% physiological saline, containing 870 mg of the biologically neutral solvent HGC (2-Hydroxypropyl-γ-cyclodextrin, RBI Nattick), to a final concentration of 10 mg/ml/kg, resulting in a molar ratio of spironolactone to HGC of 1:8. Spironolactone (Sigma) was injected subcutaneously in a dose of 10 mg/kg body weight.

Experiment 1

In 20 rats, two electrodes were implanted per rat, one electrode contralateral to the other. On Days 8, 10, and 12 after implantation an attack threshold was determined at one randomly chosen side, followed by a pause of 3 days. Subsequently, on Days 15, 17, and 19 an attack threshold was determined at the contralateral side in the same animals. If attack threshold decreases are due to some local adaptation to the passage of current, attack thresholds at the later tested contralateral side should decrease in the same way. If not, that hypothesis should be rejected. The time line of the procedure is illustrated in the upper panel Figure 1A and 1B.

Experiment 2

To assess how fast in initially inexperienced rats, attack thresholds decrease upon repeated testing, 50 rats were contralaterally implanted with two electrodes, following a similar design as in Experiment 1. However, in this experiment only one attack threshold was determined in the first tested side, whereas the threshold at the contralateral site was determined immediately afterward, in such a way that both thresholds in each rat were determined within 1 hr. See upper panel Figure 2.

Experiment 3

After electrode implantation 50 rats were randomly divided in two groups. One group received 10 mg/kg subcutaneous spironolactone 60 min before the first test, on Day 8 after implantation. The other group received vehicle (HGC). On Days 10 and 12, both groups received vehicle 60 min before their 2nd and 3rd threshold determinations. On Day 28, the drug treatment given on Day 8 was reversed. At that time point the "control" rats received spironolactone 60 min before the attack thresholds whereas the other group received vehicle. See upper panel Figure 3.

Experiment 4

To further explore the time domain in which the corticosterone surge accompanying aggression exerts its effect, spironolactone was given 15 rather than 60 min before the first aggression test in naive rats on Day 8 following implantation. Attack thresholds were determined immediately afterward. A second attack threshold was determined in the same electrodes on Day 10 following an injection of vehicle 15 min before the threshold. See upper panel Figure 4.

Behavioral Testing

Testing took place outside the home cage in a 100 cm high, cylindrical transparent Perspex cage, with diameter of 60 cm. A 4-cm thick circular mat of closed foam covered the floor to minimize injuries and damage. Subjects could move around unimpeded by the connecting wires. An opponent treated with a high dose of morphine was introduced into the cage directly before stimulation. Each opponent was used only once. To assess whether an electrode evoked attacks in a naive, untested rat, pulse trains of 60 s, alternating with 60-s pauses, with 40 Hz biphasic current pulses with phase duration of 0.2 ms and a phase interval of 12.3 ms were used. Current intensity was increased from zero in 25 μAmpere steps until aggressive behavior was first evoked. In the absence of attacks, stimulation stopped when upper limit of 250 μAmpere (μA) was reached or responses incompatible with aggression were obtained. Immediately after the induction of aggression at a site, the first attack threshold was determined at that site while the animal and opponent remained in the same cage.

Threshold Determinations

Threshold determinations started at a current intensity just below the intensity at which the first attack was evoked. Pulse

