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Strain differences in the neurochemical response to chronic restraint stress in the rat: Relevance to depression

Cliona M. O'Mahony [a], Gerard Clarke [b], Sinead Gibney [b], Timothy G. Dinan [b,c], John F. Cryan [a,b,d,*]

[a] Neuropharmacology Research Group, Dept of Pharmacology and Therapeutics, University College Cork, Cork, Ireland
[b] Laboratory of Neurogastroenterology, Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland
[c] Dept of Psychiatry, University College Cork, Cork, Ireland
[d] School of Pharmacy, University College Cork, Cork, Ireland

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ABSTRACT

The neurochemical basis of depression focuses on alterations in the monoaminergic and amino acid neurotransmitter systems. Moreover, decreases in serum levels of the neurotrophin brain-derived neurotrophic factor (BDNF) have led to the more recent neurotrophic hypothesis of depression. Chronic stress is one of the major predisposing factors to developing the disorder and thus we investigated the impact of chronic restraint stress on the levels of several neurotransmitters and their metabolites in a genetic animal model of depression, the Wistar Kyoto (WKY) rat. Behavioural analysis of WKY rats indicated both a depressive and anxiety-like phenotype compared to their Sprague Dawley (SD) controls. WKY animals showed similar stress-induced decreases in hippocampal GABA, noradrenaline and dopamine as their SD counterparts while exhibiting a divergent decrease in 5-HT, 5-HIAA and DOPAC. WKY rats also showed a stress-dependant increase in GABA concentrations in the amygdala compared to the SD animals. Moreover, WKY but not SD rats had a chronic stress-induced decrease in serum BDNF levels. Together these data show that there are specific strain-dependent changes in neurotransmitter and neurotrophin levels in response to chronic stress which may predispose WKY animals to a depressive-like phenotype.

1. Introduction

Life stress and genetic predisposition are considered to be key factors in the development of psychiatric illnesses including major depression (Charney and Manji, 2004). Stress may be defined as a real or interpreted threat to the physiological or psychological integrity of an individual that results in physiological and/or behavioural responses (McEwen, 2007). However, excessive stress induces abnormal changes in brain function and physiology that impair its ability to appropriately regulate physiological and behavioural responses to subsequent stressors. The mechanisms underlying this stress × genetic interaction in vulnerable populations still remain elusive. Animal studies have shown that chronic stress induces dysfunction at a multisystem level including morphological changes in the hippocampus and the amygdala (McEwen, 2005; McLaughlin et al., 2007; Vyas et al., 2002), alterations in a variety of neurotransmitters (Adell et al., 1988; Sunanda et al., 2000; Torres et al., 2002), changes in the expression of both hippocampal and serum brain-derived neurotrophic factor (BDNF) (Bergstrom et al., 2008; Smith et al., 1995; Vollmayr et al., 2001) , alterations in behaviour and reductions in body weight (Bravo et al., 2009).

In parallel, ongoing efforts using animal models and clinical neuroimaging aim to illuminate which brain structures are involved in depression. Current data implicate key limbic structures (e.g. prefrontal cortex, and hippocampus), the hypothalamus and the anterior temporal cortex in depression (Davidson et al., 2002; Singewald, 2007). From a neurotransmitter standpoint the monoamine theory of depression has long purported a specific role of serotonin (5-HT), noradrenaline (NA) and dopamine (DA) in clinical depression (Coppen, 1967; Cryan and Leonard, 2000; Nestler and Carlezon, 2006; Nutt, 2002; O'Leary and Cryan, 2010). However, increasing evidence also suggests a role for γ-aminobutyric acid (GABA) in the disorder (Cryan et al., 2005a; Sanacora et al., 2004; Slattery and Cryan, 2006).

A variety of clinical studies have shown that serum levels of the neurotrophin BDNF are decreased in patients with major depressive disorder (Kim et al., 2007, Sen et al., 2008; Shimizu et al., 2003) giving rise to a neurotrophin hypothesis of depression (Duman and Monteggia, 2006) (but see Groves, 2007) . These findings are bolstered by data from animal studies, with most but not all, demonstrating that central BDNF is downregulated by chronic stress (Bravo et al., 2009; Nibuya et al., 1999; Smith et al., 1995) and elevated by chronic antidepressant treatment (Nibuya et al., 1995; Shirayama et al., 2002).

* Corresponding author. School of Pharmacy, Cavanagh Pharmacy Building, University College Cork, Cork, Ireland. Tel.: +353 21 4901676; fax: +353 21 4901656.
E-mail address: j.cryan@ucc.ie (J.F. Cryan).

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Genetically inbred rodent strains are one of the most useful tools in dissecting the genetic basis of complex stress-related disorders (Jacobson and Cryan, 2007; Ramos et al., 1997) .The Wistar Kyoto (WKY) rat is an inbred strain that has been proposed to be a genetic model of depression (Lopez-Rubalcava and Lucki, 2000; Will et al., 2003) compared to the outbred Sprague Dawley (SD) rat strain, commonly used as control (Malkesman and Weller, 2009). WKY rats have dysregulation of the hypothalamic pituitary adrenal (HPA) axis, (Rittenhouse et al., 2002; Solberg et al., 2001) and show marked differences in their behaviour in response to acute stress such as the open field (Drolet et al., 2002; Pardon et al., 2002) and the forced swim test (Lahmame et al., 1997b; Tejani-Butt et al., 2003; Will et al., 2003). The WKY also has an increased susceptibility to chronic stress-induced gastric ulceration (Paré and Redei, 1993).

In this study we sought to investigate if WKY rats respond differently to chronic restraint stress in terms of central neurotransmitter levels, plasma corticosterone and serum BDNF concentrations. We hypothesise that WKY animals will have an aberrant neurochemical response to stress that may predispose them to elevations in depression and anxiety-like behaviour.

