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Immunity and emotions: Lipopolysaccharide increases defensive behaviours and potentiates despair in mice

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Abstract

Many studies have pointed out the relationships between immunity and depression, supporting a neuroimmune hypothesis of depressive disorders. However, despite the growing interest for such a hypothesis and the amount of clinical and experimental data available, the precise nature of this relationship between immunity and depression remains unclear. The present study aimed to investigate further the link between depression and immunity in mice using the modified version of the forced-swimming test. Based on a two-session test, results from our first experiment showed that endotoxin enhanced active defensive behaviours in mice during the first exposure to water, but was associated with increased immobility (i.e., 'behavioural despair') in the subsequent session. In our second experiment, we showed that these effects were blocked by a chronic antidepressant treatment with imipramine. Finally, we suggest a link between immunity and depression, based on the behavioural context in which immune activation takes place. We hypothesize that immune activation, by enhancing reactivity to the negative features of a given situation, increases defensive motivation of subjects, but therefore makes them more vulnerable to the deleterious emotional consequences of failure in defensive strategies.

Keywords: Immunity; Behaviour; Forced-swimming test; Depression; Emotion; Mice; Lipopolysaccharide; Imipramine

1. Introduction

Several lines of evidence indicate a relationship between stress, depression, and cytokines, and thus a possible immunologic influence on emotions (Dantzer, 2001; Miller et al., 2005). In humans, a positive correlation has been observed between the development of an infectious episode and a transient depressive state (Brown et al., 1992; Greenwood, 1987; Hendler, 1987; Meijer et al., 1988). A chronic activation of the cytokines network (e.g., multiple sclerosis and rheumatoid arthritis) has also been correlated with a propensity to develop a depressed mood (Marshal, 1993; Minden and Schiffer, 1990; Parker et al., 1992). Finally, a correlation has also been shown in women between the increased liberation of cytokines occurring at childbirth and a post-partum depressive mood (Parry, 1995). These results form the basis of the hypothesis of a dysthymical symptomatology (i.e., mood disorders characterized by chronic depression, but with less severity than a major depression) resulting from an overexpression of cytokines (Hall and Smith, 1996; Malek-Ahmadi, 1996; Yirmiya, 1996).

Further studies have shown that the administration of cytokines such as interleukin 2 (IL-2) or interferon (IFN) induces depressive symptoms in cancer patients (Healy et al., 1991; Nemeroff et al., 1990; Sluzewska et al., 1994, 1995, 1996). Furthermore, it has been found that patients suffering from major depression show an increased level of central cytokines (Levine et al., 1999; Maes, 1995; Maes et al., 1993). Other studies have reported different abnormalities concerning the immune function in depressed patients, but with various results: some describe increased immune activation in depressed patients while others found a decrease in immune function (see review by Kronfol, 2002). The hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis observed in

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response to cytokines (Besedovsky et al., 1991) is reminiscent of the activation of this axis during some depressive states (Linkowski et al., 1985; Maes, 1995; Maes et al., 1993).

Experimentally, administration of bacterial endotoxin (lipopolysaccharide, a potent cytokines inducer) has been argued to induce anhedonic symptoms in rats (i.e., decrease in preference for sucrose; Yirmiya, 1996) which are blocked by chronic treatment with imipramine, a tricyclic antidepressant.

This neuro-immune hypothesis of depression has raised considerable interest, with many neuro-pharmacological studies attempting to understand better and give further support to the relationship between immunity and depression (Dunn et al., 2005). It has been shown for instance that exposure to various stressors induces a central production of cytokines (Dunn, 2001; Nguyen et al., 1998), and that this higher level of immune mediators may have a negative effect on the serotonin (5-HT) level in the brain which could induce depressive-like symptoms (Capuron and Dantzer, 2003).

Although a relationship between immunity and depression has been clearly established, the nature of such a link is far from being fully understood and no direct causal relationship between cytokines and depression has yet been demonstrated.

Besides this convincing set of data, it has been shown that cytokine-induced behavioural changes (i.e., sleepiness, lethargy, hypophagia, and social disinterest) could be considered as a part of an adaptive strategy developed by the organism to fight against pathogens (Hart, 1988). Sickness has therefore been argued to constitute a specific condition of behavioural reorganization that aims to reinforce the efficiency of immune and physiological responses to infection. Further studies support this theoretical proposal as they have shown that cytokine-induced sickness could trigger a specific motivational state defining new priorities (e.g., rest and self-recovery) to enhance healing processes (Aubert, 1999a,b). The behavioural expression of sick animals remains flexible and adaptable to environmental changes, a feature excluded from depressive-like conditions (Aubert, 1999a,b; Aubert et al., 1997). Moreover, a recent study by Aubert and Dantzer (2005) has shown that endotoxin-treated rats fully express hedonic facial responses to an appetitive solution of sucrose, contradicting an anhedonic interpretation of the reduced sugar intake in sick animals. However, endotoxin-treated rats were not exempt from changes in affective facial expressions as they displayed more aversive and less hedonic responses when tasting a mixed bitter-sweet solution. Such modifications were referred to as alliesthesia (i.e., a given stimulus arouses either pleasure or displeasure according to the internal state of the stimulated subject; Cabanac, 1971). It has been argued that a negative alliesthesia could account for endotoxin-induced changes (Aubert and Dantzer, 2005). One could speculate a generalization of such an effect to the affective state of treated subjects and hypothesize that cytokines could increase emotional reactivity to negative features of a stimulus or situation.

One of the most common experimental tools used to assess depressive-like effects in laboratory rodents is the forced-swimming test (FST), originally developed by Porsolt et al. (1977). In this test, rodents forced to swim in a narrow inescapable tank of water will develop a characteristic immobility that has been argued to represent "behavioural despair." This effect is reversed by a wide range of clinically active antidepressant drugs. Surprisingly, there are few data in the literature on the effects of cytokines or immune stimulation using the FST (Dunn et al., 2005) and many studies have shown contradictory results. For example, data obtained after administration of IL-1 reported an increase in depressive-like behaviour in one case, and a decrease in another (see review by Dunn et al., 2005). A recent study by Deak et al. (2005) investigated the behavioural consequences in the FST of a lipopolysaccharide (LPS) treatment administered prior to the first exposure and found no differences with saline-treated rats in immobility, climbing or swimming durations. These results contradict previous findings by Makino et al. (1998) who found increased immobility during FST with mice treated with human interferon, but using only a single exposure to water. Such discrepancies, both in results and methodology, stress the need for further investigations. The aim of the present study was therefore to further explore the link between depression and immunity using a modified version of the FST for mice (Lucki, 1997), since its simplicity and reliability make it a valuable tool to investigate such effects in rodents. Such a procedure focuses not only on immobility, but also on active defensive behaviours, such as climbing and swimming.