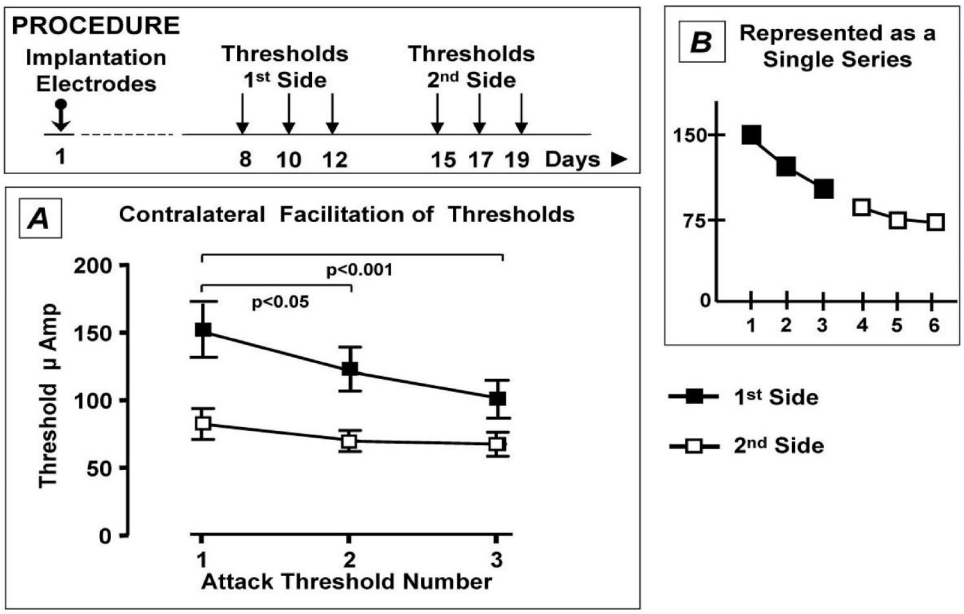

Figure 1. A: Contralateral facilitation of subsequent hypothalamic attack thresholds in 20 rats having an attack-evoking electrode in both the ipsilateral and the contralateral hypothalamus. Contralateral side (open squares, 2nd side) tested 3 days after the last threshold determination in the ipsilateral side in the same animal (filled squares, 1st side). Upper panel shows time-line of procedure. Thresholds are shown as mean ± SEM. B: Same data as in 1A depicted as one single series disregarding that the 2nd series of 3 thresholds derive from the activation of the contralateral HAA in the same animal. Lines connecting squares connect repeated threshold measurements in the same group of ipsilateral or contralateral electrodes.

frequency and duration remained unchanged, but stimulation trains of 10 s separated by 50-s pauses were used. Current intensity was semiautomatically preset according to an up-and-down procedure (Wetherill, 1966); that is, if an attack was observed within the 10 s of a stimulation train, the intensity of the next train was lowered by a fixed step of 10 μA (or 20 μA if the first attack was elicited above 120 μA). If no attack occurred, stimulation intensity of the next train was increased by the same fixed step. The threshold procedure was stopped after six so-called "response changes." A response change was scored if a train with an intensity that did evoke an attack was followed by a train with an (lower) intensity that failed to evoke an attack, or if an intensity that failed to evoke an attack was followed by an (higher) intensity that did evoke an attack. From the current intensities at which six response changes occurred, the threshold for attack was calculated (Wetherill, 1966). Such a threshold is an estimate of the theoretical current intensity at which 50% of the trains would evoke an attack. The threshold obtained from one particular electrode depends in part on its precise position within the HAA (Kruk et al., 1983). The entire procedure from the first test for aggression to the end of the first attack threshold lasted less than 30 min on average.

Statistical Analysis

Thresholds have the dimension of micro Ampere (μA) and are presented in figures as mean and standard error. Data were analyzed with GraphPad Prism version 4.0. Normality of results was tested using the d'Agostino & Pearson Omnibus Test. Data from two experiments (Figures 1 and 3) did not deviate from normality ($p > .3$, in all cases) and were analyzed using a one-way analysis of variance (ANOVA) for repeated measurements, followed by Tukey's post hoc multiple comparison test and a test for linear regression over time points. Raw data from one experiment (Figures 2) failed to pass the normality test ($p < .02$, in both samples) but did pass the test on log-transformed threshold values. Hence, Experiment 2 was analyzed by a paired t test on the transformed values. Sample size in the last experiment (Figure 3) was too small to assess normality, therefore it was analyzed with Wilcoxon's matched pairs test. The difference in the number of electrodes successfully evoking aggression in spironolactone-treated and vehicle-treated animals was tested with Fisher's exact probability test.