2. Experimental procedures

2.1. Animals

Male Sprague Dawley (SD) and Wistar Kyoto (WKY) were acquired from Harlan Laboratories (Oxon, UK). All animals were given at least 1 week to habituate in the animal facility with food and water *ad libitum,* on a 12/12 h reversed dark–light cycle with temperature 22 ± 1 °C (lights on 7 am). All animals were group housed (5/6 per cage). Three separate cohorts of animals were used in this study. (I) Forced swim test (weight of group; 330–430 g), (ii) Open field group (weight of group; 283–401 g), (iii) neurotransmitters, body weight and serum BDNF in response to restraint stress (weight of group; 285–345 g). Animals from both strains were of similar weight at the time sacrifice in each cohort and daily body weight was noted. All experiments were conducted following institutional ethics guidelines and were in full accordance with the European Community Directive (86/609/EEC).

2.2. Restraint stress

Restraint is a well validated method to induce stress (Buynitsky and Mostofsky, 2009).The duration of stressor used is based on the work of Chattarji and colleagues (Mitra et al., 2005). Rats were exposed to 2 h restraint stress for 10 days, using a transparent plastic tube with air holes to increase ventilation. Rats were then immediately returned to their home cage. Control animals were left undisturbed in their home cage. The restraint procedure was performed between 8.00 am and 12.30 pm.

2.3. Corticosterone ELISA

Plasma corticosterone levels were measured using a Corticosterone Immunoassay Kit (Assay Designs, Ann Arbor, MI, USA). Briefly, plasma samples were diluted in assay buffer and incubated with a sheep polyclonal corticosterone antibody on a 96-well plate. Following washes, bound antigen was incubated with *p*-nitrophenyl phosphate substrate and alkaline phosphatase. Following colour development, the reaction was stopped using trisodium phosphate. Wells in the plate were read at 405 nm and the optical density was calculated. The concentration of each sample was extrapolated from a standard curve. Sensitivity of this assay is 26.99 pg/ml.

2.4. Detection of serum BDNF

Trunk blood was collected 2 h after last restraint stress into serum tubes, allowed to clot for 30 min and centrifuged at 1000 × *g* for 15 min. The serum was then removed and stored at −80 °C until analysis was performed. Serum BDNF concentrations was measured using a quantitative enzyme immunoassay technique (Quantikine, R&D Systems, Inc., USA). The assay was performed in duplicate according to the manufacturer's instructions. Briefly, standards and samples were pipetted into the wells of a microplate that was pre-coated with a monoclonal antibody specific for BDNF. Any BDNF present is bound by the immobilized antibody. An enzyme-linked monoclonal antibody (specific for BDNF) was then added to the wells. Any unbound antibody-enzyme reagent was removed by washing the 96-well plate and a substrate solution was then pipetted into wells, that allowing colour development to occur in proportion to the concentrations of BDNF which was bound in the initial step. Colour development was stopped after 30 min with the addition of the stop solution and the intensity of the colour was immediately measured by a microplate reader (Bio-tek instruments-Synergy HT), set for 450 nm with a correction at 540 nm. Four parameter logistic curve-fit was used to analysis the data using Gen 5 software.

2.5. Forced swim test

The modified forced swim test was conducted as previously described (Bissiere et al., 2006; Cryan et al., 2002, 2005a,b). The rats were placed individually in 21 cm × 46 cm Pyrex cylinders filled to a 30 cm depth with 21–25 °C water. Two swimming sessions were conducted: a 15 min pre-test followed by a 5 min test 24 h later. After 15 min on day 1, the rats were removed, towel dried and placed back in their home cage. The water in each cylinder was changed between animals. Twenty-four hours after their first exposure, rats were placed back in the swim apparatus for 5 min and a video camera positioned above the cylinders was used to monitor and record the animals for subsequent analysis. The total duration of predominant behaviour in each 5 s period of the 300 s test was scored on day 2. Swimming, immobility and active climbing were the main behaviours scored with swimming described as horizontal movements throughout all 4 quadrants of the cylinder, climbing was defined as vigorous fore-paw movements directed toward the walls of the apparatus and immobility defined as floating, with only enough movement necessary for the rat to keep its head above water (see Cryan et al., 2002 for pictorial representations). The experiments were carried out between 9.00 am and 12.30 pm and analysed by two observers blind to the experimental conditions.

2.6. Open field

The open field test was performed as previously described (O'Mahony et al., 2009). Briefly, the open field consisted of a circular white arena, 90 cm in diameter, 40 cm in height, 900 lux light. Testing was conducted between 9:00 a.m. and 1:00 p.m. At the beginning of each trial the rat was placed gently into the centre of the arena and allowed to explore the arena for 10 min. The behaviour of the animals was recorded by an overhanging camera that was attached to a personal computer. Ethovision 3.0 (Noldus, The Netherlands) was used to track the movement of the animal. The total distance moved in the arena, time spent and distance moved in the inner zone of the arena were recorded. The number of fecal pellets present in the arena at the end of the 10 min trial was also recorded. When rats are anxious they usually display freezing behaviour and stay close to the sides of the arena, which will result in a reduction in the amount of time spent in the brightly lit inner zone and distance travelled. An increased number of fecal pellets can also be indicative of the anxious state of the animal.

2.7. Determination of neurotransmitter levels in brain regions using high performance liquid chromatography

2.7.1. Sample preparation

Animals were lightly anaesthetised using halothane and decapitated. It is reported that the effects of halothane evoke minor activation in corticosterone (de Haan et al., 2002) . A study by Harvey et al. reported no significant changes in hippocampal levels of DA, NA and 5-HT(Harvey et al., 2006); however, they did show changes in NA and DA in the frontal cortex. Moreover, previous studies have used halothane in investigating neurotransmitters in response to a restraint stress (Sunanda et al., 2000). The right hemisphere of the brain was dissected on an ice-cold plate as previously described (O'Mahony et al., 2008). The prefrontal cortex, frontal cortex, hippocampus, hypothalamus and the amygdala were removed, weighed and placed in 1.5 ml microcentrifuge tubes which contained 1 ml of chilled homogenisation buffer (mobile phase spiked with 2 ng/20 µl of *N*-methyl 5-HT (Sigma Chemical Co., UK) as internal standard). Samples were sonicated until suspended in the tube and centrifuged at 14,000 rpm for 15 min at 4 °C. The supernatant was drawn off and two aliquots were taken, one for monoamine analysis and one for amino acid analysis, respectively. Aliquots were stored at −80 °C until analysis.

