Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuroscience and Biobehavioral Reviews 30 (2006) 346–355
NEUROSCIENCE AND BIOBEHAVIORAL REVIEWS
www.elsevier.com/locate/neubiorev

Pleiotropic contributions of nitric oxide to aggressive behavior

Randy J. Nelson[a,*], Brian C. Trainor[a], Silvana Chiavegatto[b], Gregory E. Demas[c]

[a]*Departments of Psychology and Neuroscience, The Ohio State University, Columbus, OH 43210, USA*
[b]*Department and Institute of Psychiatry, and Laboratory of Genetics and Molecular Cardiology, Heart Institute (InCor), University of Sao Paulo Medical School, Sao Paulo, SP, Brazil*
[c]*Department of Biology, Program in Neural Science, and Center for the Integrative Study of Animal Behavior, Indiana University, Bloomington, IN 47405, USA*

Received 29 October 2004; revised 22 February 2005; accepted 25 February 2005

Abstract

Male mice with targeted deletion of the genes encoding the neuronal ($NOS^{-1-/-}$ or $nNOS^{-/-}$) isoform of nitric oxide synthase display altered aggressive behaviors. Male $nNOS^{-1-/-}$ mice are more aggressive than wild-type (WT) mice in all testing paradigms. Testosterone is necessary, but not sufficient, for evoking the persistent aggression, and that serotonin (5-HT) metabolism is altered in male $nNOS^{-1-/-}$ mice. The specific deletion of the $nNOS^{-1}$ gene not only results in a lack of $nNOS^{-1}$ protein, but in common with many genes, affects several 'downstream' processes. In this review, we address whether the elevated aggression in male $nNOS^{-1-/-}$ mice reflects pleiotropic effects of the $nNOS^{-1}$ gene on pain sensitivity, 'anxiety-like', or 'depressive-like' behaviors. For example, male $nNOS^{-1-/-}$ mice display increased sensitivity to painful stimuli, which may prolong aggressive interactions. Despite elevated corticosterone concentrations, $nNOS^{-1}$ knockout mice appear to be less 'anxious' or fearful than WT mice. Male $nNOS^{-1-/-}$ mice display longer latencies to right themselves on an inverted platform and spend more time in the center of an open field than WT mice. Because of reduced serotonin turnover, the excessive aggressiveness displayed by $nNOS^{-1-/-}$ mice may be symptomatic of a depressive-like syndrome. However, $nNOS^{-1-/-}$ mice rarely display behavioral 'despair' when assessed with the Porsolt forced swim test; rather, $nNOS^{-1-/-}$ mice show vigorous swimming throughout the assessment suggesting that the aggressive behavior does not represent depressive-like behavior. Importantly, aggressive behavior is not a unitary process, but is the result of complex interactions among several physiological, motivational, and behavioral systems, with contributions from the social as well as the physical environment. Lastly, the multiple, and often unanticipated, effects of targeted gene disruption on aggressive behavior are considered.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Nitric oxide; Aggression; Violence; Serotonin; Gene knockout; Anxiety; Depression; Pain; Perseveration

1. Introduction

Aggression is a highly complex behavior displayed by virtually all living organisms, and serves a broad range of adaptive functions. Aggression, however, especially when it is excessive or prolonged, is also observed in many non-adaptive or even pathological settings as well. Aggression has traditionally been defined as overt behavior with the intention of inflicting physical damage upon another individual (Moyer, 1971). Of course, 'intention' must always be assumed among non-human animals. The possibility for aggressive behavior exists whenever the interests of two or more individuals conflict (Svare, 1983). These conflicts usually arise over limited resources including territories, food, and mates. Indeed, the ubiquitous resident-intruder aggression test is intended to model rodent territorial aggression. In nature, the social interaction decides which animal gains access to the contested resource. In many cases, a submissive posture or gesture on the part of one animal avoids the necessity of actual combat over a resource. Animals may also participate in psychological intimidation by engaging in threat displays or ritualized combat in which dominance is determined, but no physical damage is inflicted (Moyer, 1971).

Because several types of aggressive behavior exists, ranging from predatory behavior to maternal defense, to territorial aggression, the likelihood that a single common

* Corresponding author. Tel.: +1 614 538 9540; fax: +1 614 451 3116.
E-mail address: rnelson@osu.edu (R.J. Nelson).

0149-7634/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neubiorev.2005.02.002

brain circuit underlies all forms of aggressive behavior seems remote. Nonetheless, a major goal of aggression research from a biomedical or veterinary medical perspective is to develop pharmacological interventions suitable to control aggression in violent individuals. The results of this endeavor have been mixed. Probably the best example of successful pharmacological control of aggression was realized over a half century ago with the development and use of neuroleptics. These pharmaceuticals dramatically changed how clinicians manage violence in people suffering from one or more disorders including psychotic, depressive, intellectual, or organic brain disorders, as well as managing violence in people abusing alcohol or amphetamine (Citrome and Volavka, 1997; Citrome and Volavka, 1997). Indeed, the effectiveness of chlorpromazine and haloperidol in reducing violent behavior continues to set the standard in the evaluation of novel compounds for this purpose. Although the first-generation neuroleptics worked primarily by sedating the patients and suppressing most behaviors, more recently developed drugs produce fewer negative side-effects such as tardive dyskinesia during chronic treatment (Swann, 2003).

One traditional, yet simplified, neurochemical model of aggressive behavior suggests that serotonin (5-HT) inhibits, whereas dopamine permits or enables, aggressive behavior. Recently, gamma-aminobutyric acid (GABA) and other neurotransmitters or neuromodulators have been included in this model. Pharmacological interventions have been less successful when based upon this simple model. For example, although increasing 5-HT via specific 5-HT reuptake inhibitors (SSRIs) decreases aggressive behaviors in the majority of laboratory or clinical settings (Walsh and Dinan, 2001; New et al., 2004), a significant minority of individuals treated with SSRIs *increase* aggression (Spigset, 1999). Similarly, although benzodiazepines, which enhance ligand binding to $GABA_A$ receptors, reduce aggression in most individuals, a significant minority display a paradoxical elevation in violence after benzodiazepine treatment (e.g. Azcarate, 1975). Such individual differences in aggressive responsiveness to drugs are critical to understand and target for any successful pharmacological treatment for violence.

Clearly, a very specific pharmacological approach will be necessary to regulate violence in veterinary or human medicine. Our laboratory has been studying the role of nitric oxide (NO), a signaling molecule in the nervous system, in the regulation of aggression. In this review, the behavioral effects of genetically or pharmacologically depleted NO are reviewed. In addition to the dramatic aggressive behavioral phenotypic effects, we have recently discovered several additional and subtle effects of NO that interact to affect aggressive behavior. For example, mice missing NO in neurons are less 'anxious' or 'fearful' than wild-type (WT) mice, traits that may contribute to their aggressiveness. Mice lacking NO from neurons also display increased sensitivity to painful stimuli, which may also affect aggressive interactions. Additionally, these animals persist in several behaviors including mounting anestrous females, attack behaviors, and swimming behavior in the Porsolt test. Aggressive behavior is not a unitary process, but is the result of complex interactions among several physiological, motivational, and behavioral systems, with contributions from the social and physical environment. Taken together, any pharmacological tools developed to reduce aggression most likely affect several molecular signaling pathways simultaneously to be effective. NO is a candidate molecule that may affect several behavioral, sensory, perceptual, motivational, and arousal mechanisms leading to increased rate and sustained duration of aggressive encounters.