Based on a two-session test, the results of our first experiment show that endotoxin enhanced active defence during the first exposure to water, but was associated with increased immobility in the subsequent session. In our second experiment, we show that these effects were blocked by a sub-chronic antidepressant treatment with imipramine. Finally, we propose a link between immunity and depression, based on the behavioural context in which immune activation takes place.

2. Methods

2.1. Subjects

Eighty male CD1-Swiss mice (Harlan, France) were used in this study. Mice were 60–80 days old and weighted 38–45 g at the start of experiments. Animals were housed in groups of five in standard transparent plastic cages, in a temperature-controlled room maintained at 24 ± 2 °C and displaying a reversed 08:00–20:00 light/dark schedule. Mice were kept on an ad libitum regimen for food and water throughout the experiments.

2.2. Treatments

Depending on the experimental design, mice were submitted to various pharmacological treatments consisting in (i) physiological saline (0.9% apyretic NaCl), (ii) lipopolysaccharide (200 μg/kg; from *Escherichia coli*, serotype 0127:B8; Sigma, St. Louis, MO, USA), and (iii) imipramine hydrochloride (20 mg/kg; Sigma, St. Louis, MO, USA).

We selected a 200 μg/kg dose of LPS for its ability to reliably induce sickness in rats (Kent et al., 1992) and since it corresponds to the dose we use in our behavioural studies (Aubert, 1999a,b; Aubert and Dantzer, 2005). We used a dose of 20 mg/kg of imipramine since doses ranging between 10 and 30 mg/kg are quite common doses in psychopharmacological studies, and have been shown to reliability induce antidepressant effects in various depression models as forced-swim test (e.g., Subarnas et al., 1993), learned helplessness (e.g., Chourbaji et al., 2005) or chronic-mild stress (e.g., Azpiroz et al., 1999).

All injections were administered intraperitoneally. LPS was administered 90 min prior the first test session. Imipramine was administered throughout a chronic program consisting in a daily injection over a 14-day period and finishing one day before the first exposition to the experimental apparatus allowing a 24-h washout period. Control treatments followed the same experimental design, i.e., a daily physiological saline injection over a 14-day period followed by a 24-h washout.

The effects of LPS were evaluated not only by the general appearance and posture of treated mice (i.e., piloerection, curled-up posture, and eyes closed), but also by the measure of body weight and rectal temperature. Indeed, a decrease in rectal temperature and body weight were expected to assert LPS-induced inflammation in subjects. Temperature was collected using an adapted probe (RET-2) connected to a digital thermometer accurate to 0.1 °C (Physitemp, Thermalert). Three mice did not reach these criteria and were rejected from analysis.

2.3. Forced-swimming test

Mouse behavioural despair test was conducted as previously described by Porsolt et al. (1978) with some modifications. These modifications include a more extensive set of behavioural variables compared to the original procedure (see below for a full description), and a deeper and larger cylinder to facilitate the behavioural expression of subjects, and consequently behavioural analysis. Finally, data were collected from the full test sessions.

The apparatus consisted in a cylindrical transparent plastic tank (26 cm height × 16 cm diameter) filled with 24 ± 1 °C water. The recipient was filled at a depth of 20 cm so that mice could not support themselves by touching the bottom with their rear paws. On the first day, mice were gently placed in the water for a 10-min period (pre-test phase). Twenty-four hours later, animals were exposed to the same conditions as described above but for only a 5-min period (test phase). At the end of each swim session, mice were removed from the water, partially dried with a towel, and placed in a heated cage for 10 min before returning to their home cage. A VHS-C camera (SONY) recorded the whole tests sessions while the experimenter observed the session in an adjacent room.

2.4. Behavioural scoring

Behaviour was scored by an individual who was blind to the treatments. Behavioural data consisted in calculating total duration of 'Immobility' (i.e., mouse displayed only those movements required to keep its head above the water), as it is described in the original procedure (Porsolt et al., 1977, 1978). However, since this method fails to fully account for the behavioural processes in progress, this study included the measure of the duration of active behaviours (Detke et al., 1995; Lucki, 1997): 'Swimming' (i.e., the mouse displayed coordinated movements with its four paws and move around the cylinder in a horizontal or near-horizontal position) and 'Climbing' (i.e., the mouse produced vigorous movements with its forepaws in contact with the walls). Moreover, this ethogram was completed by the measures of the frequencies of 'Diving' (i.e., the mouse submerge its entire body and swim toward the bottom of the apparatus) (Pare, 1989) and 'Head-Shaking' (i.e., the mouse vigorously shake its head laterally) (Pare, 1989).

2.5. General procedure

The present study reports the data of two experiments.

In a first experiment, the effects of LPS on defensive behaviour were examined. The experiment was composed of two tests. In the first test, subjects were submitted to the forced-swimming procedure as described above 90 min after a saline ($n = 9$) or LPS ($n = 10$) treatment. The delay was chosen to allow the full onset of inflammatory processes in mice. In the second test, the delayed effects of a previous inflammatory episode on defensive behaviour in mice were evaluated. The same mice used in the first test were submitted to the same FST procedure after a 24-h delay. The absence of actual effects of LPS at the time of testing was assessed through the absence of changes in rectal temperature and body weight compared to control animals.

The second experiment was designed to check the influence of an antidepressant treatment on LPS-effects. Four experimental groups were compared, all of them following the same forced-swimming procedure as describe above. Two groups received LPS 90 min before the first test session, after a chronic treatment to imipramine ($n = 11$) or saline ($n = 9$). The two others received saline prior the first exposition, after a chronic treatment to imipramine ($n = 10$) or saline ($n = 10$).

2.6. Statistics

Data were analyzed using non-parametric procedures, that are specially adapted to the statistical analysis of small samples ($n < 30$). These statistical procedures have two main advantages relative to their parametric counterparts: (1) they do not depend on population's assumptions and thus do not imply any risk of violation of specific prerequisites (Siegel and Castellan, 1988), and (2) they have better relative power-efficiency for small samples (Bridge and Sawilowsky, 1999). Independent two-group comparisons were analyzed with the Mann–Whitney U test. When more than two groups were concerned, a first global analysis was done following the Kruskal–Wallis 'ANOVA-on-ranks' procedure, followed when justified (i.e., $p < .05$) by Dunn–Sidak post hoc analyses (Siegel and Castellan, 1988) including the correction for multiple comparisons.

3. Results

On the overall, diving behaviour was never observed throughout the entire study, and therefore is not commented any further.

3.1. Experience 1: Effects of LPS on forced-swimming induced despair in mice

3.1.1. First test session

At the beginning of this first test session, all LPS-treated mice considered for further analyses displayed clinical signs of inflammation (i.e., lethargy, closed eyes, and curled posture) as well as a drop in rectal temperature and body weight. As mentioned earlier, three mice did not reach these criteria and were rejected from analysis.