2.7.2. Determination of monoamine concentration

Monoamine analysis was carried out by HPLC coupled to electrochemical detection as described previously (O'Mahony et al., 2008). Briefly, the mobile phase contained 0.1 M citric acid, 0.1 M sodium dihydrogen phosphate, 0.01 mM EDTA (Alkem/Reagecon, Cork), 5.6 mM octane-1-sulphonic acid (Sigma) and 9% (v/v) methanol (Alkem/Reagecon), and was adjusted to pH 2.8 using 4 N sodium hydroxide (Alkem/Reagecon). 20 µl of the supernatant was injected onto the HPLC system which consisted of a SCL 10-Avp system controller, LC-10AS pump, SIL-10A autoinjector (with sample cooler maintained at 4^0 C), CTO-10A oven, LECD 6A electrochemical detector (Shimadzu) and an online Gastorr Degasser (ISS, UK). A reverse-phase column (Synergi 4u MAX-RP 80A, 250×4.6 mm, Phenomenex) maintained at 30 °C was employed in the separation (Flow rate 2 ml/min), the glassy carbon working electrode combined with an Ag/AgCL reference electrode (Shimdazu) was operated a +0.8 V and the chromatograms generated were analysed using Class-VP 5 software (Shimadzu). The neurotransmitters 5-HT, dopamine noradrenaline, and metabolites 5-H1AA (5-hydroxyindoleacetic acid), dihydroxyphenylacetic acid (DOPAC) and HVA were identified by their characteristic retention times as determined by standard injections which were run at regular intervals during the sample analysis. Analyte: Internal standard peak height rations were measured and compared with standard injections and results were expressed at ng of neurotransmitter per g fresh weight of tissue.

2.7.3. Determination of GABA concentration

Sample analysis was carried out by HPLC coupled to fluorescence detection as described previously (Clarke et al., 2007). The derivatisation reaction was carried out as the following: 100 µl of sample supernatant, 900 µl of borate buffer (0.1 M, pH 9.5), 200 µl of potassium cyanide (10 mM) and 200 µl of NDA (6 mM) were added to a single reaction tube, vortex mixed and the allowing the reaction to proceed at ambient temperatures in the absence of light. A 20 µl aliquot of the derivative was injected onto the HPLC system.

The HPLC system consisted of a Waters 510 pump, 717plus Autosampler, a Hewlett Packard 1046A fluorescent detector, a waters bus SAT/IN module and a Croco-Cil column oven. System components were used in conjunction with Waters Empower software. All samples were injected onto a reversed phase Luna 3 u C18 (2) 150 mm×2 mm column (Phenomenex), which was protected by krudkatcher disposable pre-column filters and SecurityGuard cartridges (Phenomenex).

The mobile phase used on the system was composed of a mixture of 0.1 M di-sodium hydrogen orthophosphate/50 µM EDTA (pH 5.3, 1 M orthophosphoric acid) and HPLC grade methanol (35:65). Mobile phase was filtered and vacuum degassed prior to use. Compounds were eluted isocratically over a 20 min runtime at a flow rate of 0.1 ml/min. The fluorescent detector was set at an excitation wavelength of 420 nm, an emission wavelength of 480 nm and a PMT gain of 5. GABA was identified by its characteristic retention time as determined by standard injections run at regular intervals during sample analysis. Sample peak heights were measured and compared with standard injections in order to quantify the amino acid. The limits of detection as a signal–noise ration of 2:1 were determined as 1.1 ng/injection for GABA. The limits of quantitation at a signal-to-noise ratio of 5:1 were determined as 2.8 ng/injection for GABA.

2.8. Stress ulcers

As WKY animals have been shown to be susceptible to stress-induced ulcers (Pare and Redei, 1993; Paré and Redei, 1993) it was important to assess whether our stress regimen influenced ulceration. After decapitation of the animals, the stomach was quickly removed and visually inspected for ulcers.

2.9. Statistical analysis

Statistical analysis was performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA) statistical software. Two-way repeated measures ANOVA was performed to determine any difference in body weight between the two strains and compared to their relative controls in response to stress. A 2-way ANOVA was performed to determine any significant difference between the SD and WKY rats in response to restraint stress. Fisher's least significant difference (LSD) post hoc tests were conducted where appropriate. *A priori* pair-wise comparisons were conducted using Student's t-test. Differences were considered significant for $p<0.05$.

3. Results

3.1. Increased depression and anxiety-related behaviours in WKY rats

3.1.1. Forced swim test

WKY rats demonstrated a significant increase in immobility when compared to the SD rats (Fig. 1A) ($p<0.001$). In addition, the WKY strain showed a significant decrease in both swimming ($p<0.001$) and climbing ($p<0.05$) behaviours.

3.1.2. Open field

When exposed to the open field test, WKY rats displayed a decrease in the total distance travelled around the open field when compared to the SD animals ($p<0.01$, Fig. 1B). When the open field was divided into inner and outer zones, the WKY animals also demonstrated significantly reduced movement but no difference in time spent in the inner zone when compared to the SD animals ($p<0.05$, Fig. 1B). Furthermore, WKY animals showed increased defecation in the open field when compared to the SD animals ($p<0.05$, Fig. 1B).

3.2. Body weight

Restraint stress significantly altered body weight gain with both stress groups gaining less weight than their respective controls, as shown in Fig. 2. A 2-way repeated measures ANOVA, revealed an overall effect of stress and a clear persistent over time-day*stress ($F_{(9,324)} = 4.372$, $p = 0.001$). *Post-hoc* tests revealed a significant effect of stress on days 2, 3, 4, 5, 6, 7, 9 and day 10 in WKY rats and on days 5, 6, 7, 9 and day 10 in SD rats (see Fig. 2).