2. Nitric oxide

Nitric oxide (NO) was initially identified as an endogenous regulator of blood vessel tone (Ignarro, 1990; Moncada and Higgs, 1993). Since its initial characterization, NO was also discovered to mediate the bactericidal and tumoricidal actions of macrophages (Nathan, 1992; Nathan and Xie, 1994), and act as a neuronal messenger in the central and peripheral nervous systems (Dawson and Snyder, 1994; Dawson and Dawson, 1996). NO is an endogenous gas that has biochemical properties of a free-radical. NO is very labile, with a half-life of <5 s; consequently, many studies have manipulated NO indirectly by affecting its synthetic enzyme, NO synthase (NOS), that transforms arginine into citrulline and NO. Three distinct isoforms of NOS have been discovered in: (1) the endothelial tissue of blood vessels (eNOS or NOS-3), (2) macrophages as an inducible form (iNOS or NOS-2), and (3) neurons (nNOS or NOS-1), although they are not exclusively found in these locations. Suppression of NO formation by either elimination of arginine or by *N*-methyl-arginine, a potent NOS inhibitor, affects all three isoforms of NOS. Neurons containing nNOS are discretely localized throughout the brain. High densities of nNOS positive cells are localized in the limbic system, which regulates emotional behavior and aggression, particularly the lateral septal nuclei, posterior hypothalamus, entorhinal cortex, and amygdala (Albert and Walsh, 1984; Vincent and Kimura, 1992).

2.1. Neuronal nitric oxide synthase (nNOS) and aggression

An initial role of nNOS in the regulation of aggression was discovered serendipitously during the establishment of a breeding colony of mice lacking the nNOS gene ($nNOS^{-/-}$). We conducted a series of behavioral studies (Nelson et al., 1995). In both studies of aggression and mating behavior, male $nNOS^{-/-}$ mice displayed a dramatic loss of behavioral inhibition reflected by persistent fighting and mounting behavior despite obvious [to the human observers] signals of submissiveness or disinterest,

respectively, by their test partners. Nulliparous female nNOS$^{-/-}$ mice displayed neither elevated aggressiveness, nor inappropriate mating behaviors. Prolonged inappropriate aggressiveness and mating behavior among males are often associated with elevated blood concentrations of testosterone (Simon, 2002); however, no differences in testosterone concentrations were detected between WT and nNOS$^{-/-}$ males (Nelson et al., 1995). However, castration of nNOS$^{-/-}$ mice results in a marked reduction in aggression and testosterone replacement therapy restores aggression to pre-castration levels (Kriegsfeld et al., 1997). These results suggest that testosterone is necessary, but not sufficient, to support the high levels of aggression in nNOS$^{-/-}$ mice as compared to WT mice. No sensory or motor deficiencies were observed that could account for the elevated aggression or mounting behavior among the nNOS$^{-/-}$ mice.

Both reproductive and aggressive behaviors in males are generally modulated, if not regulated, by androgens, presumably because defense of resources and competition are critical for reproductive success (Simon, 2002). However, non-gonadal mechanisms may have evolved to regulate aggression in animals living in habitats that require competition outside of the breeding season (e.g. Soma and Wingfield, 2001). For example, Siberian hamsters (*Phodopus sungorus*) and Syrian hamsters (*Mesocricetus aruatus*) display an 'atypical' seasonal pattern of aggressive behavior. Males that reduce gonadal size and function after exposure to short, winter-like days have low circulating testosterone concentrations, but display elevated aggression as compared to animals with large functional gonads and relatively high testosterone values (Garrett and Campbell, 1980; Jasnow et al., 2000; Jasnow et al., 2002). Furthermore, exogenous administration of testosterone (T) does not increase aggression in short-day males as occurs in males of other species (Jasnow et al., 2000).

In a recent study, nNOS expression in the brains of long- and short-day Siberian hamsters (*P. sungorus*) was examined after assessment of aggressive behavior (Wen et al., 2004). The reproductive response to short days varies among individuals of this species: Short-day responsive hamsters inhibit reproductive function and have undetectable testosterone concentrations, whereas short-day non-responsive hamsters display fully functional gonads and long-day testosterone blood concentrations. Regardless of gonadal response to short days, all hamsters housed in short photoperiods were more aggressive than long-day animals. These results support the hypothesis that the short-day mediated aggression is independent of circulating testosterone, because testes size and testosterone concentrations of the short day non-responders were similar to long-day animals. Short-day Siberian hamsters, again regardless of reproductive response, also displayed significantly fewer nNOS-immunoreactive cells in several amygdala regions compared to long-day animals. The results are also consistent with the hypothesis that reductions in NO drive elevated aggression. Together, these results suggest seasonal aggression in male Siberian hamsters is regulated by photoperiod, probably independently from gonadal steroid hormones, and may be regulated by nNOS.

Female mice do not typically show much territorial aggression, and aggression in nulliparous female nNOS$^{-/-}$ mice was equivalent to WT animals. However, female mice are more likely to engage in aggression when guarding their nest. In contrast to our predictions, female nNOS$^{-/-}$ mice were much less likely to display maternal aggression (but not other maternal behaviors) than WT females (Gammie and Nelson, 1999; Gammie et al., 2000). Again, there were no dramatic sensorimotor deficits among the mutant mice to account for the changes in aggressive behavior. Taken together, these results suggest that neuronal NO has important, but opposite, effects in the mediation of aggression in male and female mice. Although there are no sex differences in NOS activity in the cortex, cerebellum, amygdala, or hypothalamus, androgens generally inhibit and estrogens generally increase NOS activity in the brain (Weiner et al., 1994; Singh et al., 2000).

2.2. Endothelial nitric oxide synthase (eNOS) and aggression

In addition to the effects of NO generated from nNOS on aggression, we also evaluated the role of NO from eNOS on aggression. Specifically, we hypothesized that NO from the endothelial tissue could also contribute to aggressive behavior. To test this hypothesis mice with targeted disruption of the gene encoding the endothelial isoform of NOS (eNOS$^{-/-}$) were examined (Huang et al., 1995; Demas et al., 1999). Because NO was originally identified as endothelium-derived relaxing factor (Ignarro, 1990), and eNOS is localized in the endothelial lining of vascular smooth muscle, affected blood pressure was the first phenotype investigated in these mutant mice (Huang et al., 1995). eNOS$^{-/-}$ mice exhibit ~35% increase in basal blood pressure relative to WT mice (Huang et al., 1995).

Initial anecdotal observations in our laboratory suggested that eNOS knockout mice were very docile. To examine this observation more rigorously, animals were tested using two behavioral paradigms. First, animals were tested using the resident-intruder model; eNOS$^{-/-}$ mice displayed virtually no aggressive encounters and a dramatic decreased duration of agonistic encounters relative to WT mice when a WT intruder was placed into their home cage. Likewise, when tested in a neutral arena with a WT stimulus male, eNOS$^{-/-}$ mice displayed many fewer attacks and a greatly increased latency to attack the stimulus male relative to WT mice (Demas et al., 1999). These results were not simply due to hypertension because pharmacological normalization of blood pressure did not affect the absence of aggression in eNOS$^{-/-}$ mice. These data, in combination with the nNOS$^{-/-}$ data, suggest that the two isoforms of NOS may normally act to increase (eNOS$^{-/-}$) and decrease

($nNOS^{-/-}$) aggressive behavior in vivo. Thus, WT mice with normal concentrations of both isoforms of NOS display only moderate levels of aggression.