During the first exposition to the test, LPS-treated mice displayed less immobility behaviour than saline's ($U = 129.5$; $p < .001$) and were therefore globally more active than controls (cf. Fig. 1). Regarding active defensive behaviours, LPS-treated mice expressed less swimming behaviour ($U = 125$; $p < .005$) but more climbing ($U = 45$; $p < .001$) relative to control mice (cf. Fig. 1). Finally, the frequency of head-shakings displayed by LPS-treated mice was not significantly different from those displayed by saline (33 ± 7 vs. 30.5 ± 4 as median ± semi-interquartile range, respectively, for saline- and LPS-treated animals; $U = 84$; $p = .653$).

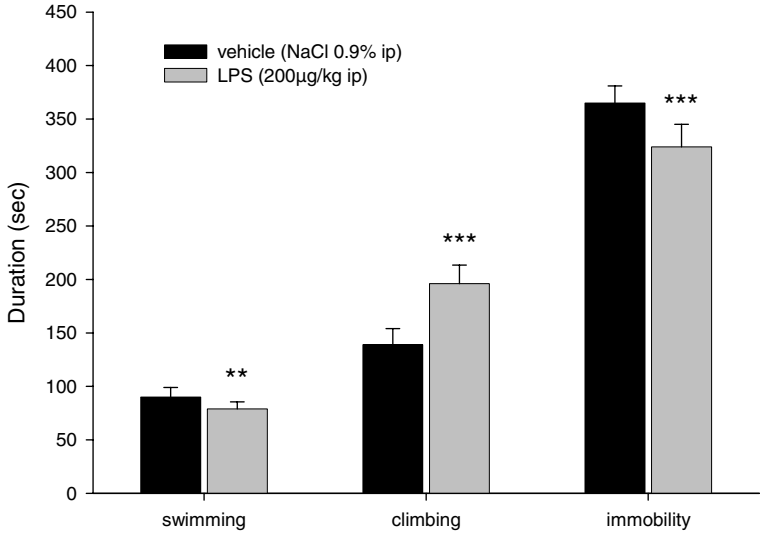

Fig. 1. Median ± SIQR (semi-interquartile range) duration of swimming, climbing, and immobility of mice during the first session of the forced-swim test. Black columns: control subjects (0.9% NaCl, ip); grey columns: LPS-treated mice (200 µg/kg LPS, i.p.). $^{**}p < .01$; $^{***}p < .001$.

3.1.2. Second test session

During this phase (i.e., 24 h after treatments), mice from the 'LPS group' did not show any signs of inflammation as defined earlier.

As shown in Fig. 2, previously LPS-treated subjects were more passive than controls ($U = 58$; $p < .01$ for the duration of immobility). They expressed significantly less climbing behaviour compared to controls ($U = 120$; $p < .016$) and a marginal tendency to express less swimming ($U = 113$; $p < .066$) (cf. Fig. 2). Finally, head-shaking frequency was also lower in these experimental animals relative to controls (23 ± 3 vs. 12 ± 1, respectively, for controls and previously LPS-treated animals; $U = 131$; $p < .001$).

3.2. Experience 2: Effects of a chronic antidepressant treatment on LPS enhancing effects of forced-swimming induced despair in mice

3.2.1. First test session

As explained for Experiment 1, all LPS-treated mice used for analyses expressed the whole set of inflammatory signs (i.e., lethargy, closed eyes, curled posture, decrease in rectal temperature, and body weight).

Fig. 3 summarizes results for durations of swimming, climbing, and immobility in mice during the first test session. Immobility was significantly different between the four groups tested ($H(3) = 8.18$; $p < .042$). Post hoc analyses confirm results of Experiment 1 since immobility was lower

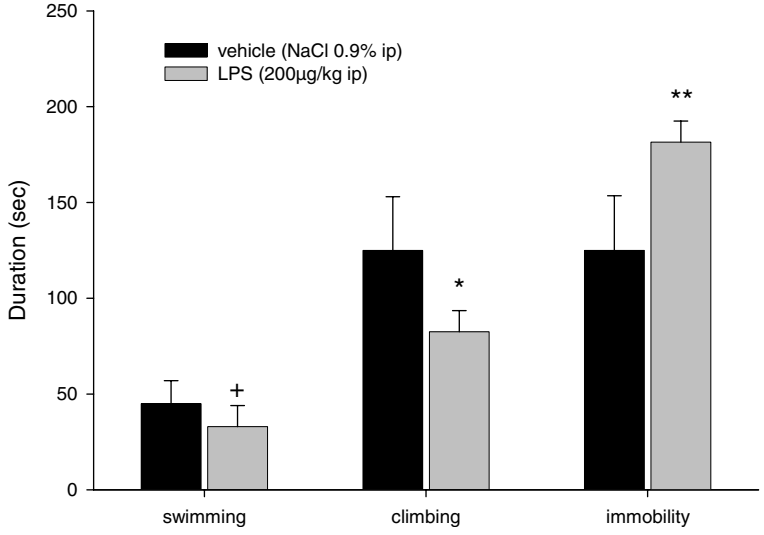

Fig. 2. Median ± SIQR (semi-interquartile range) duration of swimming, climbing, and immobility of mice during the second session of the forced-swim test (i.e., 24 h after the first session and treatments). Black columns: control subjects (0.9% NaCl, i.p.); grey columns: LPS-treated mice (200 µg/kg LPS, i.p.). $^{+}p < .066$; $^{*}p < .05$; $^{**}p < .01$.

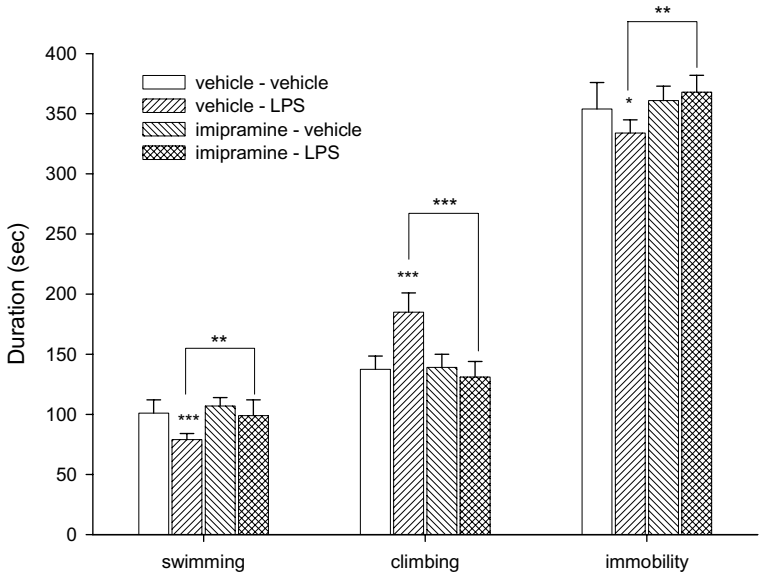

Fig. 3. Median ± SIQR (semi-interquartile range) duration of swimming, climbing, and immobility of mice during the first session of the forced-swim test of the second experiment. White columns: vehicle–vehicle (0.9% NaCl i.p. during 14 days and 0.9% NaCl i.p. 90 min before testing); ascending-hatch columns: vehicle–LPS (0.9% NaCl i.p. during 14 days and 200 μg/kg LPS i.p. 90 min before testing); descending-hatch columns: imipramine–vehicle (20 mg/kg imipramine during 14 days and 0.9% NaCl 90 min before testing); Crossed-columns: imipramine-LPS (20 mg/kg imipramine during 14 days and 200 μg/kg LPS i.p. 90 min before testing). Post hoc tests: $*p < .05$, $**p < .01$, $***p < .001$.