Results

Experiment 1

Figure 1 shows that the effect of attack-threshold decreases on repeated stimulation obtained at one site in an animal already facilitate the attack threshold at the contralateral site in the same animal. Hence, the facilitation is probably not caused by a local change in the tissue around the first electrode tip. In rats with one electrode in the HAA (closed squares) and another in the contralateral HAA, (open squares) the thresholds at the first tested side show the expected threshold decrease upon repeated testing ($n = 8$), $F(2, 14) = 12.09$, $p = .0009$, with a linear trend over subsequent thresholds (slope $= -26.0$; $R^2 = .165$; $p = .0002$). The first

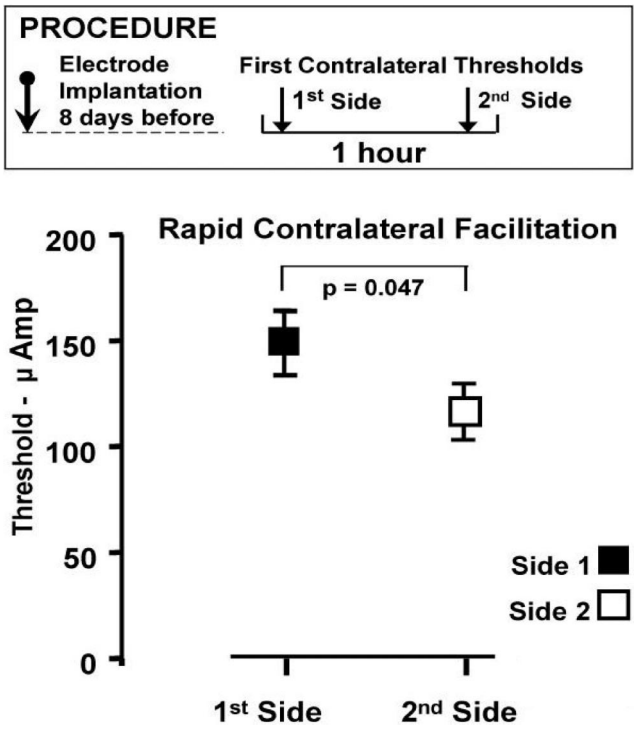

Figure 2. Rapid contralateral decrease of subsequent hypothalamic attack thresholds in 28 rats that had an attack evoking electrode in both the ipsilateral and the contralateral hypothalamus. Contralateral side (open squares) tested within 1 hr after the last threshold determination in the ipsilateral side in the same animal (filled squares). Thresholds shown as mean ± SEM.

threshold being higher than the second and third ($p < .05$ and $p < .001$, Tukey's post hoc). When the contralateral side is tested 3 days later than the ipsilateral thresholds, the thresholds at the contralateral side (open symbols) are already low and decrease only marginally ($n = 8$), $F(2, 14) = 2.73$, $p = .099$ (slope = −7.4; $R^2 = .05$; $p = .48$). In Figure 1B, the same data are plotted against the rank order of testing in time, disregarding that the last three thresholds derive from a contralateral electrode in these animals. The series of average attack thresholds leveling out at about 50% of the initial value suggests that the progressive facilitation of attack thresholds ($n = 8$), $F(5, 35) = 5.482$, $p = .0008$ (slope = −8.561; $R^2 = .36$; $p < .0001$) is a characteristic of the animal rather than a change of the tissue around the electrode tip.

Experiment 2

Figure 2 shows that the average threshold at the second tested (contralateral) side is already 23% lower than the average attack thresholds at the first tested side when the first thresholds from both ipsilateral and contralateral electrodes are determined after an hour (see Figure 2) $t(27) = 2.084$, $p = .047$.

Experiment 3

Figure 3 shows that blocking the mineralocorticoid receptor (MR) by a subcutaneous injection of 10 mg/kg spironolactone 60 min before the first attack test prevents the subsequent gradual decrease of attack thresholds that is observed in control animals receiving vehicle. Attack thresholds of animals that received spironolactone before any aggression testing remained unchanged and high for the duration of the experiment ($n = 14$), $F(3, 39) = 0.594$, $p = .62$. Attack thresholds of the control animals that received vehicle prior to aggression testing decreased on repeated testing ($n = 21$), $F(3, 60) = 8.26$, $p < .0001$. When spironolactone was finally, after 21 days, given to the control group that received only vehicle before, it did not increase attack thresholds. Figure 5 shows that in the group receiving vehicle before the first test, 21 electrodes out of 26 electrodes evoked aggression, whereas only 10 out of 24 electrodes in the group receiving spironolactone evoked aggression (Fisher's exact probability $p = .008$). These results suggest that an MR-mediated corticosteroid signal during the first aggressive conflict in naive animals is required for the subsequent long-lasting facilitation of aggression.