2.3. Cautionary aside regarding knockout mice

Although the results of the studies discussed above clearly implicate a role for NO in aggression, a conceptual problem with behavioral studies of mice with targeted genetic deletions, that is shared with all ablation studies, is that behavioral tests reveal the effects of the *missing* gene (and gene product), not the effects of the gene directly (Nelson, 1997). This conceptual short-coming can be overcome in the same way that it is overcome in other types of ablation studies, by collecting convergent evidence from a variety of methods. For example, if similar behavioral deficits are obtained after pharmacological, lesion, and genetic manipulation of the same factor, then it is reasonable to conclude that the missing factor is involved in the behavior, especially if the behavioral deficit is ameliorated when the missing factor is restored. One significant advantage to using knockout animals in behavior research is that the effects of the gene product can be abolished without the side-effects of drugs (Crawley, 2000). This is particularly important in manipulation of NO because many drugs that non-specifically block synthesis of NO affect nitrogen-dependent physiological processes, and have pronounced effects on blood pressure (Dawson and Snyder, 1994; Dawson and Dawson, 1996). Such physiological effects can obviously confound behavioral processes mediated by NO. It is also important to validate behavioral findings on mutant mice on non genetically manipulated animals.

In order to investigate whether the increased aggressive behavior of the mutants was due to the missing gene during the development of the brain with subsequent activation of compensatory mechanisms (Nelson, 1997), WT C57/B6 male mice were treated with 7-nitroindazole (7-NI) (50 mg/kg ip), a relatively specific drug that blocks nNOS activity in vivo (Demas et al., 1997). Indeed, a relative lack of NOS activity in brain homogenates of 7-NI-treated animals was revealed by immunocytochemical staining for citrulline, an indirect marker for the NO synthesis (Demas et al., 1997). Male mice treated with 7-NI exhibited substantially increased aggressive behavior in two different aggression tests (neutral arena and resident-intruder test) as compared to control animals, with no alteration in other locomotor activities, implying an specific effect in aggression and ruling out the contribution of strain differences in the knockout mice in the aggressive behavioral phenotype (Demas et al., 1997). These pharmacological data extend the behavioral results obtained in $nNOS^{-/-}$ mice and confirm a role of NO in aggression.

3. Serotonin and aggression

Both pharmacological and clinical approaches have identified serotonin (5-HT) as an important central neurotransmitter system involved in aggression and impulsivity. For example, elevated aggression in humans is correlated with low cerebrospinal fluid concentrations of 5-hydroxyindoleacetic acid (5-HIAA) (5-HT metabolite) and a blunted prolactin response to a 5-HT agonist (fenfluramine challenge) (reviewed in Manuck et al., 1998; Miczek et al., 2001; Nelson and Chiavegatto, 2001). Aggressive laboratory animals display reduced brain 5-HT turnover (reviewed in Miczek et al., 2001; Nelson and Chiavegatto, 2001). The inverse relationship between 5-HT system activity and aggressive behavior may be causally linked as suggested by pharmacological manipulation of brain 5-HT concentrations. Drugs that inhibit 5-HT synthesis, tryptophan-free diets, or lesions of 5-HT neurons decrease 5-HT levels and elevate aggression in laboratory animals (reviewed Chiavegatto et al., 2001). In contrast, decreased aggression occurs after treatment with 5-HT precursors, 5-HT reuptake inhibitors, 5-HT releasing agents, in addition to 5-HT_{1A} and 5-HT_{1B} agonists (Chiavegatto et al., 2001; Olivier et al., 1995; de Almeida et al., 2001; Miczek and de Almeida, 2001). Gene targeting strategies in mice that either directly or indirectly affect the functional integrity of the 5-HT system have generally supported the hypothesis that 5-HT modulates aggression (reviewed by Miczek et al., 2001; Nelson and Chiavegatto, 2001).

Serotonin neurotransmission was hypothesized to be disrupted in the aggressive nNOS null mice because of the inverse relation of 5-HT system activity and aggression. Serotonin metabolism, analyzed by the ratio of the metabolite 5-HIAA and 5-HT levels by HPLC, was significantly reduced in several brain regions including the cortex, hypothalamus, midbrain, and cerebellum of $nNOS^{-/-}$ in comparison to WT mice (Chiavegatto et al., 2001). The alterations in 5-HT turnover were due to increased concentrations of 5-HT with no changes in its metabolite in most brain regions studied. The disturbed neurochemical profile appears specific to the 5-HT system, because norepinephrine, dopamine, and metabolites were generally unaffected. Although monoamine oxidase has been implicated in aggression, the normal values of norepinephrine and dopamine data suggest that it is unlikely that alterations in monoamine oxidase account for the 5-HT abnormalities in the nNOS knockout mice (Chiavegatto and Nelson, 2003). Further work is necessary to understand the relationship between 5-HT and NO in mediating aggression.

3.1. Anxiety and aggression

Assessing emotional behavior in non-human animals presents special challenges because it is not possible to directly infer what a laboratory animal is experiencing in a given situation. Despite this limitation, the use of carefully

designed tests can allow investigators to examine behavioral responses that mimic a component or symptom of human conditions (Crawley, 2000). Common tests used to measure anxiety-related behaviors are the elevated plus maze, light/dark box, and the exploration the center of an open-field. These tests assess the motivation to explore novel areas versus the preference to avoid brightly lit and/or open areas. Mice that exhibit low levels of anxiety-related behavior spend more time in open arms of the plus maze, more time in the brightly lit chamber of the exploration box, and the central area of the open-field. Behavioral responses in these tests are modulated by benzodiazepines (Crawley, 1981), which are used to alleviate anxiety in humans.

Studies trying to correlate anxiety-related and aggressive behavior are not conclusive. For example, there is evidence that elevated aggression is related to low anxiety (reviewed in Olivier et al., 1995; Miczek et al., 2003). Genetically selected aggressive mice display fewer anxiety-like behaviors on standard tests than mice selected to be non-aggressive (Nyberg et al., 2003). On the other hand, there is also evidence that high anxiety provokes elevated aggression (reviewed in Blanchard et al., 2001). Unpredictable chronic mild stress increases aggression both in a resident-intruder test and between cage-mates (Mineur et al., 2003). Behavioral assessment of adenosine A_1 receptor knockout mice ($A_1R^{-/-}$) revealed elevated anxiety-like behaviors and increased aggressiveness in the resident-intruder test (Gimenez-Llort et al., 2002). The ambiguous relationship between aggression and anxiety is reflected in studies examining the relationship between anxiety behavior and NO signaling.

Changes in nitric oxide signaling have been reported either to increase or decrease anxiety-related behavioral responses in elevated plus-maze and light/dark exploration tests. Systemic injections of N-nitro-L-arginine methyl ester (L-NAME), which interferes with several arginine-dependent processes including production of NO, increased anxiety-like behaviors in elevated plus maze, light-dark, and hole-board tests (Czech et al., 2003), suggesting that the observed changes in anxiety-like behavior were not artifacts of general motor deficits. A study examining the effects of an NO donor found similar results, as intracerebroventricular (icv) treatment of 3 and 1 μg of 3-morpholinosyndnonimine (SIN-1) increased the time spent in the light compartment in a light/dark exploration test (Li and Quock, 2002). In contrast, acute (Volke et al., 1998) and sub-chronic (Dunn et al., 1998) systemic treatment with the NOS inhibitor, 7-nitroindazole (7-NI), increased the time spent in the open arms of a plus maze. Intraperitoneal injections of NO donors and precursors also blocked the anxiolytic effects of diazepam (Volke et al., 1998) and morphine (Shin et al., 2003) in the elevated plus maze. There have also been some variable results in elevated plus maze tests of $nNOS^{-/-}$ mice. Two studies reported no differences between $nNOS^{-/-}$ and WT mice in the plus maze (Nelson et al., 1995; Bilbo et al., 2003;), whereas a third study found that $nNOS^{-/-}$ mice spent less time in the open arms than WT mice (Bilbo et al., 2003; Weitzdoefer et al., 2004). Although the direction of the effects is unclear, it seems likely that NO signaling has a role in the regulation of anxiety-like behavior in rodents.