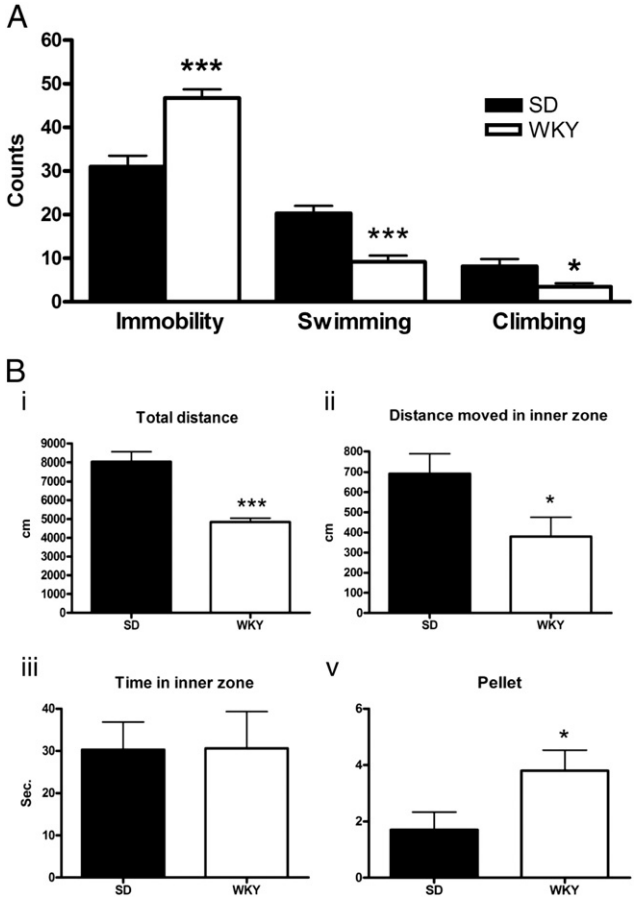

Fig. 1. (A) Forced swim test for WKY and SD rats. Graph showing swimming, climbing and immobility behaviours during a 5 min FST for SD ($n=10$) and WKY ($n=11$) rats. ***Immobility = $p<0.001$ compared to SD, ***Swimming = $p<0.001$ compared to SD, *Climbing = $p<0.05$ compared to SD. Data represent mean ± SEM. (B) Anxiety related behaviour on the open field of SD and WKY rats. (i) Represents the total distance travelled in the OF. (ii) Illustrates the total distance travelled in the inner zone of the OF. (iii) Demonstrates the amount of time spent in the inner zone. (v) Displays the number of faecal pellets excreted during the 5 min test. ***$p<0.001$ SD compared to WKY, *$p<0.05$ SD compared to WKY. Data represent mean ± SEM, ($n=10$ for all groups).

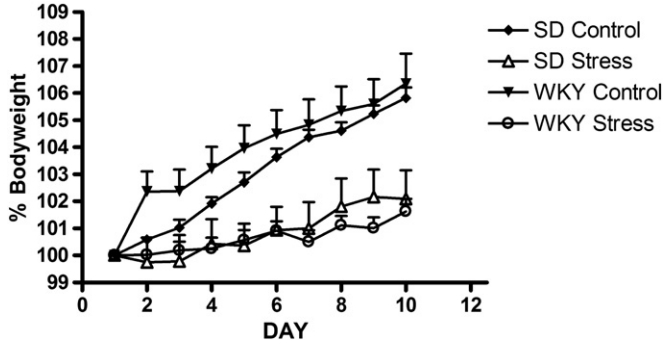

Fig. 2. Percentage body weight of SD and WKY rats in response to stress over a 10 day period. Day 2; WKY control compared to WKY stress *$p<0.05$. Day 3; WKY control compared to its relative control *$p\leq0.05$. Day 4; WKY compared to its relative control *$p<0.01$. Day 5; SD control compared to SD stress *$p<0.05$, WKY control compared to WKY stress ***$p<0.001$. Day 6; SD control compared to SD stress **$p<0.01$, WKY control compared to WKY stress ***$p<0.001$. Day 7 SD control compared to SD stress **$p<0.01$, WKY compared to SD stress ***$p<0.001$. Day 9; SD control compared to SD stress **$p<0.01$, WKY control compared to WKY stress ***$p<0.001$. Day 10; SD control compared to SD stress **$p\leq0.01$, WKY control compared to WKY stress, compared to relative control ***$p<0.001$. Data represent mean ± SEM. ($n=10$ for all groups).

Fig. 3. Graphical representation of corticosterone levels of SD and WKY rats in response to stress. Data represent mean ± SEM ($n=9$–10).

3.3. Plasma corticosterone levels

As shown in Fig. 3, no significant difference was found in baseline corticosterone levels or in response to stress between SD and WKY rats (Strain, $F(1, 39)=3.457$, $p=0.071$; Stress, $F(1,39)=1.674$, $p=0.204$.; Interaction $F(1,39)=1.674$, $p=0.126$). For technical reasons plasma from one SD control animal was not included in the analysis.

3.4. Serum BDNF levels

Fig. 4. illustrates the effects of chronic stress on serum BDNF levels. A 2-way-ANOVA revealed that there was trend toward a significant effect of strain ($F(1, 39)=3.457$, $p=0.071$) but no effect of stress ($F(1, 39)=1.674$, $p=0.204$) or interaction between stress and strain ($F(1, 39)=2.459$, $p=0.126$). *A priori* pair-wise comparisons revealed a significant decrease in BDNF levels in stressed WKY rats relative to their controls ($p<0.05$). No stress-induced alteration in BDNF levels were found in SD animals.

3.5. Neurotransmitter concentrations in the hippocampus

3.5.1. GABA

Statistical analysis revealed an overall effect of stress ($F(1, 39)=21.456$, $p=0.001$) in the GABA levels in the hippocampus, however there was no effect of strain or interaction between stress and strain in GABA levels in the hippocampus (Strain, $F(1, 39)=0.001$, $p=0.985$; Interaction $F(1, 39)=0.044$, $p=0.836$). *Post hoc* analysis revealed that both strains reacted similarly to stress when compared to their relative unstressed controls, showing a decrease in GABA levels (Fig. 5).

Fig. 4. Illustrates serum BDNF levels of SD and WKY animals in response to a chronic 10 day restraint stress. *$p<0.05$ WKY stressed compared to control, #$p<0.05$ SD stress compared to WKY stress. Data represent mean ± SEM ($n=10$ for all groups).