in 'vehicle–LPS' animals compared to 'vehicle–vehicle' mice ($p < .05$). Moreover, imipramine did not influence immobility scores in saline-treated mice ('vehicle–vehicle' vs. 'imipramine–vehicle' comparisons: $p > .05$). However, imipramine treatments significantly blocked the effects of LPS on immobility duration ('imipramine–LPS' vs. 'vehicle–LPS' mice; $p < .01$).

Swimming and climbing behaviours were also significantly influenced by treatments ($H(3) = 12.83$; $p < .005$ and $H(3) = 20.38$; $p < .001$, respectively). In accordance to Experiment 1, 'vehicle–LPS' mice expressed more climbing and less swimming relative to 'vehicle–vehicle' subjects (p's $< .001$). Moreover, post hoc tests showed that imipramine treatment did not change swimming or climbing durations in control mice ('imipramine–vehicle' vs. 'vehicle–vehicle' comparisons; p's $> .05$). However, imipramine significantly changed swimming and climbing durations in LPS-treated mice ('imipramine–LPS' vs. 'vehicle–LPS' comparisons; $p < .01$ and $p < .001$, respectively, for swimming and climbing). Finally, treatments did not significantly change frequencies in head-shaking ($H(3) = 1.83$; $p = .608$).

3.2.2. Second test session

As explained for Experiment 1, no previously LPS-treated mice (i.e. 24 h earlier) expressed any of the signs of sickness at the beginning of the second test session.

Fig. 4 summarizes results for durations of swimming, climbing, and immobility in mice during the second test session. Kruskal–Wallis analyses revealed that treatments changed significantly immobility, swimming, and climbing behaviours ($Hs(3) = 32.27$, 35.41, and 20.49, respectively; p's $< .001$).

Confirming its antidepressant properties, imipramine significantly blocked the increased immobility in mice ('imipramine–vehicle' vs. 'vehicle–vehicle' mice; $p < .001$). Comparing the same groups, post hoc analyses revealed that imipramine induced longer swimming duration ($p < .001$) but did not changed climbing duration ($p > .05$). As in Experiment 1, post hoc tests showed that immobility in 'vehicle–LPS' mice was longer than 'vehicle–vehicle' controls ($p < .01$), while durations in swimming and climbing was lower (p's $< .01$). Imipramine chronic treatment significantly prevented the increase in immobility in previously LPS-treated mice: immobility duration between 'imipramine–LPS' and 'imipramine–vehicle' mice was not significantly different, but significantly lower compared to their respective control (i.e., 'vehicle–LPS' and 'vehicle–vehicle,' respectively, p's $< .001$). Moreover, imipramine effects revealed to be the same on climbing and swimming behaviours between LPS-treated animals and controls (i.e. 'imipramine–LPS' vs. 'imipramine–vehicle' comparisons; p's $> .05$). Imipramine induced longer swimming duration both in LPS- and saline-treated mice relative to their controls ('vehicle–LPS' and 'vehicle–vehicle' respectively; p's $< .001$). Furthermore, climbing was only changed by imipramine treatment in LPS-treated mice: 'imipramine–vehicle' vs 'vehicle–vehicle', $p > .05$ and 'imipramine–LPS' vs. 'vehicle–LPS', $p < .01$. Finally, treatments did not have a significant effect on frequencies in head-shaking ($H(3) = 0.97$; $p = .721$).

4. Discussion

Studies using the FST do not usually focus on the first exposure to the water tank. It has been argued that two

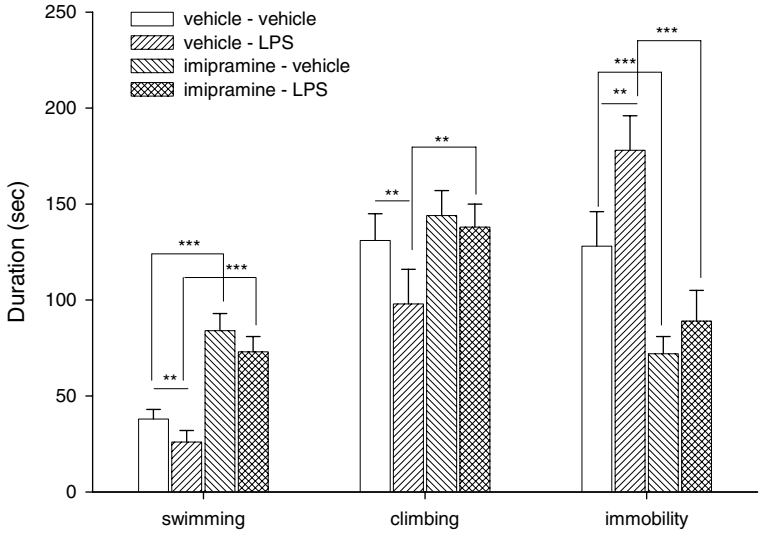

Fig. 4. Median ± SIQR (semi-interquartile range) duration of swimming, climbing, and immobility of mice during the second session of the forced-swim test of the second experiment (i.e., 24 h after first session and treatments). Columns design is the same as in Fig. 3. Post hoc tests: $^{**}p < .01$, $^{***}p < .001$.

exposures are necessary for the subjects to find out that escape is impossible (Borsini and Meli, 1988) and that all defence strategies are ineffective. The first part of our study aimed to assess both the immediate impact of an immune challenge on defensive behaviours (first exposure), and the delayed consequences in subsequent confrontations with the same situation (second exposure).

During the first exposure, treated mice were observed while under the immune effects of LPS, which had been administered 90 min before the beginning of the test, as confirmed by the presence of postural, temperature and body-weight changes.

During this phase of the experiment, LPS-treated subjects generally displayed more active defences and less immobility than controls. The mode of defence was also different in experimental subjects, for example, LPS-treated mice expressed more climbing and less swimming behaviour than controls. According to Lucki (1997), climbing behaviour could be considered as an initial panic-like response to the test situation, while swimming could be interpreted as a secondary exploratory behaviour (Cryan et al., 2005; Lucki, 1997). If so, the increased expression of climbing in LPS-treated mice could be interpreted as an emotional over-reaction (i.e., a panic-like response) to immersion in water.