One step in understanding the precise contribution of NO signaling to anxiety-like behavior may be to use more anatomically precise manipulations. Nitric oxide synthase has been identified in areas of the hypothalamus, amygdala, and hippocampus that regulate both aggressive behavior and responses to anxiogenic stimuli (Vincent and Kimura, 1992). When NG-nitro-L-arginine (NOARG), another inhibitor of NO production, was infused into either the amygdala or hippocampus of rats, a decrease in the time spent in the open arms of the plus maze was observed (Monzon et al., 2001). Given the wide distribution of NOS in the brain, it is likely that the production of NO in different parts of the brain differentially affects behavior.

3.2. HPA axis, stress, and aggression

Considerable evidence in rodents suggests the hypothalamo-pituitary-adrenal (HPA) axis regulates both stress responses and aggression in mammalian species (Haller and Kruk, 2003). Both increased and decreased activity of the HPA axis can affect aggressive behavior. Chronic activation of the HPA axis and the subsequent release of glucocorticoids (GC) generally appears to curtail aggressive behavior (Haller and Kruk, 2003). For example, animals exposed to prolonged stressors display chronically elevated circulating corticosterone concentrations and decreased aggression, whereas animals with GC hypofunction display pathologically high levels of aggression (Haller and Kruk, 2003). In contrast, acute activation of the HPA axis can increase aggression in rodents; e.g., stimulation of hypothalamic brain regions can evoke aggression in addition to rapid activation of the HPA axis (Kruk et al., 1998). The switch point between acute and chronic HPA activation, however, remains unspecified. Because the relationship between NO and the HPA axis and the relationship between the HPA axis on aggressive behavior, it seems reasonable to suggest that NO may be involved in mediating aggression via the HPA axis.

Despite substantial research, the role of NO in the regulation of the HPA axis remains undetermined (Givalois et al., 2002). NOS is present in discrete hypothalamic areas (i.e., supraoptic nuclei (SON) and paraventricular nuclei (PVN)) that regulate neuroendocrine responses (Huang et al., 1993), and stimuli that affect pituitary hormone release (i.e., stress, food deprivation, gonadectomy, exposure to endotoxin) can up-regulate nNOS expression. HPA activity is regulated primarily by the actions of the hypothalamic neuropeptide corticotropin releasing hormone (CRH), which acts on the pituitary to trigger the release of the tropic hormone ACTH. ACTH in turn, regulates the release of glucocorticoids (GCs) from the adrenal cortex. Numerous factors act at the level of hypothalamus or

pituitary to affect the release of CRH or ACTH, respectively. For example, the cytokines interleukin-1 (IL-1), IL-1β, IL-2, and IL-2β increase CRH release both in vitro and in vivo (McCann et al., 2000). In vivo treatment with IL-1β increases hypothalamic CRH release, as well as plasma ACTH and corticosterone concentrations; IL-1β-induced activation of the HPA can be attenuated, however, by pre-treatment with the NOS inhibitor L-NAME (Rivier and Shen, 1994).

In addition to the effects of cytokines on HPA activity, several neurotransmitters/ neurohormones (e.g., acetylcholine, norepinephrine, prostaglandins) affect CRH secretion and NO has been implicated as a potential neuroendocrine mediator (Nelson et al., 1997). In contrast, endotoxin-induced increases in plasma ACTH and corticosterone are enhanced by central pre-treatment with L-NAME (Imaki and Rivier, 1999; Rivier, 2000). Despite the contradictory results regarding the specific effects of NO on HPA activity, NO exerts a significant role at the level of the HPA, and NO may modulate CRH release differentially depending on whether the stressor is extrinsic or intrinsic (Bilbo et al., 2003).

The effects of restraint on antigen-specific delayed-type-hypersensitivity (DTH) responses in the skin are blunted in $nNOS^{-/-}$ mice, and they lost less body mass after stress than WT mice (Bilbo et al., 2003). Neuronal NO appears to be involved in the neuroendocrine-immune response to stress, perhaps via GC regulation. Despite elevated corticosterone concentrations, nNOS knockout mice appear less 'anxious' or 'fearful' than WT mice, which may contribute to their aggressiveness; e.g., male $nNOS^{-/-}$ mice spend more time in the center of the open field than WT mice (Bilbo et al., 2003).

3.3. Pain and aggression

The relationship between pain and aggression appears to be bi-directional; pain often increases aggression (Bigi et al., 1993; Matto et al., 1998), and aggressive encounters often alters pain perception (Kulling et al., 1988; Vivian and Miczek, 1999). Alterations in neurochemical expression at many levels of this pathway could result in contextually inappropriate aggressive behavior, accompanied by altered pain perception. To determine the interaction among NO, pain, and aggression, $nNOS^{-/-}$ mice were tested on a traditional model of pain perception, *viz.*, the timed hot plate. nNOS knockout mice display increased sensitivity to painful stimuli on a hotplate (Rivera and Nelson, unpublished observations). The extent to which this increased pain responsiveness contributes to the aggressive phenotype of nNOS knockout mice remains to be determined.

3.4. Depression and aggression

Elevated aggression is often associated with depression (Harvey et al., 2003). Growing evidence suggests that NO might mediate the link between aggression and depression (McLeod et al., 2001). In humans, postmortem comparisons of brains of depressed people reveal less nNOS staining in the suprachiasmatic (SCN) and paraventricular nuclei (PVN) than in brains from age-matched people who had no history of psychiatric or neurological diseases (Bernstein et al., 2002). Blood NO concentrations were also decreased in patients with major depression (Selley, 2004).

Several tests have been developed to assess depression-like behavior in rodent model systems. The most common test is the Porsolt forced swim test (Crawley, 2000; Porsolt et al., 1977). Mice are introduced into a pool of water, and they generally swim vigorously, apparently looking for an exit. Some mice eventually stop swimming and float in the water, appearing that they are 'giving up'. A second paradigm is the learned helplessness test in which mice are first exposed to inescapable shocks (Maier, 1984; Shanks and Anisman, 1993). The mice are then tested in a standard active avoidance test where shocks can be avoided by moving from one chamber to an adjacent chamber. The failure to avoid the shocks in the active avoidance test is interpreted as behavioral despair. Antidepressant drugs reduce the amount of time spent floating in the Porsolt test and increase active avoidance of shocks in the learned helplessness paradigm, suggesting a link with aspects of human behavior. Similar to antidepressants, the selective nNOS inhibitors 7-NI and 1-(2-trifluoromethylphenyl) imidazole (TRIM) decreased the immobility time in the forced swimming test (Yildiz et al., 2000; Volke et al., 2003). The magnitude of the effect mimicked that of the tricyclic antidepressant imipramine (Volke et al., 2003). Treatment with a non-specific NOS inhibitor also decreases immobility time in the forced swim test (Harkin et al., 1999). Male $eNOS^{-/-}$ mice were more likely to avoid aversive stimuli in learned helplessness tests, but did not differ from WT mice in forced-swim or dark/light box tests (Reif et al., 2004).

