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Central monoamine levels differ between rat strains used in studies of depressive behavior

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Abstract

Previous studies have shown that the Wistar-Kyoto (WKY) rat strain may be a genetic model of depression when their behaviors are compared to Sprague-Dawley (SD) or Wistar (WIS) rats. Significant differences in dopamine (DA), serotonin (5-HT), and norepinephrine (NE) transporter site densities have been reported when comparing WKY to both SD and WIS rats. Susceptibility of WKY rats to anxiety and depressive behavior may be related to underlying differences in monoamine levels in various regions of the brain. Levels of monoamines (DA, 5-HT and NE) and their metabolites were measured in monoaminergic cell body, cortical and limbic brain regions using HPLC with electrochemical detection and compared between WKY, WIS and SD rats. In regions where strain differences in monoamine levels were observed (the basolateral amygdala, subregions of the hippocampus and the nucleus accumbens shell), WKY rats consistently had lower levels than SD rats. Similarly, WKY rats had lower monoamine levels compared to WIS, although these differences were observed in a more restricted number of brain regions. Interestingly, WIS rats showed reduced levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in several regions including the prefrontal cortex, subregions of the hippocampus and subregions of the hypothalamus, suggesting decreased 5-HT turnover when compared to both WKY and SD rats. Overall, these results imply that decreased monoamine levels, combined with alterations in transporter sites, may be related to the predisposition of WKY rats towards depressive behavior.

Keywords

HPLC; Depression; Serotonin; Norepinephrine; Dopamine; Animal Model; Depression

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1. Introduction

The Wistar Kyoto (WKY) rat strain has been proposed as an animal model of depressive behavior because it differs from other outbred rat strains in behavioral, physiological and neuroendocrine responsiveness to environmental as well as pharmacological challenges (Pare et al., 1989; Redei et al., 1994; Tejani-Butt et al., 2003). Behaviorally, WKY rats are less active in an open-field test compared to Wistar (WIS) rats (Pare, 1989), and more sensitive to chronic stress compared to Sprague-Dawley (SD) rats (Pare and Tejani-Butt, 1996). The WKY rat strain also shows increased depressive behavior when compared to either SD or WIS rats (Tejani-Butt et al., 2003). For example, WKY rats exhibit decreased struggling (Lahmame et al., 1997) and increased immobility (Rittenhouse et al., 2002; Lopez-Rubalcava and Lucki, 2000) in the forced-swim test when compared to the SD strain.

Clinical depression has been associated with a disruption in the normal function of the central monoamines norepinephrine (NE), serotonin (5-HT), and dopamine (DA) (Gold et al., 1988; Bonhomme and Esposito et al., 1998). Likewise, WKY rats differ in markers of monoaminergic function compared to SD and WIS rats. Specifically, the density of monoamine transporter sites differ in WKY compared to control strains, although the brain regions in which these differences are observed depend upon the strain of rat used as the control (Tejani-Butt et al., 1994; Pare and Tejani-Butt., 1996; Jiao et al., 2003). For example, decreases in dopamine transporter (DAT) binding in WKY rats have been reported in the central nucleus of the amygdala (CeA), ventral tegmental area (VTA), substantia nigra pars compacta (SNc) and nucleus accumbens (NAc); and increases have been seen in the lateral hypothalamus (LH) and dorsal hypothalamus (DH) when compared to SD rats. Decreased DAT binding was also seen in WKY rats in the medial amygdala (MeA), SNc and the NAc shell when compared to the WIS strain (Jiao et al., 2003). Altered DAT binding in WKY is likely to impact the clearance and metabolism of DA in these rats. Dopamine levels and activity in non-stressed WKY rats have been assessed previously (De La Garza and Mahoney, 2004) in brain regions such as the medial prefrontal cortex (mPFC), striatum, NAc shell and basolateral amygdala (BLA). Of these regions, only the NAc shell was reported to show decreased DAT binding in WKY compared to WIS rats (Jiao et al., 2003). Interestingly, DA turnover was higher in the NAc shell of non-stressed WKY rats compared to WIS rats (De La Garza and Mahoney, 2004), suggesting a relationship between altered DAT levels and DA activity. To date, comparison of DA levels or activity between WKY, SD and WIS rats have not been explored in areas of the brainstem or hypothalamus where DAT differences are apparent.