In the second exposure to the water-tank (i.e., 24 h after the first session), subjects that had previously been treated with LPS (but no longer under the actual effects of endotoxin, i.e., no clinical signs of inflammation (piloerection, curled-up posture, and eyes closed), nor changes in rectal temperature or body weight loss relative to controls), displayed a longer duration of immobility and less climbing and swimming than controls. Therefore, the increase in immobility between the two exposures, referred to as "behavioural despair" (Porsolt et al., 1977) increased in the 'LPS' group, which could therefore be considered as experiencing higher levels of depressive-like effects.

Dunn et al. (2005) recently discussed the possible adaptive strategies displayed in response to LPS or IL-1. According to these authors, if the sickness behaviour is adaptative, the appropriate response to the FST would be to increase floating, not swimming. In that case, this increase in floating time (i.e., immobility) would not be considered as 'behavioural despair' but as the optimal strategy to increase the chance of survival (i.e., saving energy while the experiment was in progress). However, in our study, LPS did not increase immobility during the first exposure (i.e., while LPS was physiologically active), but did so during the second exposure (i.e., in the absence of clinical signs of LPS bioactivity).

Some authors have argued that the increased immobility during FST is the result of a learning process, whereby subjects wait to be removed from the water (De Pablo et al., 1989; West, 1990). Not only has this point of view been rejected as being based on anthropomorphic arguments (Cryan et al., 2005), but it ignores the fact that the FST represents a highly stressful situation for rodents, and especially mice. Such a situation ought to elicit a high emotional reaction, supporting defensive responses. Inhibiting such a reaction in a single session would imply highly developed emotional control systems in subjects. Such control systems are known to be present in the central nervous system (i.e., frontal cortical regions) and are unlikely to be sufficiently developed in the mouse brain to account for immobility in FST.

Recently, Dunn and Swiergiel (2005) used 1 and 5 μg/mouse i.p., and found that both doses increased immobility in the FST. These findings are in contrast with those we obtained. It is hard to pretend to fully explain this contrast considering the scarcity of such studies. However, two methodological differences between the study by Dunn and Swiergiel and ours could account for these discrepancies. First, these authors used a one-exposure procedure, in

which the last 4 min were analysed from a 6-min exposure to the water. However, it has been shown that immobility duration increases during the first forced-swimming experience, lasting less than 40% of the time in the first part of the test, taking more than 80 and 90% of the time on the second and third period of observation (Barros and Ferigolo, 1998). Second, in the study by Dunn and Swiergiel, water temperature was 30 °C while it was 24 °C in our study. It has been shown that water temperature drastically modulates forced-swim reactivity. In a study by Drugan et al. (2005), subjects submitted to 20–25 °C water were more reactive (i.e., displayed more active defensive reactions) than those submitted to a 30 °C water. Therefore, Dunn and Swiergiel tested their subjects under conditions favourable to the rapid development of immobility, and under observation conditions (i.e., the second and third part of the test session) where immobility is predominant. These two factors could, at least partly, explain the contrast between the study by Dunn and Swiergiel (2005) and ours.

In another recent study, Deak et al. (2005) used the FST with LPS-treated rats. Interestingly, their results indicated no significant behavioural changes in LPS-injected subjects. By contrast, our study revealed significant differences between saline- and LPS-treated subjects.

A possible explanation for this discrepancy could be the species involved. The study by Deak et al. (2005) used *Rattus norvegicus* rats, while the present study used *Mus musculus domesticus* mice. Each species has evolved its own behavioural repertoire, shaped by its unique evolutionary history. Consequently, different species may react differently to the same stimulus or manifest different behavioural displays in response to the same internal drive (Crews, 1997). In rodents, important differences exist between rats and mice in terms of their natural habitats: rats live in subterranean burrows near water, on riverbanks for example, which makes them natural swimmers as reflected in their greater competence in experiments involving swimming. By contrast, house mice live in dryer zones, and it has been shown for instance that even if they can learn water-based tasks, their learning performance is worse than rats (Whishaw, 1995). With regard to behaviours displayed in the water-tank, rats commonly dive, whereas this behaviour has not been observed in mice. It is therefore highly conceivable that immersion in the same water-tank is more stressful for mice than for rats.

Another possible explanation for differences between Deak et al.'s study and our own could simply be the dose of LPS, as Deak et al. used a lower dose (100 μg/kg) than we did (200 μg/kg). For our part, we used this dose as it corresponds to the concentration usually injected in our subjects since it is known to reliably induce sickness in rodents.

In the second experiment, we tested the ability of a chronic antidepressant treatment (i.e., imipramine) to block potentiating effects of LPS on behavioural despair.

Results reveal that imipramine clearly abolished the increase in defensive reactions in LPS-treated mice during the first exposure to the apparatus. Moreover, results from the second exposure revealed the absence of increased immobility and decreased swimming and climbing in previously LPS-treated rats receiving chronic imipramine treatment.

As shown in Fig. 3, chronic treatment with imipramine did not change behaviour mice during the first exposure to the water tank (based on 'imipramine-vehicle' vs. 'vehicle–vehicle' comparisons). Results from the second exposure (cf. Fig. 4) show that imipramine-treated subjects displayed less immobility, and were therefore more active than controls. These effects are the core arguments for the antidepressant effect of imipramine. However, imipramine influenced differentially swimming and climbing behaviours. It was demonstrated that 5-HT acting antidepressants increase swimming, while on the other hand, noradrenergic reuptake blockers increase climbing movements (Cryan et al., 2005; Detke et al., 1995; Lucki, 1997). Imipramine is a tricyclic antidepressant, but the potency and selectivity for the inhibition of the uptake of norepinephrine, serotonin, and dopamine vary greatly among tricyclic agents (i.e., tertiary amine tricyclics inhibit predominantly the serotonin uptake pump, whereas the secondary amine ones are better in switching off the norepinephrine pump). For instance, it well known that imipramine is a potent blocker of serotonin transport (increased levels of serotonin in hypothalamus and amygdala), while desipramine inhibits the uptake of norepinephrine. Therefore, since imipramine acts predominantly as a 5-HT reuptake inhibitor, our results (i.e., increase of swimming but not climbing after imipramine treatment) are in accordance with the literature.

One could argue that the enhancing effect of LPS in FST-induced immobility reported in our first experiment could be due to an increased exhaustion of LPS-treated mice after the first exposure, leaving them with too little energy to display the same amount of defensive behaviour as controls. However, the fact that a previous chronic imipramine treatment blocked such an effect confirms that experiencing failure to escape a stressful event while under the effects of LPS-induced sickness increases its despairing effects (i.e., depression-like).

These results, showing increased defensive reactions and enhanced despairing effects of LPS-treated subjects in the FST, are both in accordance with the motivational conception of sickness and the cytokine hypothesis of depression.