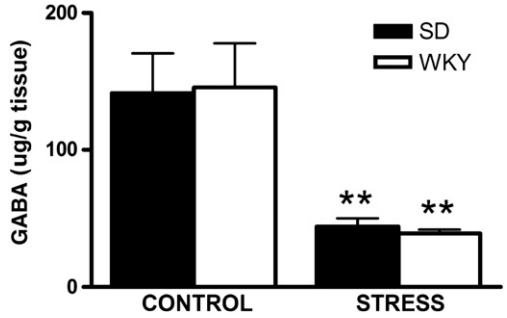

Fig. 5. Graphical representation of stress-induced alterations of amino acids GABA in the hippocampus of SD and WKY rats. **$p<0.01$ compared to control. Data represent mean ± SEM ($n=10$ for all groups).

3.5.2. Monoamines

As seen in Fig. 6A a 2-way-ANOVA revealed an overall effect of stress ($F(1, 39)=10.652$, $p=0.002$), however, there was no effect of strain or interaction between stress and strain in 5-HT levels (Strain, $F(1, 39)=0.479$, $p=0.493$; Interaction $F(1, 39)=1.256$, $p=0.270$). *Post-hoc* analysis revealed a significant difference in serotonin levels between SD control compared to SD stressed animals ($p<0.01$), no significance was found between stressed WKY animals compared to their respective controls.

Statistical analysis revealed that an overall effect of stress occurred ($F(1, 39)=8.068$, $p=0.007$) on hippocampal 5-HIAA levels, however, there was no effect of strain ($F(1, 39)=0.086$, $p=0.771$) or interaction between stress and strain ($F(1, 39)=0.582$, $p=0.451$) (Fig. 6B). *Post-hoc* analysis revealed a significant difference in 5-HIAA levels in SD unstressed rats compared to stress SD animals ($p<0.05$). However, no differences were found in WKY stressed animals compared with their respective controls.

In the context of hippocampal 5-HT turnover there was an overall significant effect of stress ($F(1, 39)=6.668$, $p=0.014$) (Fig. 6C). However, there was no effect of strain or interaction between stress and strain (Strain, $F(1, 39)=2.217$, $p=0.145$; Interaction $F(1, 39)=2.906$, $p=0.097$). *Post-hoc* analysis revealed a significant increase in 5-HT turnover levels between SD controls and SD stressed animals ($p<0.01$). No significantly different effects were found between WKY controls compared to WKY stress animals. However, *a priori* pair-wise comparisons that stressed WKY animals had significantly lower 5-HT turnover compared to stressed SD animals ($p<0.05$).

2-way-ANOVA revealed an overall effect of stress on noradrenaline levels in the hippocampus ($F(1, 39)=13.448$, $p=0.001$), however, there was no effect of strain ($F(1, 39)=0.51$, $p=0.823$) or interaction between stress and strain ($F(1, 39)=0.423$, $p=0.520$) (Fig. 6D). Both strains reacted similarly to stress when compared to their relative unstressed controls, resulting in a decrease in noradrenaline levels. *Post-hoc* analysis revealed a significant difference when both rat strains were compared to their relative controls, SD control compared to SD stress ($p<0.01$), WKY control compared to WKY stress ($p<0.05$).

3.6. Neurotransmitter levels in the prefrontal cortex (PFC), frontal cortex (FC), hypothalamus, hippocampus and the amygdala

There was a significant difference found in the amygdala in regard to GABA concentrations. However, no significant differences were found in monoamine concentrations. An overall effect of stress was revealed by a 2-way-ANOVA ($F(1, 34)=5.263$, $p=0.029$). However, there was no effect of strain ($F(1, 34)=2.277$, $p=0.141$) or interaction between stress and strain in GABA levels in the amygdala ($F(1, 34)=2.504$, $p=0.124$) (Table 1). *Post-hoc* tests revealed a significant difference in GABA levels between WKY control compared to WKY stress ($p<0.01$), with no significance found between SD control compared to SD stress. A significant difference was also found between SD stress compared to WKY stress ($p<0.01$).

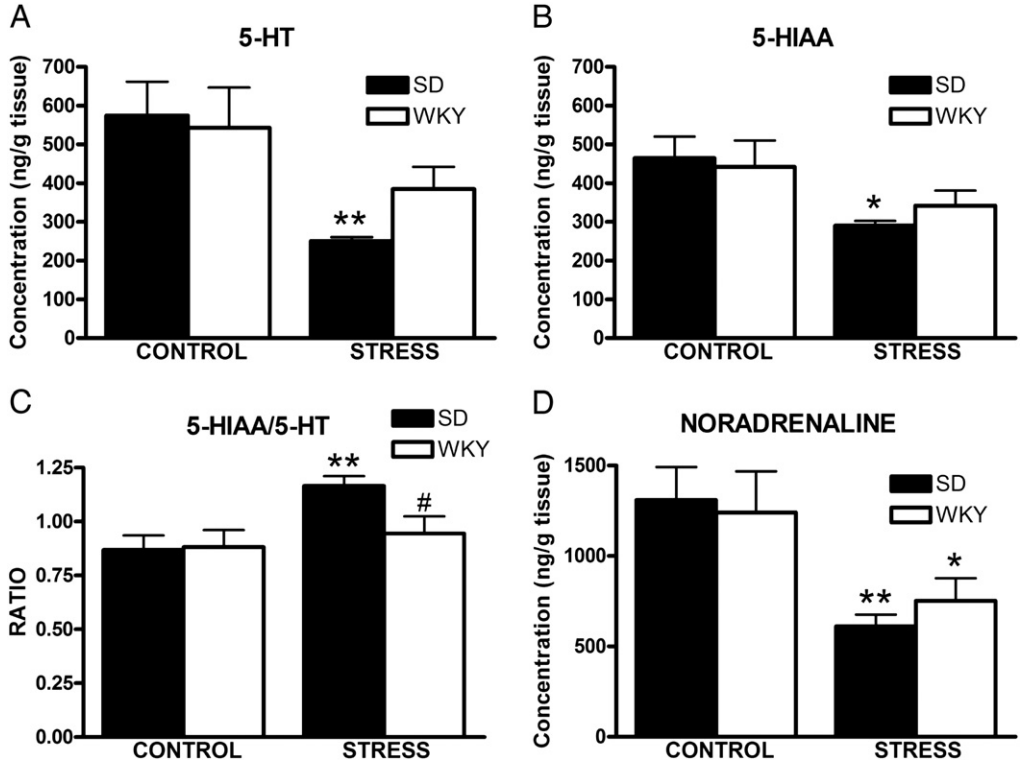

Fig. 6. Graphical representation of monoamine alterations in the hippocampus of SD and WKY rats in response to chronic restraint stress. (A) serotonin, (B) 5-HIAA, (C) 5-HIAA/5-HT, (D) Noradrenaline.*$p<0.05$ compared to relative control. **$p<0.001$ compared to relative control. #$p<0.05$ compared to strain. Data represent mean ± SEM ($n=10$ for all groups).