Experiment 4

Figure 4 shows that spironolactone injected 15 min before the first attack test did not prevent a decrease of the second attack thresholds determined 2 days later in the same sites. (Wilcoxon signed-ranks test, matched pairs $n = 6$, $p = .031$). Apparently, the 15-min period between injection of the antagonist and testing is too short to block the corticosteroid mediated effect on aggression. These findings suggest that the MR-mediated corticosteroid surge

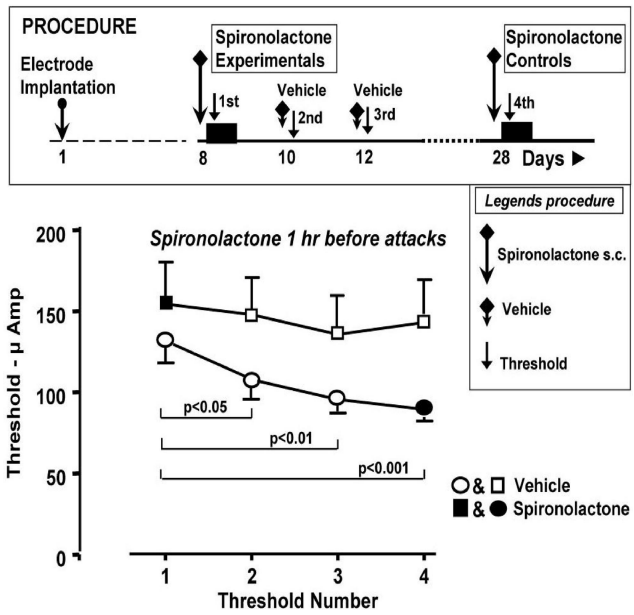

Figure 3. Absence of subsequent attack threshold decreases caused by mineralocorticoid receptor blockade during a first conflict in 14 animals inexperienced with hypothalamic conflict (squares), contrasted with the effects of a similar blockade after 3 weeks in 21 - initially equally inexperienced - animals in which thresholds already have been decreasing on repeated testing (circles). Filled symbols: thresholds determined under MR blockade, open symbols: thresholds determined under vehicle condition. Injections were given 1 hr before the threshold determination. Thresholds shown as mean ± SEM. Upper panel: timeline procedures.

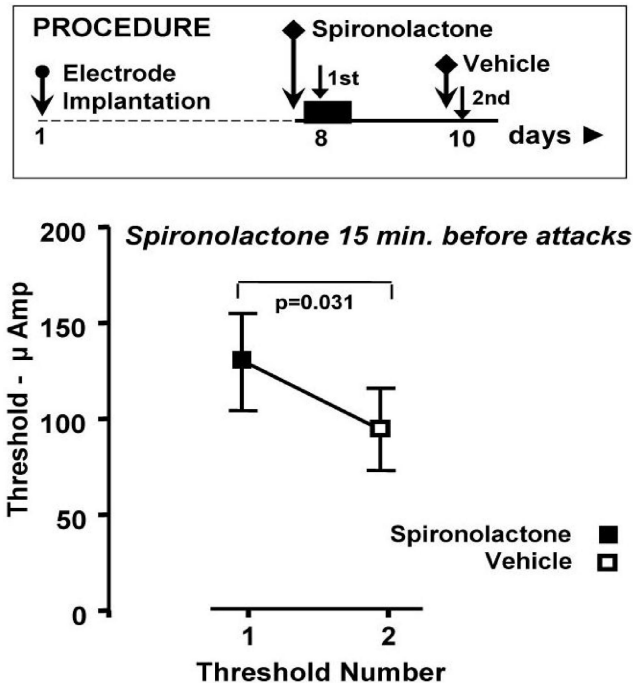

Figure 4. First and second hypothalamic attack threshold determined at a 2 day's interval in 6 inexperienced animals that received spironolactone 15 min before the first threshold determination. Thresholds shown as mean ± SEM. Upper panel: timeline procedures.

that initializes the lasting facilitation of attack acts during the early stages of the novel conflict.