These data are in fact in line with previous findings where LPS increased aversive responses and decreased hedonic expressions to a mixed bittersweet solution (Aubert and Dantzer, 2005). In humans, several findings relate mood changes with transitory respiratory tract infections. For instance, it has been shown that infection with the *influenza* virus is associated with negative mood and increased irascibility (Hall and Smith, 1996).

Taken together, these results could indicate that LPS-induced sickness would potentiate the reactivity of subjects to negative features of a given stimulus or situation. In a pilot study, we exposed LPS-treated mice to the

forced-swim test 24-h after treatments (Aubert, 1999b). It has been found that these LPS-treated mice did not behaviourally differ from controls (i.e., saline-treated mice tested 24-h after injections). Moreover, LPS-treated mice tested only 24-h after injections did not display reduced immobility and increased climbing as LPS-injected mice tested 90-min after treatment (Aubert, 1999b). These results are in accordance with the interpretation regarding a LPS-induced potentiation of despair on the second exposure to water, based on over-reaction to the first exposure to the threat.

Such hyper-reactivity could easily be interpreted as a part of motivational changes, triggering increased defensive behaviours to a potential or actual threat. However, the higher emotional response that was elicited by the threat would imply more severe consequences in the case of a failure to cope with the situation. On one hand, higher emotional reactivity of LPS-treated subjects increases their defensive motivation (and therefore promotes their immediate survival skills), but on the other hand, it makes them more vulnerable to the deleterious emotional consequences of a failure. Indeed, from the relative current consensus, emotional processes imply a cognitive (i.e., situational appraisal) and a physiological (emotional arousal) part, combining to support and direct behaviour. According to this setting, depressive-like symptoms (i.e., behavioural despair or helplessness) would correspond to an impaired evaluation of his capacity to cope with the current threat (cf. Figs. 5A and B).

The possible mechanisms underlying such phenomena could be at least partly understood through the metabolic and physiological consequences of inflammation. It is now well established that one of the prominent physiological responses to LPS-induced cytokine release is the potent activation of the hypothalamic–pituitary–adrenal (HPA) axis, at CRH, ACTH, and glucocorticoïds levels (for review, see Haddad et al., 2002). Not only is LPS-induced sickness known to induce HPA axis activation involved in the physiology of stress (Besedovsky et al., 1991), but physiological aspects of inflammation have also been argued to be stress-related (Maier and Watkins, 1998). Moreover, it has been demonstrated that a single administration of interleukin-1 increased CRH and ACTH mRNA in the hypothalamic paraventricular nucleus, paralleling a long-lasting sensitization to novelty stress (Schmidt et al., 2003). Finally, it has been shown that a chronic antidepressant treatment attenuated LPS-induced increased of plasma ACTH and corticosterone in rats (Castanon et al., 2003). Therefore, since the HPA axis is the key player in stress response, and proinfammatory cytokines and cytokine-inducer as LPS stimulate it, it is likely that the LPS-induced potentiation of despair we describe here could be physiologically supported by HPA/cytokine interactions.

Such a convergence between stress and inflammation recalls earlier work by Dolf Zillman. Zillman's arousal theory (Zillman, 1978, 1983) focuses on the immediate effects that a given situation may have on behavioural responses in a subsequent situation. For example, naïve human subjects taunted with insults in a gymnasium responded more aggressively if the provocation occurred after training (i.e., after physiological arousal due to physical exercise) than if they were provoked before the beginning of their training session. However, such a transfer of arousal is non-specific. Thus, other factors (i.e., contextual and situational) will determine what these behavioural outcomes will be: sexual, aggressive, or as in our case, depressive-like (Berkowitz, 1993). In our case, peripheral effects of LPS administration

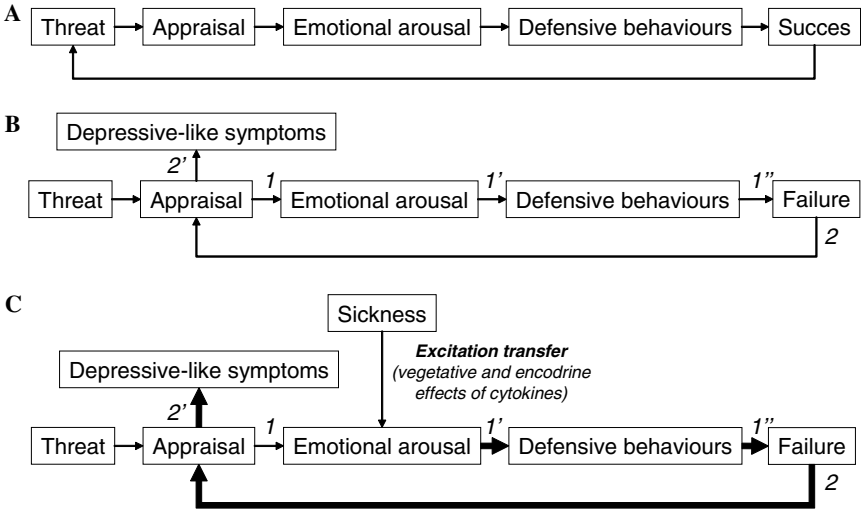

Fig. 5. Ongoing sequence of event as the subject is confronted to a threatening event (A). In the first place, event is evaluated as a threat. This appraisal induces an emotional state, which implies a general arousal that will sustain the efficient expression of defensive behaviours, which in turn, if successful, will eliminate the threat. In the case of forced-swim test, the defensive behaviours cannot eliminate the threat (immersion in a water tank). The failure of defensive strategies is evaluated as well as the persistence of the threat, and therefore induces depressive-like symptoms in subjects (B). In LPS-treated mice, the endocrine and vegetative effects of immune activation share common feature with physiological components of emotions. It therefore increases emotional arousal thus enhancing defensive urges. As defensive investment grows, the consequences of failure in eliminating threat also increase, thus facilitating the development of depressive-like symptoms (C).

(i.e., endocrine and vegetative changes) would enhance the emotional arousal created by the situation (i.e., forced swimming), and therefore would increase the overall emotional response to the threat as well as the defensive behaviour associated with such a reaction. Consequently, as the defences and underlying emotional responses are increased, the behavioural and emotional consequences of the failure of subjects' defensive strategies are increased (cf. Fig. 5C).

In conclusion, our study shows both an increase in defensive reactions to an actual threat in LPS-treated mice as well as a potentiated despair in subsequent exposure to the same stressful situation. This therefore offers a possible link between sickness and depression, not dependent on a direct causal effect of liberated cytokines, but through the vulnerabilization of subjects to contextual cues (e.g., defeat and coping failure) due to an increased emotional arousal (Aubert, 2005; Olff, 1999). The difference in LPS capacity to induce behavioural changes in rats or mice submitted to the FST argues in favour to the contextualisation (i.e., situational significance) of the relations between immunity and depression. Finally, it could be hypothesized (and further investigated) that this increased emotional reactivity is the consequence of a transfer of arousal, due at least partly to the convergence of physiological phenomena in stress and inflammatory processes.