Table 1
Effect of stress-induced alteration in amino acids and monoamines in SD and WKY rats in the hippocampus and the amygdala.

Regions	Amygdala				Hippocampus			
	Sprague Dawley		WKY		Sprague Dawley		WKY	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
5-HT	388.6 ± 51.7	390.1 ± 48.4	375.6 ± 94.5	431.1 ± 51.0	574.4 ± 87.4	**250.8 ± 9.3****	542.8 ± 103.9	384.6 ± 57.0
5-HIAA	230.0 ± 25.0	262.8 ± 28.1	215.2 ± 33.4	282.3 ± 34.6	464.6 ± 55.4	**290.4 ± 11.9***	441.9 ± 67.9	341.5 ± 38.9
5-HIAA/5-HT	0.6 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.8 ± 0.0	**1.1 ± 0.0****	0.8 ± 0.0	**0.9 ± 0.0***
Noradrenaline	515.7 ± 59.8	498.7 ± 59.5	456.6 ± 105.5	709.2 ± 125.4	1309.6 ± 182.5	**610.5 ± 65.1****	1240.8 ± 227.6	**752.3 ± 124.4***
Dopamine	194.0 ± 37.0	155.6 ± 26.6	142.1 ± 46.4	201.8 ± 79.5	64.5 ± 13.6	**26.6 ± 4.8***	77.6 ± 18.4	**34.0 ± 5.2***
DOPAC	ND	ND	ND	ND	65.4 ± 13.3	**21.3 ± 1.6****	60.8 ± 10.8	43.0 ± 13.2
DOPAC/dopamine	ND	ND	ND	ND	0.8 ± 0.0	0.9 ± 0.2	0.8 ± 0.1	1.3 ± 0.3
HVA	ND	ND	ND	ND	ND	ND	ND	ND
HVA/Dopamine	ND	ND	ND	ND	ND	ND	ND	ND
GABA	33.1 ± 5.7	39.1 ± 6.0	32.5 ± 7.6	**65.3 ± 12.1 **##**	141.4 ± 29.0	**44.2 ± 5.9****	145.6 ± 32.3	**39.2 ± 2.7****

Hippocampus: DOPAC/dopamine-SD control ($n=7$), SD stress ($n=9$), WKY control ($n=7$), WKY stress ($n=7$).
Amygdala: GABA-all groups $n=8$. NA-SD control ($n=9$), dopamine-SD control ($n=7$), SD stress ($n=9$), WKY control ($n=9$). Animals were removed due to levels been below detection. Hypothalamus ($n=5$) for all groups except for the GABA-SD control ($n=4$), WKY control ($n=4$). Animals were removed due to technical reasons.
$^{*}p<0.05$, $^{**}p<0.01$ compared to relative control, $^{\#}p<0.05$ $^{\#\#}p<0.01$ compared to stress between strains, Data represent mean ± SEM.

2-way ANOVA analysis showed an overall effect of stress in dopamine levels in the hippocampus ($F(1, 35)=12.944$, $p=0.001$) with no effect of strain ($F(1, 35)=0.824$, $p=0.371$) or interaction between stress and strain ($F(1, 35)=0.063$, $p=0.804$) (Table 1). Both strains reacted similar to stress when compared to their relative unstressed controls resulting in a decrease in dopamine levels, a *post hoc* test revealed a significant difference when both rat strains were compared to their relative controls, SD control compared to SD stress ($p<0.05$), WKY control compared to WKY stress ($p<0.05$).

An overall effect of stress was revealed by a 2-way-ANOVA in hippocampal DOPAC levels ($F(1, 31)=9.721$, $p=0.004$), however, there was no effect of strain found in response to restrain stress ($F(1, 31)=0.741$, $p=0.397$) nor was there an interaction between stress and strain ($F(1, 31)=1.737$, $p=0.198$) (Table 1). A *post hoc* analysis revealed a significant difference when SD control was compared to SD stress group ($p<0.01$), however, no significant difference was found when WKY control was compared to WKY stress.

No significant differences were found under baseline conditions or following chronic stress in the concentrations of GABA, or of the monoamines analysed in the pre-frontal cortex, frontal cortex or the hypothalamus (Table 2).

4. Discussion

The mechanisms underlying the effects of genetic background on the response to chronic stress and the ability to cope with a stressful environment remain largely elusive. Our study shows that two strains of rat, which differ in baseline forced swim test and open field behaviour, respond to chronic stress in a disparate manner in terms of neurochemistry and serum BDNF levels. These data not only highlight the utility of the WKY strain as a model to investigate stress-induced changes relevant to depression and chronic anxiety states, but may also provide insight into the neurochemical mechanisms involved in differential responses to chronic stress.

As expected stressed animals from both strains gained weight more slowly than their control counterparts, thereby indicating that a 2 h daily restraint stress for 10 days is sufficient to induce an overall stressed state, supporting its utility as a reliable stressor. Importantly, our stress protocol was not sufficient to induce ulcers in either SD or WKY rat strains which may have confounded our data. At an endocrine level, corticosterone were not significantly different in the either of the two strains in response to chronic stress. While it is generally accepted that chronic stress increases corticosterone, these effects are time-dependent. Indeed, some studies have shown that corticosterone levels in response to stress showed no change 3 h after the onset of chronic restraint stress (Galea et al., 1997). Another study that used the same stress protocol we employed showed no difference in corticosterone levels in pair-housed animals (Lucas et al., 2007). Given that our analysis was after the peak for stress-induced corticosterone levels (approximately 2 h after cessation of the restraint stress), it may not be surprising that no change was seen. However, it may also reflect an adaptive response in corticosterone levels in the chronically stressed animals (Lucas et al., 2007; Naert et al., in press). Future studies should focus on examining different time-points following stress and investigating other aspects of the HPA-axis including hypothalamic and extra-hypothalamic CRF levels (Bravo et al., 2010).