Discussion

A first conflict in an unfamiliar setting is a stressful event. First offenders cannot be completely certain of victory, and the behavioral and metabolic costs of fighting are high. Priming effects of aggressive conflicts with longer lasting consequences for the offender have been observed in hamsters, rats, and humans (Hebert, Potegal, & Meyerhoff, 1994; Potegal, 1992; Potegal, Robison, Anderson, Jordan, & Shapiro, 2007). The data here presented suggest that an MR-mediated signal of corticosterone during a first conflict probably initializes a neuronal circuit enabling a long-lasting facilitation of aggression in subsequent conflicts (see Figures 3 and 5).

Time Window of the Effect

The period during which this MR-mediated signal is able to initiate such a long-lasting facilitation seems limited. Spironolactone has a low affinity for the MR, and it is rapidly cleared from the body. Its actions are largely due to its metabolite canrenone that reaches peak plasma concentrations in an hour after injection (Tokumura, Muraoka, Masutomi, & Machida, 2005). Therefore MR blockade is at its maximum during the first hypothalamic attack session in inexperienced rats. Spironolactone and its active metabolite canrenone, have half-lives of 43, respectively 130 min in the rat (Kaukonen, Lennernas, & Mannermaa, 1998). Two days after the first test, during the second test these blockers are completely cleared from the body. Because the MR is no longer blocked, the corticosterone surge accompanying hypothalamic attack can bind and activate to free MR. Yet in animals that have received spironolactone before their first aggression test, attack thresholds on Day 4 or even on Day 20 after the MR-blockade failed to decrease. Their attack thresholds remained at their initial high level. Thus, blocking the MR during the first conflict prevents facilitation of aggressive behavior in subsequent conflicts. Attack thresholds in the group that received vehicle before the first aggression test did decrease steadily over three subsequent tests, the largest threshold decrease being between the 1st and 2nd test (see Figure 3). Spironolactone given to vehicle-treated animals 1 hr before the fourth test, 20 days after the first test, did not increase their attack thresholds, suggesting that the first conflict initialized an irreversible progressive facilitation of aggression. Spironolactone given 15 min before the first aggression test fails to affect the second attack threshold from the same electrode 2 days after the first (see Figure 4). In this case, canrenone, the active metabolite of spironolactone, may have reached the MR only after the corticosterone surge accompanying hypothalamic attack. Therefore, it may have failed to block it in time. Together these results suggest that the MR-mediated facilitation of aggression is restricted to a short time window during the first conflict.

Both Stress Response and Experience Required

In the absence of an opponent, HAA stimulation does not evoke any visible sign of aggression, but it does induce a rapid corticosteroid response (Kruk et al., 2004; Kruk et al., 1998). However, rats repeatedly stimulated in the HAA prior to any aggression testing, show the same initial attack threshold decrease when they are subsequently repeatedly tested (Kruk et al., 1979). To initialize a long- lasting facilitation of aggressive behavior, it is apparently

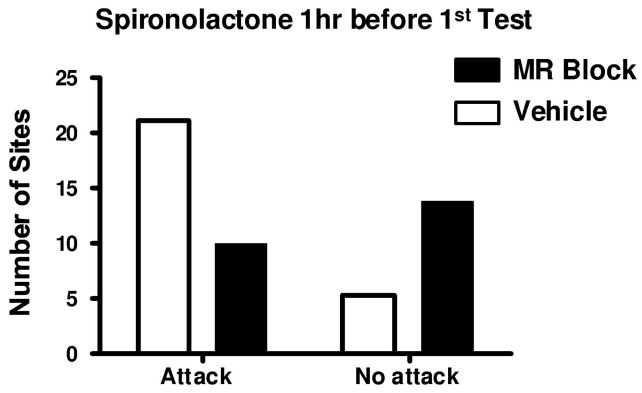

Figure 5. Decrease in the number of electrode sites that successfully induced attack after mineralocorticoid receptor blockade during a first conflict, in animals inexperienced with hypothalamic conflict as compared with animals receiving vehicle. Data from Experiment 3 (see also Figure 3). Open, white bars: tested under vehicle conditions. Filled, black bars: tested under MR blockade. The two bars to the left represent number of sites eliciting attacks. The two bars to the right represent number of sites failing to induce attacks. Data from Experiment 3. (Fisher's exact probability $p = .008$). Failures remain failures upon subsequent retesting (data not shown).