References

Aubert, A., 1999a. Sickness and behaviour in animals: a motivational perspective. Neurosci. Biobehav. Rev. 23, 1029–1036.

Aubert, A., 1999b. Potentiation by lipopolysaccharide of the effects of forced-swimming. In: 4th Symposium of the French Society for Neurosciences. May 25–28, Marseille, France

Aubert, A., 2005. Les approches comportementales en Psychoneuroimmunologie. In: Delfour, F., Dubois, M. (Eds.), Ethologie et Cognition Animale. PUL, Lyon, pp. 73–98.

Aubert, A., Dantzer, R., 2005. The taste of sickness: lipopolysaccharide-induced finickiness in rats. Physiol. Behav. 84, 437–444.

Aubert, A., Goodall, G., Dantzer, R., Gheusi, G., 1997. Differential effects of lipopolysaccharide on pup retrieving and nest building in lactating mice. Brain Behav. Immun. 11, 107–118.

Azpiroz, A., Fano, E., Garmendia, L., Arregi, A., Cacho, R., Beitia, G., Brain, P.F., 1999. Effects of chronic mild stress (CMS) and imipramine administration, on spleen mononuclear cell proliferative response, serum corticosterone level and brain norepinephrine content in male mice. Psychoneuroendocrinology 24, 345–361.

Barros, H., Ferigolo, M., 1998. Ethopharmacology of imipramine in the forced-swimming test: gender differences. Neurosci. Biobehav. Rev. 23, 279–286.

Berkowitz, L., 1993. The experience of anger as a parallel process in the display of impulsive, "angry" aggression. In: Geen, R.G., Donnerstein, E.I. (Eds.), Aggression: Theoritical and Empirical Reviews. Academic Press, Orlando, pp. 103–133.

Besedovsky, H.O., Del Rey, A., Klusman, I., Furukawa, H., Monge Arditi, G., Kabiersch, A., 1991. Cytokines as modulators of the hypothalamus–pituitary–adrenal axis. J. Steroid Biochem. Mol. Biol. 40, 613–618.

Borsini, F., Meli, A., 1988. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology 94, 147–160.

Bridge, P.D., Sawilowsky, S.S., 1999. Increasing physicians' awareness of the impact of statistics on research outcomes: comparative power of the *t* test and Wilcoxon Rank-Sum test in small samples applied research. J. Clin. Epidemiol. 52, 229–235.

Brown, R., King, M.G., Husband, A.J., 1992. Sleep deprivation-induced hyperthermia following antigen challenge due to opioid but not interleukin-1 involvement. Physiol. Behav. 51, 767–770.

Cabanac, M., 1971. Physiological role of pleasure. Science 173, 1103–1107.

Capuron, L., Dantzer, R., 2003. Cytokines and depression: the need for a new paradigm. Brain Behav. Immun. 17, S119–S124.

Castanon, N., Konsman, J.P., Médina, C., Chauvet, N., Dantzer, R., 2003. Chronic treatment with the antidepressant tianeptine attenuates lipopolysaccharide-induced Fos expression in the rat paraventricular nucleus and HPA axis activation. Psychoneuroendocrinology 28, 19–34.

Chourbaji, S., Zacher, C., Sanchis-Segura, C., Dormann, C., Vollmayr, B., Gass, P., 2005. Learned helplessness: validity and reliability of depressive-like states in mice. Brain Res. Protoc. 16, 70–78.

Crews, D., 1997. Species diversity and the evolution of behavioral controlling mechanisms. Ann. N. Y. Acad. Sci. 807, 1–21.

Cryan, J.F., Valentino, R.J., Lucki, I., 2005. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci. Biobehav. Rev. 29, 547–569.

Dantzer, R., 2001. Cytokine-induced sickness behavior: where do we stand? Brain Behav. Immun. 15, 7–24.

De Pablo, J.M., Parra, A., Segovia, S., Guillamon, A., 1989. Learned immobility explains the behavior of rats in the forced swimming test. Physiol. Behav. 46, 229–237.

Deak, T., Bellamy, C., D'Agostino, L.G., Rosanoff, M., McElderly, N.K., Bordner, K.A., 2005. Behavioral responses during the forced swim test are not affected by anti-inflammatory agents or acute illness induced by lypopolysaccharide. Behav. Brain Res. 160, 125–134.

Detke, M.J., Rickels, M., Lucki, I., 1995. Active behaviors in the rat forced-swimming test differentially produced by serotoninergic and noradrenergic antidepressants. Psychopharmacology 121, 66–72.

Drugan, R.C., Eren, S., Hazi, A., Silva, J., Christianson, J.P., Kent, S., 2005. Impact of water temperature and stressor controllability on swim stress-induced changes in body temperature, serum corticusterone, and immobility in rats. Pharmacol. Biochem. Behav. 82, 397–403.

Dunn, A., 2001. Effects of cytokines and infections on brain neurochemistry. In: Ader, R., Felten, D., Cohen, N. (Eds.), Psychoneuroimmunology. Academic Press, San Diego, pp. 649–666.

Dunn, A.J., Swiergiel, A.H., 2005. Effects of interleukin-1 and endotoxin in the forced swim and tail suspension tests in mice. Pharmacol. Biochem. Behav. 81, 688–693.

Dunn, A.J., Swiergel, A.H., de Beaurepaire, R., 2005. Cytokines as mediators of depression: what can we learn from animal studies? Neurosci. Biobehav. Rev. 29, 891–909.

Greenwood, R., 1987. Residual mental disorders after herpesvirus infections. In: Kurstak, E., Lipowski, Z.J., Morozov, P.V. (Eds.), Viruses, Immunity and Mental Disorders. Plenum Press, New-York, pp. 65–80.

Haddad, J.J., Saadé, N.E., Safieh-Garabedian, B., 2002. Cytokines and neuro-immuno-endocrine interactions: a role for the hypothalamic–pituitary–adrenal revolving axis. J. Neuroimmunol. 133, 1–19.

Hall, S., Smith, A., 1996. Investigation of the effects and after-effects of naturally occurring upper respiratory tract illnesses on mood and performance. Physiol. Behav. 59, 569–577.

Hart, B.L., 1988. Biological basis of the behavior of sick animals. Neurosci. Biobehav. Rev. 12, 123–137.

Healy, D., Cavin, J., Whitehouse, A.M., White, W., Wilton-Cox, H., Theodorou, A.E., Lawrence, K.M., Horton, R.W., Paykel, E.S., 1991. Alpha-1-acid glucoprotein in major depressive and eating disorders. J. Affect. Discord. 22, 13–20.

Hendler, N., 1987. Infectious mononucleosis and psychiatric disorders. In: Kurstak, E., Lipowski, Z.J., Morozov, P.V. (Eds.), Viruses, Immunity and Mental Disorders. Plenum Press, New-York, pp. 81–94.