We first validated that there were behavioural differences between both WKY and SD rats. Our behavioural data are consistent with other reports showing increased immobility in FST in WKY rats when compared to SD animals, signifying that WKY animals have elevated depressive-like behaviours in the modified forced swim test (Lahmame et al., 1997a; Rittenhouse et al., 2002; Tejani-Butt et al., 2003; Will et al., 2003). The WKY rats displayed hypoactivity in the inner zone and an increase in the number of faecal pellets excreted when placed in the open field, when compared to SD rat strain, which is in agreement with other studies demonstrating anxiety-like behaviour in the WKY rat (McAuley et al., 2009; Pardon et al., 2002; Ramos et al., 1997; Tejani-Butt et al., 2003).

BDNF is implicated in both the pathophysiology of stress-related mood disorders such as depression (Duman and Monteggia, 2006; Martinowich et al., 2007), and in the therapeutic effects of antidepressants (Tapia-Arancibia et al., 2004). Therefore, we chose to investigate if there were any differences in serum BDNF concentrations between the two strains under baseline conditions and in response to stress. We demonstrated that WKY stressed rats have decreased serum BDNF levels when compared to their unstressed controls, a result which is supported by clinical studies demonstrating the association between depression and low levels of BDNF in the periphery (Karege et al., 2002; Ozan et al., 2010; Shimizu et al., 2003). Most studies to date have focused on central levels of BDNF, although a number of reports have also shown alterations in serum BDNF in both clinical depression (Karege et al., 2002) and animal models of depression (Elfving et al., 2010). Moreover, correlations have been made between peripheral BDNF measures and central hippocampal levels in some (Klein et al., 2010; Sartorius et al., 2009) but not all studies (Elfving et al., 2010). However, there is no universal support for the neurotrophin hypothesis and questions remain to be addressed regarding the robustness of the findings especially in the clinical literature (Chourbaji et al., 2011). Indeed, some clinical studies did not detect any alterations in patients with depression and in response to antidepressant treatment (Basterzi et al., 2009; Matrisciano et al., 2009) while a recent study by Molendijk et. al., (2010) demonstrated a disassociation between antidepressant-induced increases in serum BDNF and the natural history of the antidepressant response in patients. On the preclinical side there have been reports showing a stress-induced increase in BDNF levels (Bergstrom et al.,

Table 2
Effect of stress-induced alteration in amino acids and monoamines in SD and WKY rats in the PFC, FC, and hypothalamus.

Regions	Prefrontal cortex				Frontal cortex				Hypothalamus			
	Sprague Dawley		WKY		Sprague Dawley		WKY		Sprague Dawley		WKY	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
5-HT	283.5 ± 49.7	304.5 ± 34.6	364.1 ± 55.8	313.4 ± 28.7	315.3 ± 41.6	298.6 ± 19.3	345.6 ± 26.2	326.3 ± 11.8	371.3 ± 60.5	387.3 ± 43.4	352.7 ± 112.8	404.2 ± 53.0
5-HIAA	174.2 ± 21.6	184.2 ± 21.1	199.7 ± 27.0	151.2 ± 13.4	198.1 ± 22.7	221.0 ± 21.0	210.3 ± 13.1	222.9 ± 8.4	284.2 ± 15.1	887.6 ± 617.4	241.2 ± 44.3	248.8 ± 35.5
5-HIAA/5-HT	0.6 ± 0.0	0.6 ± 0.6	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.8 ± 0.1	3.2 ± 2.6	0.9 ± 0.2	0.6 ± 0.0
Noradrenaline	273.4 ± 46.1	248.7 ± 59.4	425.9 ± 120.7	219.5 ± 30.8	714.6 ± 88.2	639.8 ± 56.9	797.7 ± 80.8	767.7 ± 30.7	1289.8 ± 185.2	1504.0 ± 145.7	1226.2 ± 165.6	1329.9 ± 178.6
Dopamine	79.4 ± 10.0	63.7 ± 12.0	82.2 ± 18.1	84.2 ± 4.8	466.0 ± 113.6	463.4 ± 80.1	596.8 ± 142.4	431.3 ± 134.3	95.8 ± 10.2	142.0 ± 24.5	129.1 ± 29.8	119.2 ± 4.8
DOPAC	ND	ND	ND	ND	122.7 ± 20.9	115.4 ± 16.0	136.2 ± 15.8	101.6 ± 17.9	ND	ND	ND	ND
DOPAC/dopamine	ND	ND	ND	ND	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	ND	ND	ND	ND
HVA	ND	ND	ND	ND	51.4 ± 7.3	52.6 ± 6.4	63.7 ± 6.0	54.5 ± 7.6	ND	ND	ND	ND
HVA/Dopamine	ND	ND	ND	ND	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	ND	ND	ND	ND
GABA	18.5 ± 2.6	13.7 ± 5.8	25.9 ± 7.7	18.4 ± 3.7	31.2 ± 8.2	28.0 ± 6.6	34.6 ± 7.5	34.6 ± 3.6	62.4 ± 8.3	72.3 ± 12.6	62.9 ± 8.2	68.6 ± 12.2

$n = 10$ for all groups except:
PFC: GABA-SD control ($n = 5$), SD stress ($n = 8$), WKY control ($n = 7$), WKY stress ($n = 5$), 5-HT-SD stress ($n = 9$), 5-HT turnover-SD stress ($n = 9$), Dopamine-SD control ($n = 3$), SD stress ($n = 9$), WKY control ($n = 4$), WKY stress ($n = 9$). NA-SD control ($n = 6$), WKY control ($n = 8$).
FC: GABA-SD control ($n = 9$). DOPAC-SD control ($n = 9$). DOPAC/dopamine-SD control ($n = 9$). HVA-SD control ($n = 9$), SD stress ($n = 9$), WKY control ($n = 9$), WKY stress ($n = 8$). HVA/DOPAC-SD control ($n = 9$), SD stress ($n = 9$), WKY control ($n = 9$), WKY stress ($n = 8$).