necessary that the first aggressive responses are expressed during the accompanying corticosterone surge. Aggression can also be induced by injection of glutamate agonists, GABA antagonists, or both into the hypothalamus (Adams et al., 1993; Roeling et al., 1994). It is interesting that induction fails in inexperienced animals (Haller, Abraham, et al., 1998).

Initiating a Two-Stage Process

The facilitation of hypothalamic aggression in the absence of MR-blockade cannot be reversed by spironolactone when it is given after the initial aggressive encounter has occurred. Its persistence suggests a progressive, long-lasting adaptation in the aggression controlling network. Such an adaptation probably requires an epigenetic process altering genomic activity. The facilitating effects of corticosterone in resident–intruder conflict also seem to occur in two successive stages. In the first 20 min, the facilitation is not sensitive to protein synthesis inhibition, but after that period it is (Mikics et al., 2004). Such a two-stage process may reflect a functional sequence of an immediate adaptation to the acute requirements of a novel conflict, followed by consolidation of the experience in memory in preparation for similar future conflicts.

Appraisal of a Novel Challenge

In agreement with the idea of such a functional two stage process, it has been proposed that nongenomic MRs are involved in the immediate appraisal of novel situations and in the selection of appropriate behavioral responses to a challenge (de Kloet, Karst, & Joëls, 2008; Joëls & Baram, 2009; Joëls et al., 2008). Moreover, MR mediate reactivity to spatial novelty (Oitzl, Fluttert, & de Kloet, 1994), a crucial factor in the expression of aggression in an unfamiliar environment. It is interesting to note that spironolactone administered prior to training in a fear-conditioning paradigm (Zhou et al., 2010) impaired contextual memory when tested 3 hr and 24 hr later. Similar effects were found in forebrain-specific MR knockout mice (Harris, Holmes, de Kloet, Chapman, & Seckl, 2013; Zhou et al., 2010). Furthermore, a glucocorticoid receptor (GR) antagonist only affected consolidation of the fearful experience in the memory. In mouse mutants, with engineered differential MR:GR expressions in the forebrain, the interaction between two receptor types indeed was found to control the different phases in information processing and behavioral performance. (Harris et al., 2013). In this study, hypothalamic attack is first induced in an initially novel, unfamiliar environment, where rats normally don't fight. It seems likely, therefore, that the MR-mediated function, activated by the initial corticosterone surge, operates in the appraisal and encoding of a novel conflict, whereas GR-mediated corticosteroid driven genomic events underlie the previously mentioned second stage of consolidation of the experience determined by the outcome of the conflict (Mikics et al., 2004).

Acting Outside the Hypothalamus?

Where this rapid MR effect takes place is not known. *Classical intracellular genomic* MR are rare in the HAA. It is interesting that a recent electronmicroscopic study showed MR at postsynaptic membrane densities of excitatory synapses in mammalian brain (Prager, Brielmaier, Bergstrom, McGuire, & Johnson, 2010). Whether the recently discovered membrane-bound, nongenomic MR are present in the HAA is not known. However, MR-mediated inhibition of facilitation of hypothalamic attack by spironolactone does not necessarily originate within the HAA itself. This facilitation rapidly generalizes from the first tested hypothalamic site in an animal to the second tested, contralateral site (see Figures 1 and 2). Deoxyglucose studies (Roberts & Nagel, 1996) and C-Fos studies have shown that the contralateral site is activated during stimulation of the ipsilateral side, together with many other areas in the brain (Halász, Liposits, Meelis, et al., 2002). Also, the HAA has reciprocal anatomical connections to other brain areas involved in the appraisal of conflict areas where MR are present, such as the frontal cortex and amygdala (Halász, Toth, Kallo, Liposits, & Haller, 2006; Roeling et al., 1993; Toth et al., 2010). These findings clearly suggest that the facilitation of attacks may be mediated by a process occurring elsewhere in the brain.