Additionally, indirect evidence suggests that 5-HT levels may differ within the brain of WKY compared to outbred control strains such as SD or WIS rats. The antidepressant response to selective serotonin reuptake inhibitors (SSRIs) or the 5-HT_{1A} agonist 8-OH-DPAT is blunted in WKY compared to SD rats (Lahmame and Armario, 1996; Pollier et al., 2000; Lopez-Rubalcava and Lucki 2000; Pare et al., 2001; Tejani-Butt et al., 2003; Will et al., 2003), suggesting dysfunctional central 5-HT pathways in the WKY strain of rats. However, De La Garza and Mahoney (2004) did not find any differences in serotonergic activity when comparing a number of forebrain regions in non-stressed WKY and WIS rats. Following exposure to chronic novel stressors, WKY rats exhibit increased 5-HT_{1A} receptor binding in the hippocampus and hypothalamus and decreased 5-HT transporter (5-HTT) densities in the cortex and hippocampus compared to SD controls (Pare and Tejani-Butt, 1996) which suggest altered 5-HT function. However, it is not known whether the 5-HT levels or activity actually differ between WKY and SD or WIS strains within these brain regions in the absence of prior stress or antidepressant exposure.

Unlike serotonergic antidepressants, the behavioral response of WKY rats to the noradrenergic antidepressant, desipramine appears to be similar to that seen in SD controls (Lopez-Rubalcava and Lucki, 2000; Tejani-Butt et al., 2003). However, others have reported that WKY rats are subsensitive to the effects of desipramine in the forced swim test (Lahmame & Armario, 1996). Contrasting results may be a result of genetic heterogeneity among the WKY rats (Will et al., 2003) or may be due to differing forced swim test procedures that exist between these studies, such as pre-test exposure, water height or duration of testing. De La Garza and Mahoney (2004) showed that NE levels did not differ between non-stressed WKY and WIS rats in the mPFC, striatum, NAc shell or BLA. However, Pearson et al. (2006) found an increase in expression of genes encoding for NE synthesis and metabolism in the locus coeruleus (LC) of drug or stress naive WKY rats compared to SD controls. Furthermore, decreased norepinephrine transporter (NET) expression was observed in the amygdala of WKY rats compared to WIS rats following desipramine treatment (Jeannotte et al., 2009), suggesting differences in NE systems between these two strains. Combined, these findings suggest that NE levels could be altered in the WKY rat at the level of the LC or in other regions innervated by the LC such as the hypothalamus, hippocampus and subregions of the amygdala.

Based on the use of the WKY rat as a model of depressive behavior, it is clearly important to have a comprehensive study of regional brainstem, limbic and striatal monoamine levels or activity in the WKY strain in order to assist in explaining the behavioral characteristics and sensitivity to antidepressants found in these rats. Furthermore, a comparison between the WKY strain with both of the two most commonly used outbred control strains (SD and WIS) is necessary for determining the underlying mechanisms that could lead to the overall depressive profile of the WKY rat. The goal of the current study was to measure monoamine (DA, NE and 5-HT) levels and activity in discrete brainstem, limbic and striatal brain regions of the WKY strain and compare these measures to values obtained from both SD and WIS rats. We hypothesized that WKY would differ in monoamine levels and/or activity in these regions based on previous transporter binding, differences in gene expression or antidepressant responses as described above.

2. Results

2.1 Concentrations of 5-HT and 5-HIAA in Selected Brain Regions

Differences in 5-HT levels between strains were found in multiple brain regions. In general, WKY rats had lower 5-HT concentrations in these brain regions when compared to SD or WIS rats (Figure 1). Serotonin concentrations in the BLA differed between strains (Figure 1A; $F_{(2,29)} = 3.364$, $p = 0.049$). WKY had significantly lower levels of 5-HT compared to SD rats ($p = 0.029$), but not WIS ($p = 0.106$). There were also significant differences between strains in 5-HT levels within the LH (Figure 1B; $F_{(2,28)} = 4.586$, $p = 0.019$) with WKY rats exhibiting lower concentrations of 5-HT compared to SD rats ($p = 0.023$) and WIS rats ($p = 0.030$). Concentrations of 5-HT also differed between strains in the DH (Figure 1B; $F_{(2,27)} = 5.720$, $p = 0.008$) with concentrations in WKY rats significantly lower than SD rats ($p = 0.007$) and WIS rats ($p = 0.042$).

In the NAc shell, levels of 5-HT were different between strains (Figure 1C; $F_{(2,28)} = 5.051$, $p = 0.013$). WKY rats had lower 5-HT levels compared to SD ($p = 0.019$) and WIS rats ($p = 0.019$), respectively. Within the cell body regions, a significant difference was found between the strains in the dorsal raphe nucleus (dRN) (Figure 1D; $F_{(2,28)} = 4.659$, $p = 0.018$), with lower concentrations of 5-HT in the WKY rats compared to the SD rats ($p = 0.014$) but not the WIS rats ($p = 0.222$). Significant differences between strains were also found in the SNc (Figure 1D; $F_{(2,27)} = 4.262$, $p = 0.025$) where WKY rats had lower 5-HT concentrations compared to SD rats ($p = 0.049$) and WIS rats (0.019). No significant effects

of strain were found in any other cell body or terminal region examined (Figure 1; $p > 0.05$). Furthermore, there were no significant differences in 5-HT concentrations between SD and WIS within any brain region studied (Figure 1, $p > 0.05$).