Most antidepressants used today increase 5-HT in the brain by inhibiting 5-HT reuptake, suggesting that the antidepressive effects of NO may be related to 5-HT function. Male $nNOS^{-/-}$ mice possess reduced 5-HT turnover rates and deficient serotonin 5-HT_{1A} and 5-HT_{1B} receptors function in several brain areas regulating emotion, including the hypothalamus, hippocampus, and amygdala (Chiavegatto et al., 2001) (and see below). Serotonin dysfunction may be responsible for the observed increases in aggression and impulsivity in these mice (Chiavegatto et al., 2001). Interestingly, patients exhibiting depressive symptoms, which may include hyperaggression and impulsivity, often possess 5-HT dysfunction, as well as significantly elevated cortisol concentrations and decreased negative feedback mechanisms (Harris et al., 2000; Tafet et al., 2001; Tafet et al., 2001). Unexpectedly, despite the reduced 5-HT turnover and increased plasma corticosterone concentration, $nNOS^{-/-}$ mice display fewer 'depressive-like' responses in the Porsolt swim test

(M. Gatien and R.J. Nelson, unpublished data). That is, nNOS$^{-/-}$ mice swim longer and display less 'float times' than WT mice. This may reflect their general perseveration in all behaviors, including fighting and mating, rather than a manic state.

An interesting connection between aggression and depression may involve neurogenesis in the hippocampus. There is growing evidence that variability in neurogenesis may be related to aggression. A gene chip study reported that mice bred for long attack latency in a resident-intruder aggression test had upregulated cytoskeleton transcripts (α-tubulin and cofilin) in the brain compared to mice bred to have a short attack latency (Feldker et al., 2003). These cytoskeleton transcripts are upregulated during neuronal growth, and were confirmed with in situ hybridization to be upregulated in the CA1 region of the hippocampus (Feldker et al., 2003).

Several magnetic imaging studies indicate that patients diagnosed with major depression have smaller hippocampal volumes compared to non-depressed individuals (Sheline et al., 1999; Bremner et al., 2000; Mervaala et al., 2000). One hypothesis for these results is that the neurotoxic effects of the chronically upregulated glucocorticoid activity often associated with depression could lead to increased cell death in the hippocampus (Sapolsky, 2000).

How NO signaling would mediate any connections between depression and aggression remains unspecified; different groups have reported that NO either inhibits (Packer et al., 2003; Moreno-Lopez et al., 2004) or promotes (Reif et al., 2004) neurogenesis. The increased aggression of nNOS$^{-/-}$ mice has been proposed to reflect impaired olfactory discrimination because of decreased neurogenesis in the olfactory bulb (Moreno-Lopez et al., 2004). eNOS male mice are less aggressive (Demas et al., 1999), and they display increased neurogenesis (Reif et al., 2004). Although an interesting hypothesis, nNOS$^{-/-}$ mice do not display olfactory impairments in standard tests (Nelson et al., 1995), and olfactory bulbectomy in male mice eliminates aggressive behavior (Neckers et al., 1975). Additional work is necessary to understand the relationship among NO, neurogenesis, and aggression.

3.5. Behavioral persistence

The depletion of nNOS has many behavioral effects. When the different effects are compared, it is noteworthy that behavior is often exaggerated rather than inhibited. Male nNOS$^{-/-}$ mice display persistent aggression, mating behavior, and persistent swimming in the Porsolt test. These observations suggest that a loss of nNOS functioning may affect a general system of behavioral inhibition. It has been suggested that the 5-HT system plays an important role in behavioral inhibition, such that deficits in 5-HT function can result in impulsive behavior, and aggression in particular (Krakowski, 2003). Although the term 'impulsive' is frequently used in psychological literature, there is no consensus on a definition. Most definitions agree that impulsiveness is characterized by a lack of inhibition, with a given behavioral response occurring either without assessment of context or with an inability to await larger rewards (Krakowski, 2003; Swann et al., 2002). Despite ambiguity in impulsiveness, many studies have found this trait to be associated with reduced 5-HT activity. In humans low 5-HT function is associated with a several forms of impulsive behavior including violent crime (Linnoila et al., 1989), gambling (DeCaria et al., 1996), and suicide (Mann, 1998). Free-living rhesus macaques with low cerebrospinal fluid 5-HIAA (the major metabolite of 5-HT) were more likely to take long, dangerous leaps in the forest canopy (Higley et al., 1996; Higley et al., 1996) and were more likely to die prematurely (Higley et al., 1996; Higley et al., 1996).

Impulsive-like behavior has been evaluated in rodents by testing animals under conditions in which delaying a response (e.g. bar press) leads to a larger subsequent reward than an immediate response. One frequently used test requires test subjects to respond to target lights by nose-poking or pressing a lever 5 s after the signal to receive a food reward, with premature responses yielding no reward (Robbins, 2002). Lesioning of 5-HT neurons in rats with 5,7-dihydroxytryptamine increases premature responding to target lights (Harrison et al., 1997). 5-HT$_{1B}$ receptor knockout mice consistently responded prematurely in a delayed response operant conditioning task (Pattij et al., 2003). Many of the behavioral effects of nNOS deletion can be interpreted in part as a reduction in behavioral inhibition. Given the reduced 5-HT function in nNOS knockouts, this suggests that nNOS may modulate the effects of 5-HT on impulsive-like behavior in rodents.

4. Conclusions

Aggressive behavior is not a unitary process, but is the result of complex interactions among several physiological, motivational, and behavioral systems, with contributions from both the social and physical environment. The multiple, and often unanticipated, effects of targeted gene disruption on aggressive behavior must be considered when phenotyping a gene manipulation. Although many other molecules can affect aggressive behavior (Chiavegatto et al., 2001), most agents likely influence aggression via the signaling properties of 5-HT, or possibly GABA.

Interactions between NO and the HPA axis, as well as between NO and 5-HT mechanisms have been implicated in aggressive behavior. Future studies must consider the environmental (social and physical), hormonal, cellular, contributions to aggressive behavior to understand the molecular mechanisms. Hypothetical psychological constructs such as fear, hunger, anxiety, and depression also affect the molecular mechanisms of aggression. A variety of subtle adjustments in 5-HT concentrations, turnover, and metabolism, or slight changes in receptor subtype

activation, density, and binding affinity alone or in combination, and possibly mediated by NO, can influence aggression in different ways by affecting inputs into aggression circuitries. Because of the complexity of aggressive behavior, it is likely that multiple changes in 5-HT signaling are associated with different types of aggression. The integrity of this complex pathway seems necessary for normal expression and termination of aggressive behavior (Chiavegatto and Nelson, 2003).

Future studies using gene arrays, inducible gene knock-outs and knock-ins, and RNA silencing techniques may be necessary to untangle the multiple influences of various molecules on aggressive behavior. Multiple levels of analysis, as well as comparative research approaches are necessary to completely reveal the contributions of NO to the molecular bases of aggressive behavior.

Acknowledgements

We thank Zachary Weil and Brenda Reader for bibliographic assistance, and Dr. A.C. DeVries for valuable comments. Support for the studies reported from our laboratory and for writing this review was provided by NIH Grants MH 57535 and MH57760 (RJN). SC acknowledges FAPESP-Brazil support (01/09079-1).