Perspective on a Molecular Mechanism

The molecular mechanism underlying the rapid initiation of threshold decreases is not clear, but the new data are promising. The affinity of the corticosteroid-preferring nuclear MR is such that occupancy and activation of the MR would already be complete under the conditions used in these experiments (Reul, van den Bosch, & de Kloet, 1987). It is, therefore, not likely to initiate an attack threshold facilitation. The lower affinity membrane variant of the MR mediates the rapid corticosterone-induced enhancement of synaptic transmission by increasing the frequency of miniature excitatory postsynaptic currents (mEPSCs) in the hippocampus (Karst et al., 2005; Olijslagers et al., 2008; Wiegert, Joëls, & Krugers, 2006) and amygdala (Karst, Berger, Erdmann, Schutz, & Joëls, 2010). In the amygdala, the MR-mediated enhancement of synaptic transmission is long lasting, providing an extended window of metaplasticity for encoding of emotional experiences depending on stress history (Karst et al., 2010). In this study, a similar MR-mediated increased ability to encode new information could be involved. These observations illustrate the possible molecular and cellular mechanism underling the rapid and long-lasting corticosterone effect mediated by MR and GR on the threshold of hypothalamic attack.

Appraisal and Coping by the Offender

Violent attack, as evoked by HAA stimulation or induced by repeated winning experiences in other paradigms (Bondar et al., 2009; de Boer et al., 2009; Fish et al., 2005), is the most injurious element out of the generally much more moderate behavioral repertoire expressed during conflicts. Rapid initialization of a long-lasting facilitation in naive individuals is therefore a matter of concern. Only a few drugs are able to suppress such attacks once thresholds have stabilized at a low level (Kruk, 1991). The MR-mediated effect reported here seems to initialize a rather entrenched behavioral routine. Attacking can be viewed as a serious behavioral coping response to a serious social challenge. The concept that nongenomic rapidly acting MR control an initial stress reaction that is important for appraisal and coping processes (Joëls et al., 2008) also seems to apply to the results presented

here. There is ample evidence of the adaptive as well as the detrimental effects of the adrenocortical stress response in victims of aggression. Here we show for the first time evidence that a very early adrenocortical stress response operating through the brain MR has a role in the winner of a conflict.

Initializing a Violent Routine

In an unfamiliar environment this allegedly "hardwired" but clearly modifiable aggressive brain circuit activated through the hypothalamus, apparently needs to be initialized by a MR-mediated signal. Steroid hormones are necessary as *organizing* and *activating* factors early in life and around puberty, to develop the condition required for adult aggression. However, that condition in itself may not be sufficient to develop a stable propensity for aggressive responding in a particular environment. We suggest that during a first conflict in an unfamiliar environment an MR-mediated adrenocortical signal is required as *initializing* factor to enable the facilitation of stable aggressive responses in that environment in subsequent conflicts, possibly through epigenetic modulation of gene expression (Tremblay, 2010; Tremblay & Szyf, 2010). The hypothesis that initial temporary associations between hormonal and behavioral events may have long-lasting consequences for future brain function may be worth testing for other hormones or behavioral phenomena.

Conclusion

In conclusion, our observations show that the strategic decision, which determines the future threshold for attack in an initially unfamiliar environment, requires the early and simultaneous reading of both an external as well as an internal signal. It requires a conflict with an opponent in an unfamiliar environment, as well as an early MR-mediated corticosteroid signal during that first conflict. Together these signals define the nature of the social conflict and the readiness of the available metabolic and physiological resources to cope with that conflict. It is not known whether a similar rapid MR-mediated process also has a role in humans who win a first stressful conflict. This article suggests that studying the effects of corticosteroids in humans engaged in a first stressful conflict might be a worthwhile project.

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