Kent, S., Bluthé, R.M., Kelley, K., Dantzer, R., 1992. Sickness behavior as a new target for drug development. TINS 13, 24–28.

Kronfol, Z., 2002. Immune dysregulation in major depression: a critical review of existing evidence. Int. J. Neuropsychopharmacol. 5, 333–343.

Levine, J., Barak, Y., Chengappa, K.N., Rapoport, A., Rebey, M., Barak, V., 1999. Cerebrospinal cytokine levels in patients with acute depression. Neuropsychobiology 40, 171–176.

Linkowski, P., Mendelwicz, J., LeClerq, R., Brasseur, M., Hubain, P., Golstein, J., Copinschi, G., VanGauter, E., 1985. The 24-hour profile of adrenocorticotropin and cortisol in major depressive illness. J. Clin. Endocrinol. Metab. 61, 429–438.

Lucki, I., 1997. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav. Pharmacol. 8, 523–532.

Maes, M., 1995. Evidence for an immune response in major depression: a review and hypothesis. Prog. Neuropsychopharmacol. Biol. Psychiatry 19, 11–38.

Maes, M., Scharpe, S., Meltzer, H.Y., Bosmans, E., Suy, E., Calabrese, J., Cosyns, P., 1993. Relationships between interleukin-6 activity, acute phase proteins, and function of the hypothalamic–pituitary–adrenal axis in severe depression. Psychiatry Res. 49, 11–27.

Maier, S.F., Watkins, L.R., 1998. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. Psychol. Rev. 105, 83–107.

Makino, M., Kitano, Y., Hirohashi, M., Takasuna, K., 1998. Enhancement of immobility in mouse forced swimming test by treatment with human interferon. Eur. J. Pharmacol. 356, 1–7.

Malek-Ahmadi, P., 1996. Neuropsychiatric aspects of cytokines research: an overview. Neurosci. Biobehav. Rev. 20, 359–365.

Marshal, P.S., 1993. Allergy and depression: a neurochemical threshold model of the relation between the illnesses. Psychol. Bull. 113, 23–43.

Meijer, A., Zakay-Rones, Z., Morag, A., 1988. Pos-influenzal psychiatric disorders in adolescents. Acta Psychiatr. Scand. 78, 176–181.

Minden, S.L., Schiffer, R.B., 1990. Affective disorders in multiple sclerosis. Review and recommendations for clinical research. Arch. Neurol. 47, 98–104.

Miller, A.H., Capuron, L., Raison, C.L., 2005. Immunologic influences on emotion regulation. Clin. Neurosci. Res. 4, 325–333.

Nemeroff, C.B., Krishnan, R.R., Blazer, D.G., Knight, D.L., Benjamin, D., Meyerson, L.R., 1990. Elevated plasma concentrations of alpha-1-acid glycoprotein, a putative endogenous inhibitor of tritiated imipramine binding site, in depressed patients. Arch. Gen. Psychiatr. 47, 337–340.

Nguyen, K.T., Deak, T., Owens, S.M., Kohno, T., Fleshner, M., Watkins, L.R., Maier, S.F., 1998. Exposure to acute stress induces brain interleukin-1beta protein in the rat. J. Neurosci. 18, 2239–2246.

Olff, M., 1999. Stress, depression and immunity: the role of defense and coping styles. Psychiatry Res. 18, 7–15.

Pare, W.P., 1989. "Behavioral despair" test predicts stress ulcer in WKY rats. Physiol. Behav. 46, 483–487.

Parker, J., Smarr, K., Anderson, S., Hewett, J., Walker, S., Bridges, A., Calwell, W., 1992. Relationship of changes in helplessness and depression to disease activity in rheumatoid arthrisis. J. Rheumatol. 19, 1901–1905.

Parry, B.L., 1995. Mood disorders linked to the reproductive cycle in women. In: Blomm, F.E., Kupfer, D.J. (Eds.), Psychopharmacology: The Fourth Generation of Progress. Raven Press, New-York, pp. 1029–1042.

Porsolt, R.D., Le Pichon, M., Jalfre, M., 1977. Depression: a new animal model sensitive to antidepressant treatments. Nature 266, 730–732.

Porsolt, R.D., Anton, G., Blavet, N., Jalfre, M., 1978. Behavioral despair in rats: a new model sensitive to antidepressant treatment. Eur. J. Pharmacol. 47, 379–391.

Schmidt, E.D., Aguilera, G., Binnekade, R., Tilders, F.J., 2003. Single administration of interleukin-1 increased corticotropin releasing hormone and corticotropin releasing hormone-receptor mRNA in the hypothalamic paraventricular nucleus which paralleled long-lasting (weeks) sensitization to emotional stressors. Neuroscience 116, 275–283.

Siegel, S., Castellan, N.J., 1988. Nonparametric statistics for the behavioural sciences, second ed. McGraw-Hill, New-York. 399pp.

Sluzewska, A., Wiktorrowicz, K., Mackiewicz, A., Rybakowski, J.K., 1994. The effect of short-term treatment with lithium and carbamazepine on some immunological indices in depressed patients. Lithium 5, 41–46.

Sluzewska, A., Rybakowski, J., Laciak, M., Mackiewicz, A., Sobieska, M., Wiktorrowicz, K., 1995. Interleukin-6 serum levels in depressed patients before and after treatment with fluoxetine. Ann. N. Y. Acad. Sci. 762, 474–476.

Sluzewska, A., Rybakowski, J., Bosmans, E., Sobieska, M., Berghmans, R., Maes, M., Wiktorrowicz, K., 1996. Indicators of immune activation in major depression. Psychiatr. Res. 64, 161–167.

Subarnas, A., Tadano, T., Oshima, Y., Kisara, K., Ohizumi, Y., 1993. Pharmacological properties of beta-amyrin palmitate, a novel centrally acting compound, isolated from *Lobelia inflata* leaves. J. Pharm. Pharmacol. 45, 545–550.

West, A.P., 1990. Neurobehavioral studies of forced swimming: the role of learning and memory in the forced swim test. Prog. Neuropsychopharmacol. Biol. Psychiatry 14, 863–877.

Whishaw, I.Q., 1995. A comparison of rats and mice in a swimming pool place task and matching to place task: some surprising differences. Physiol. Behav. 58, 687–693.

Yirmiya, R., 1996. Endotoxin produces a depressive-like episode in rats. Brain Res. 711, 163–174.

Zillman, D., 1978. Attribution and misattribution of excitatory reactions. In: Harvey, J.H., Ickes, W., Kidd, R.F. (Eds.), New Directions in Attribution Research, vol. 2. Erlbaum, Hillsdale, NJ.

Zillman, D., 1983. Transfer of excitation in emotional behaviour. In: Cacciopo, T.J., Petty, R.E. (Eds.), Social Psychophysiology: A Sourcebook. Guilford Press, New-York, pp. 215–240.