References

Albert, D.J., Walsh, M.L., 1984. Neural systems and the inhibitory modulation of agonistic behavior: a comparison of mammalian species. Neurosci. Biobehav. Rev. 8, 5–24.

Azcarate, C.L., 1975. Minor tranquilizers in the treatment of aggression. J. Nerv. Ment. Dis. 160, 100–107.

Bernstein, H.G., Heinemann, A., Krell, D., Mawrin, C., Bielau, H., Danos, P., Diekmann, S., Keilhoff, G., Bogerts, B., Baumann, B., 2002. Further immunohistochemical evidence for impaired NO signaling in the hypothalamus of depressed patients. Ann. NY Acad. Sci. 973, 91–93.

Bigi, S., De Acetis, L., De Simone, R., Aloe, L., Alleva, E., 1993. Neonatal capsaicin exposure affects isolation-induced aggressive behavior and hypothalamic substance P levels of adult male mice (*Mus musculus*). Behav. Neurosci. 107, 363–369.

Bilbo, S.D., Hotchkiss, A.K., Chiavegatto, S., Nelson, R.J., 2003. Blunted stress responses in delayed type hypersensitivity in mice lacking the neuronal isoform of nitric oxide synthase. J. Neuroimmunol. 140, 41–48.

Blanchard, D.C., Griebel, G., Blanchard, R.J., 2001. Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. Neurosci. Biobehav. Rev. 25, 205–218.

Bremner, J.D., Narayan, M., Anderson, E.R., Staib, L.H., Miller, H.L., Charney, D.S., 2000. Hippocampal volume reduction in major depression. Am. J. Psychiatry 157, 115–118.

Chiavegatto, S., Nelson, R.J., 2003. Interaction of nitric oxide and serotonin in aggressive behavior. Horm. Behav. 44, 233–241.

Chiavegatto, S., Dawson, V.L., Mamounas, L.A., Koliatsos, V.E., Dawson, T.M., Nelson, R.J., 2001. Brain serotonin dysfunction accounts for aggression in male mice lacking neuronal nitric oxide synthase. Proc. Natl Acad. Sci. U.S.A. 98, 1277–1281.

Citrome, L., Volavka, J., 1997a. Psychopharmacology of violence: Part I: Assessment and acute treatment. Psychiatr. Ann. 27, 691–695.

Citrome, L., Volavka, J., 1997b. Psychopharmacology of violence: Part II: Beyond the acute episode. Psychiatr. Ann. 27, 696–703.

Crawley, J.N., 1981. Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines. Pharmacol. Biochem. Behav. 15, 695–699.

Crawley, J.N., 2000. What's Wrong With My Mouse. Wiley, New York.

Czech, D.A., Jacobson, E.B., LeSueur-Reed, K.T., Kazel, M.R., 2003. Putative anxiety-linked effects of the nitric oxide synthase inhibitor L-NAME in three murine exploratory behavior models. Pharmacol. Biochem. Behav. 75, 741–748.

Dawson, T.M., Dawson, V.L., 1996. Nitric oxide synthase: role as a transmitter/mediator in the brain and endocrine system. Annu. Rev. Med. 47, 219–227.

Dawson, T.M., Snyder, S.H., 1994. Gases as biological messengers: nitric oxide and carbon monoxide in the brain. J. Neurosci. 14, 5147–5159.

de Almeida, R.M., Nikulina, E.M., Faccidomo, S., Fish, E.W., Miczek, K.A., 2001. Zolmitriptan-a 5-HT_{1B}/D agonist, alcohol, and aggression in mice. Psychopharmacology (Berl) 157, 131–141.

DeCaria, C.M., Hollander, E., Grossman, R., Wong, C.M., Mosovich, S.A., Cherkasky, S., 1996. Diagnosis, neurobiology, and treatment of pathological gambling. J. Clin. Psychiatry 57 (Suppl. 8), 80–83 discussion 83–84.

Demas, G.E., Eliasson, M.J., Dawson, T.M., Dawson, V.L., Kriegsfeld, L.J., Nelson, R.J., Snyder, S.H., 1997. Inhibition of neuronal nitric oxide synthase increases aggressive behavior in mice. Mol. Med. 3, 610–616.

Demas, G.E., Kriegsfeld, L.J., Blackshaw, S., Huang, P., Gammie, S.C., Nelson, R.J., Snyder, S.H., 1999. Elimination of aggressive behavior in male mice lacking endothelial nitric oxide synthase. J. Neurosci. 19, RC30.

Dunn, R.W., Reed, T.A., Copeland, P.D., Frye, C.A., 1998. The nitric oxide synthase inhibitor 7-nitroindazole displays enhanced anxiolytic efficacy without tolerance in rats following subchronic administration. Neuropharmacology 37, 899–904.

Feldker, D.E., Datson, N.A., Veenema, A.H., Meulmeester, E., de Kloet, E.R., Vreugdenhil, E., 2003. Serial analysis of gene expression predicts structural differences in hippocampus of long attack latency and short attack latency mice. Eur. J. Neurosci. 17, 379–387.

Gammie, S.C., Nelson, R.J., 1999. Maternal aggression is reduced in neuronal nitric oxide synthase-deficient mice. J. Neurosci. 19, 8027–8035.

Gammie, S.C., Olaghere-da Silva, U.B., Nelson, R.J., 2000. 3-Bromo-7-nitroindazole, a neuronal nitric oxide synthase inhibitor, impairs maternal aggression and citrulline immunoreactivity in prairie voles. Brain Res. 870, 80–86.

Garrett, J.W., Campbell, C.S., 1980. Changes in social behavior of the male golden hamster accompanying photoperiodic changes in reproduction. Horm. Behav. 14, 303–318.

Gimenez-Llort, L., Fernandez-Teruel, A., Escorihuela, R.M., Fredholm, B.B., Tobena, A., Pekny, M., Johansson, B., 2002. Mice lacking the adenosine A1 receptor are anxious and aggressive, but are normal learners with reduced muscle strength and survival rate. Eur. J. Neurosci. 16, 547–550.

Givalois, L., Li, S., Pelletier, G., 2002. Central nitric oxide regulation of the hypothalamic-pituitary-adrenocortical axis in adult male rats. Brain Res. Mol. Brain Res. 102, 1–8.

Haller, J., Kruk, M.R., 2003. Neuroendocrine stress responses and aggression. In: Mattson, M.P. (Ed.), Neurobiology of Aggression: Understanding and Preventing Violence. Humana Press, New Jersey, pp. 93–118.

Harkin, A.J., Bruce, K.H., Craft, B., Paul, I.A., 1999. Nitric oxide synthase inhibitors have antidepressant-like properties in mice. 1. Acute treatments are active in the forced swim test. Eur. J. Pharmacol. 372, 207–213.

Harris, T.O., Borsanyi, S., Messari, S., Stanford, K., Cleary, S.E., Shiers, H.M., Brown, G.W., Herbert, J., 2000. Morning cortisol as a risk factor for subsequent major depressive disorder in adult women. Br. J. Psychiatry 177, 505–510.

Harrison, A.A., Everitt, B.J., Robbins, T.W., 1997. Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: interactions with dopaminergic mechanisms. Psychopharmacology (Berl) 133, 329–342.

Harvey, B.H., McEwen, B.S., Stein, D.J., 2003. Neurobiology of antidepressant withdrawal: implications for the longitudinal outcome of depression. Biol. Psychiatry 54, 1105–1117.