2008; Larsen et al., 2010; Marmigère et al., 2003; Naert et al., in press), or no changes (Kuroda and McEwen, 1998; Razzoli et al., 2009). Moreover, there are also conflicting results reported between antidepressant-induced alterations in protein and mRNA levels of BDNF, although it is generally accepted that treatment increases levels, these discrepancies may be due to the type, dose and treatment period of the antidepressant as well as the methodology used for analysis (Coppell et al., 2003; De Foubert et al., 2004; O'Leary and Castrén, 2010).

The hippocampus is known as a crucial region in both the manifestation of depression-related behaviour and integration of the stress response (Airan et al., 2007; Sapolsky, 2003). On the other hand, the amygdala is also known to be involved in stress-related disorders as it plays a pivotal role in emotional responses such as fear (Cullinan et al., 1995; De Kloet et al., 2005; Figueiredo et al., 2002). Given that chronic stress and depression are associated with alterations in central neurotransmitter levels, it was important to investigate whether there were specific stress-induced changes in WKY animals compared to SD rats in these regions. Interestingly, the hippocampus and the amygdala showed significant alterations in a variety of neurotransmitters (GABA, 5-HT, 5-HIAA, 5-HT turnover, noradrenaline, dopamine and DOPAC) in response to stress (see Figs. 4, 5 and Table 2).

In terms of the major inhibitory neurotransmitter GABA, decreased levels in the hippocampus of both SD and WKY in response to stress were observed when compared to their relative controls as seen in Fig. 3. Clinical studies have also shown reduced levels of GABA in cerebrospinal fluid of depressed subjects (Gold et al., 1980). In animal studies, GABAA and GABAB receptors are both abundantly expressed in the hippocampus and studies have shown that chronic stress alters levels of $GABA_A$ hippocampal receptors (Cullinan and Wolfe, 2000; Montpied et al., 1993; Slattery, 2010). In terms of stress-induced alterations in the concentration of GABA itself, an increase in extracellular GABA hippocampal levels were found in response acute novelty stress (Bianchi et al., 2003). However, in line with our current data another study showed a decrease in extracellular GABA levels in response to chronic mild stress (Grønli et al., 2007). On the other hand 6 h restraint stress for 21 days revealed no change in the levels of GABA, therefore it appears that the sensitivity of the GABAergic system to chronic stressors may be dependent on the specific stressor employed (Sunanda et al., 2000).

In terms of the amygdala we have demonstrated a stress-induced increase in GABA in the amygdala of the WKY rat but not SD rats (Table 2). Given that amygdalar GABA is important in manifestation of fear and anxiety (Sanders and Shekhar, 1995) the maladaptive response seen following chronic stress may play a role in the development of altered cortico-amygdalar circuits responsible for stress-induced psychopathology.

Several studies have also focused on the role of monoamines specifically serotonin, noradrenaline and dopamine, and have shown that alterations of monoamines have a strong association with clinical depression (Cryan and Leonard, 2000; Elhwuegi, 2004; Nemeroff, 1998; Nestler and Carlezon, 2006; Nutt, 2002). Preclinical studies have also demonstrated a clear association between chronic stress and depression-related behaviour, and alterations of 5-HT, NA, DA, their respective metabolites, turnover ratios and transporters (Elhwuegi, 2004; Graeff et al., 1996; Harro and Oreland, 2001; Mallo et al., 2007; Tonissaar et al., 2008). Our data demonstrate that hippocampal 5-HT and its metabolite are decreased in stressed SD rats concomitant with an increase in 5-HT turnover when compared to their relative controls. WKY rats did show a similar trend to that of SD but this did not reach significance with regard to 5-HT and 5-HIAA. To date there are conflicting results regarding chronic stress-induced alterations in monoamine concentrations. Our results are consistent with studies demonstrating a decrease in 5-HT in the hippocampus in response to chronic restraint stress (Sunanda et al., 2000; Torres et al., 2002) and other stressors (Tannenbaum et al., 2002). However, it should be noted other studies have shown an increase in 5-HT, 5-HIAA, NA (Adell et al.,

1988; Hayley et al., 2001) and no changes have also been recorded in response to stress (forced swim test) (Connor et al., 1997). Reasons for this may be due to differences in the protocols being applied, in terms of duration and type of stressor and the time post stress cessation at which the samples were collected.

Regarding dopamine, in the present study both strains exhibited similar alterations in response to stress with a decrease in dopamine in the hippocampus compared to their relative controls. However, only SD rats demonstrated a significant decrease in its metabolite DOPAC. On the other hand, no alterations of overall dopaminergic turnover were observed as expressed by HVA/DA and DOPAC/DA ratios. Our results are consistent with previous studies showing a decrease in dopamine in response to chronic restraint stress in rats (Sunanda et al., 2000; Torres et al., 2002).

Noradrenergic mechanisms have long been implicated in manifestation of the stress response (Morilak et al., 2005). Our study shows a significant reduction in levels of noradrenaline in the hippocampus of both strains in response to stress. Our findings are consistent with those of previous studies demonstrating a decrease in this neurotransmitter following chronic stress (Sunanda et al., 2000). However, it should also be noted that other studies have shown an increase in noradrenaline in response to chronic stress (Adell et al., 1988; Beck and Luine, 2002).

Regarding the neurochemical data care must be taken not to over-interpret tissue neurotransmitters levels, a measurement that does not distinguish between intracellular and extracellular pools and the results reported here do not clarify whether or not the active pool of neurotransmitter is modulated by the stress procedures employed. However, it should also be acknowledged that alterations in the tissue concentrations of neurotransmitters are unlikely to be without consequences for neuronal signaling. Given the alterations seen between both strains, future studies employing in vivo microdialysis are now warranted.

5. Conclusions

Taken together, our data demonstrate that both strains show key similarities and differences in the neurochemical responses to stress. Specifically, while both SD and WKY strains have similar reductions in hippocampal GABA, noradrenaline and dopamine levels, WKY animals have a blunted decrease in hippocampal 5-HT and its metabolite 5-HIAA coupled with an increase in amygdala GABA levels. Moreover, WKY but not SD rats showed a decrease in serum BDNF levels. These data confirm that while there were no baseline differences in neurotransmitter levels between WKY and SD rats, selective neurochemical deficits are unmasked following exposure to chronic stress. These may contribute to the depression and anxiety related phenotype in the WKY rat.

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