Higley, J.D., Mehlman, P.T., Higley, S.B., Fernald, B., Vickers, J., Lindell, S.G., Taub, D.M., Suomi, S.J., Linnoila, M., 1996a. Excessive mortality in young free-ranging male nonhuman primates with low cerebrospinal fluid 5-hydroxyindoleacetic acid concentrations. Arch. Gen. Psychiatry 53, 537–543.

Higley, J.D., Mehlman, P.T., Poland, R.E., Taub, D.M., Vickers, J., Suomi, S.J., Linnoila, M., 1996b. CSF testosterone and 5-HIAA correlate with different types of aggressive behaviors. Biol. Psychiatry 40, 1067–1082.

Huang, P.L., Dawson, T.M., Bredt, D.S., Snyder, S.H., Fishman, M.C., 1993. Targeted disruption of the neuronal nitric oxide synthase gene. Cell 75, 1273–1286.

Huang, P.L., Huang, Z., Mashimo, H., Bloch, K.D., Moskowitz, M.A., Bevan, J.A., Fishman, M.C., 1995. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature 377, 239–242.

Ignarro, L.J., 1990. Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu. Rev. Pharmacol. Toxicol. 30, 535–560.

Imaki, T., Rivier, C.L., 1999. Nitric oxide and carbon monoxide have a stimulatory role in the paraventricular nucleus following lipopolysaccharide injection 821, 2244–2253.

Jasnow, A.M., Huhman, K.L., Bartness, T.J., Demas, G.E., 2000. Short-day increases in aggression are inversely related to circulating testosterone concentrations in male Siberian hamsters (*Phodopus sungorus*). Horm. Behav. 38, 102–110.

Jasnow, A.M., Huhman, K.L., Bartness, T.J., Demas, G.E., 2002. Short days and exogenous melatonin increase aggression of male Syrian hamsters (*Mesocricetus auratus*). Horm. Behav. 42, 13–20.

Krakowski, M., 2003. Violence and serotonin: influence of impulse control, affect regulation, and social functioning. J. Neuropsychiatry Clin. Neurosci. 15, 294–305.

Kriegsfeld, L.J., Dawson, T.M., Dawson, V.L., Nelson, R.J., Snyder, S.H., 1997. Aggressive behavior in male mice lacking the gene for neuronal nitric oxide synthase requires testosterone. Brain Res. 769, 66–70.

Kruk, M.R., Westphal, K.G., Van Erp, A.M., van Asperen, J., Cave, B.J., Slater, E., de Koning, J., Haller, J., 1998. The hypothalamus: cross-roads of endocrine and behavioural regulation in grooming and aggression. Neurosci. Biobehav. Rev. 23, 163–177.

Kulling, P., Frischknecht, H.R., Pasi, A., Waser, P.G., Siegfried, B., 1988. Social conflict-induced changes in nociception and beta-endorphin-like immunoreactivity in pituitary and discrete brain areas of C57BL/6 and DBA/2 mice. Brain Res. 450, 237–246.

Li, S., Quock, R.M., 2002. Effects of a nitric oxide donor on behavior and interaction with nitrous oxide in the mouse light/dark exploration test. Eur. J. Pharmacol. 447, 75–78.

Linnoila, M., DeJong, J., Virkkunen, M., 1989. Monoamines, glucose metabolism, and impulse control. Psychopharmacol. Bull. 25, 404–406.

Maier, S.F., 1984. Learned helplessness and animal models of depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 8, 435–446.

Mann, J.J., 1998. The neurobiology of suicide. Nat. Med. 4, 25–30.

Manuck, S.B., Flory, J.D., McCaffery, J.M., Matthews, K.A., Mann, J.J., Muldoon, M.F., 1998. Aggression, impulsivity, and central nervous system serotonergic responsivity in a nonpatient sample. Neuropsychopharmacology 19, 287–299.

Matto, V., Skrebuhhova, T., Allikmets, L., 1998. The effect of antidepressants on rat aggressive behavior in the electric footshock and apomorphine-induced aggressiveness paradigms. Methods Find. Exp. Clin. Pharmacol. 20, 329–337.

McCann, S.M., Kimura, M., Karanth, S., Yu, W.H., Mastronardi, C.A., Rettori, V., 2000. The mechanism of action of cytokines to control the release of hypothalamic and pituitary hormones in infection. Ann. NY Acad. Sci. 917, 4–18.

McLeod, T.M., Lopez-Figueroa, A.L., Lopez-Figueroa, M.O., 2001. Nitric oxide, stress, and depression. Psychopharmacol. Bull. 35, 24–41.

Mervaala, E., Fohr, J., Kononen, M., Valkonen-Korhonen, M., Vainio, P., Partanen, K., Partanen, J., Tiihonen, J., Viinamaki, H., Karjalainen, A.K., Lehtonen, J., 2000. Quantitative MRI of the hippocampus and amygdala in severe depression. Psychol. Med. 30, 117–125.

Miczek, K.A., de Almeida, R.M., 2001. Oral drug self-administration in the home cage of mice: alcohol-heightened aggression and inhibition by the 5-HT_{1B} agonist anpirtoline. Psychopharmacology (Berl) 157, 421–429.

Miczek, K.A., Maxson, S.C., Fish, E.W., Faccidomo, S., 2001. Aggressive behavioral phenotypes in mice. Behav. Brain Res. 125, 167–181.

Miczek, K.A., Fish, E.W., De Bold, J.F., 2003. Neurosteroids, GABAA receptors, and escalated aggressive behavior. Horm. Behav. 44, 242–257.

Mineur, Y.S., Prasol, D.J., Belzung, C., Crusio, W.E., 2003. Agonistic behavior and unpredictable chronic mild stress in mice. Behav. Genet. 33, 513–519.

Moncada, S., Higgs, A., 1993. The L-arginine-nitric oxide pathway. N. Engl. J. Med. 329, 2002–2012.

Monzon, M.E., Varas, M.M., De Barioglio, S.R., 2001. Anxiogenesis induced by nitric oxide synthase inhibition and anxiolytic effect of melanin-concentrating hormone (MCH) in rat brain. Peptides 22, 1043–1047.

Moreno-Lopez, B., Romero-Grimaldi, C., Noval, J.A., Murillo-Carretero, M., Matarredona, E.R., Estrada, C., 2004. Nitric oxide is a physiological inhibitor of neurogenesis in the adult mouse subventricular zone and olfactory bulb. J. Neurosci. 24, 85–95.

Moyer, K.E., 1971. The Physiology of Hostility. Markham, Chicago.

Nathan, C., 1992. Nitric oxide as a secretory product of mammalian cells. FASEB J. 6, 3051–3064.

Nathan, C., Xie, Q.W., 1994. Nitric oxide synthases: roles, tolls, and controls. Cell 78, 915–918.

Neckers, L.M., Zarrow, M.X., Myers, M.M., Denenberg, V.H., 1975. Influence of olfactory bulbectomy and the serotonergic system upon intermale aggression and maternal behavior in the mouse. Pharmacol. Biochem. Behav. 3, 545–550.

Nelson, R.J., 1997. The use of genetic knockout mice in behavioral endocrinology research. Horm. Behav. 31, 188–196.

Nelson, R.J., Chiavegatto, S., 2001. Molecular basis of aggression. Trends Neurosci. 24, 713–719.

Nelson, R.J., Demas, G.E., Huang, P.L., Fishman, M.C., Dawson, V.L., Dawson, T.M., Snyder, S.H., 1995. Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase. Nature 378, 383–386.

Nelson, R.J., Kriegsfeld, L.J., Dawson, V.L., Dawson, T.M., 1997. Effects of nitric oxide on neuroendocrine function and behavior. Front. Neuroendocrinol. 18, 463–491.

New ,A.S., Buchsbaum, M.S., Hazlett, E.A., Goodman, M., Koenigsberg, H.W., Lo, J., Iskander, L., Newmark, R., Brand, J., 2004. Fluoxetine increases relative metabolic rate in prefrontal cortex in impulsive aggression. Psychopharmacology (Berl) 176, 451–458.

Nyberg, J.M., Vekovischeva, O., Sandnabba, N.K., 2003. Anxiety profiles of mice selectively bred for intermale aggression. Behav. Genet. 33, 503–511.

Olivier, B., Mos, J., van Oorschot, R., Hen, R., 1995. Serotonin receptors and animal models of aggressive behavior. Pharmacopsychiatry 28 (Suppl. 2), 80–90.

Packer, M.A., Stasiv, Y., Benraiss, A., Chmielnicki, E., Grinberg, A., Westphal, H., Goldman, S.A., Enikolopov, G., 2003. Nitric oxide negatively regulates mammalian adult neurogenesis. Proc. Natl. Acad. Sci. U.S.A. 100, 9566–9571.

Pattij, T., Broersen, L.M., van der Linde, J., Groenink, L., van der Gugten, J., Maes, R.A., Olivier, B., 2003. Operant learning and differential-reinforcement-of-low-rate 36-s responding in 5-HT_{1A} and 5-HT_{1B} receptor knockout mice. Behav. Brain Res. 141, 137–145.

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

Reif, A., Schmitt, A., Fritzen, S., Chourbaji, S., Bartsch, C., Urani, A., Wycislo, M., Mossner, R., Sommer, C., Gass, P., Lesch, K.P., 2004. Differential effect of endothelial nitric oxide synthase (NOS-III) on the regulation of adult neurogenesis and behaviour. Eur. J. Neurosci. 20, 885–895.

Rivier, C.L., 2000. Nitric oxide modulates the response of the hypothalamic-pituitary-adrenal axis to physico-emotional stressors in rats. J. Neurosci. 14, 2244–2253.

Rivier, C., Shen, G.H., 1994. In the rat, endogenous nitric oxide modulates the response of the hypothalamic-pituitary-adrenal axis to interleukin-1 beta, vasopressin, and oxytocin. J. Neurosci. 14, 1985–1993.

Robbins, T.W., 2002. The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. Psychopharmacology (Berl) 163, 362–380.

Sapolsky, R.M., 2000. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch. Gen. Psychiatry 57, 925–935.

Selley, M.L., 2004. Increased (*E*)-4-hydroxy-2-nonenal and asymmetric dimethylarginine concentrations and decreased nitric oxide concentrations in the plasma of patients with major depression. J. Affect Disord. 80, 249–256.

Shanks, N., Anisman, H., 1993. Escape deficits induced by uncontrollable foot-shock in recombinant inbred strains of mice. Pharmacol. Biochem. Behav. 46, 511–517.

Sheline, Y.I., Sanghavi, M., Mintun, M.A., Gado, M.H., 1999. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. J. Neurosci. 19, 5034–5043.

Shin, I.C., Kim, H.C., Swanson, J., Hong, J.T., Oh, K.W., 2003. Anxiolytic effects of acute morphine can be modulated by nitric oxide systems. Pharmacology 68, 183–189.

Simon, N., 2002. Hormonal systems regulating the development and expression of aggression. In: Pfaff, A., Rubin, R., Fahrbach, B. (Eds.), Hormones, Brain and Behavior. Academic Press, San Diego, pp. 39–85.

Singh, R., Pervin, S., Shryne, J., Gorski, R., Chaudhuri, G., 2000. Castration increases and androgens decrease nitric oxide synthase activity in the brain: physiologic implications. Proc. Natl Acad. Sci. U.S.A. 97, 3672–3677.

Soma, K.K., Wingfield, J.C., 2001. Dehydroepiandrosterone in songbird plasma: seasonal regulation and relationship to territorial aggression. Gen. Comp. Endocrinol. 123, 144–155.

Spigset, O., 1999. Adverse reactions of selective serotonin reuptake inhibitors: reports from a spontaneous reporting system. Drug Saf. 20, 277–287.

Svare, B., 1983. Hormones and Aggressive Behavior. Plenum, New York.

Swann, A.C., 2003. Neuroreceptor mechanisms of aggression and its treatment. J. Clin. Psychiatry 64 (Suppl 4), 26–35.

Swann, A.C., Bjork, J.M., Moeller, F.G., Dougherty, D.M., 2002. Two models of impulsivity: relationship to personality traits and psychopathology. Biol. Psychiatry 51, 988–994.

Tafet, G.E., Idoyaga-Vargas, V.P., Abulafia, D.P., Calandria, J.M., Roffman, S.S., Chiovetta, A., Shinitzky, M., 2001. Correlation between cortisol level and serotonin uptake in patients with chronic stress and depression. Cogn. Affect Behav. Neurosci. 1, 388–393.

Tafet, G.E., Toister-Achituv, M., Shinitzky, M., 2001. Enhancement of serotonin uptake by cortisol: a possible link between stress and depression. Cogn. Affect Behav. Neurosci. 1, 96–104.

Vincent, S.R., Kimura, H., 1992. Histochemical mapping of nitric oxide synthase in the rat brain. Neuroscience 46, 755–784.

Vivian, J.A., Miczek, K.A., 1999. Interactions between social stress and morphine in the periaqueductal gray: effects on affective vocal and reflexive pain responses in rats. Psychopharmacology (Berl) 146, 153–161.

Volke, V., Soosaar, A., Koks, S., Vasar, E., Mannisto, P.T., 1998. L-Arginine abolishes the anxiolytic-like effect of diazepam in the elevated pluz-maze test in rats. Eur. J. Pharmacol. 351, 287–290.

Volke, V., Wegener, G., Bourin, M., Vasar, E., 2003. Antidepressant- and anxiolytic-like effects of selective neuronal NOS inhibitor 1-(2-trifluoromethylphenyl)-imidazole in mice. Behav. Brain Res. 140, 141–147.

Walsh, M.T., Dinan, T.G., 2001. Selective serotonin reuptake inhibitors and violence: a review of the available evidence. Acta. Psychiatry Scand. 104, 84–91.

Weiner, C.P., Lizasoain, I., Baylis, S.A., Knowles, R.G., Charles, I.G., Moncada, S., 1994. Induction of calcium-dependent nitric oxide synthases by sex hormones. Proc. Natl Acad. Sci. U.S.A. 91, 5212–5216.

Weitzdoerfer, R., Hoeger, H., Engidawork, E., Engidawork, E., Engelmann, M., Singewald, N., Lubec, G., Lubec, B., 2004. Neuronal nitric oxide synthase knock-out mice show impaired cognitive performance. Nitric Oxide 10, 130–140.

Wen, J.C., Hotchkiss, A.K., Demas, G.E., Nelson, R.J., 2004. Photoperiod affects neuronal nitric oxide synthase and aggressive behavior in male Siberian hamsters (*Phodopus sungorus*). J. Neuroendocrinol. 16, 916–921.

Yildiz, F., Erden, B.F., Ulak, G., Utkan, T., Gacar, N., 2000. Antidepressant-like effect of 7-nitroindazole in the forced swimming test in rats. Psychopharmacology (Berl) 149, 41–44.