Concentrations of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) also differed between the three strains in a number of brain regions as illustrated by Figure 2. In contrast to 5-HT levels, differences were also observed between SD and WIS strains, with SD rats consistently exhibiting greater 5-HIAA levels (Figure 2). Concentrations of 5-HIAA differed between SD, WKY and WIS rats in the dorsal hippocampus (dHipp) (Figure 2A; $F_{(2,29)} = 5.639$, $p = 0.009$), with significantly higher levels of 5-HIAA in SD compared to WIS ($p = 0.007$) but not WKY rats ($p = 0.073$). The same pattern was observed in the ventral hippocampus (vHipp), (Figure 2A; $F_{(2,29)} = 3.862$, $p = 0.033$) with higher 5-HIAA levels in SD rats compared to WIS ($p = 0.034$) but not WKY rats ($p = 0.063$). Within the hypothalamus, differences in 5-HIAA levels were observed between strains in the ventral hypothalamus (VH), (Figure 2B; $F_{(2,29)} = 4.848$, $p = 0.015$) with significantly lower levels of 5-HIAA in the WIS rats compared to both SD rats ($p = 0.016$) and WKY rats ($p = 0.018$). Differences were also found between strains in the DH (Figure 2B; $F_{(2,28)} = 7.073$, $p = 0.003$), where SD rats had greater 5-HIAA concentrations compared to WKY ($p = 0.004$) and WIS rats ($p = 0.009$).

Differences in 5-HIAA levels were also found between SD, WKY and WIS rats in the NAc shell (Figure 2C; $F_{(2,29)} = 6.389$, $p = 0.005$) where SD rats had higher 5-HIAA levels when compared to WKY rats ($p = 0.005$) and WIS rats ($p = 0.029$). Within the lateral striatum (LStr), significant difference in 5-HIAA levels was also observed between the strains (Figure 2C; $F_{(2,29)} = 5.930$, $p = 0.007$). In this region, WIS rats had lower levels of 5-HIAA compared to both SD ($p = 0.039$) and WKY rats ($p = 0.005$). Similarly, WIS rats had lower 5-HIAA levels in the mPFC (Figure 2C; $F_{(2,28)} = 4.229$, $p = 0.025$) compared to SD ($p = 0.025$) and WKY rats ($p = 0.028$).

In the monoamine cell body regions, 5-HIAA concentrations only differed between strains within the dRN (Figure 2D; $F_{(2,29)} = 5.519$, $p = 0.009$). Levels of 5-HIAA were significantly higher in SD rats compared to WKY ($p = 0.011$) and WIS rats ($p = 0.018$).

Serotonin turnover, estimated from the ratio of 5-HIAA to 5-HT, was calculated for all brain regions. Based on this index, WIS rats generally had lower 5-HT turnover rates when compared to either SD or WKY rats. In the amygdaloid complex, significant differences in 5-HT turnover were observed between strains in the MeA (Figure 3A; $F_{(2,29)} = 4.601$, $p = 0.018$), with lower turnover in WIS compared to SD ($p = 0.017$) and WKY rats ($p = 0.021$). WIS rats also had lower 5-HT turnover in the BLA (Figure 3A; $F_{(2,29)} = 3.939$, $p = 0.031$) compared to WKY rats ($p = 0.025$) but not SD rats ($p = 0.061$).

Strain differences in 5-HT turnover were observed in all hypothalamic regions tested (Figure 3B). Serotonin turnover was significantly different between strains in the LH (Figure 3B; $F_{(2,28)} = 5.152$, $p = 0.012$), with WIS rats exhibiting lower turnover compared to WKY rats ($p = 0.009$). Similarly, differences in 5-HT turnover was found between strains in the VH (Figure 3B; $F_{(2,29)} = 4.537$, $p = 0.019$) with lower 5-HT turnover in the WIS rats compared to WKY ($p = 0.016$) but not SD rats ($p = 0.169$). In the DH (Figure 3B; $F_{(2,29)} = 4.738$, $p = 0.017$), WIS rats had lower 5-HT turnover than either WKY ($p = 0.014$) and SD rats ($p = 0.030$).

Within the NAc, significant differences were found in 5-HT turnover between strains in the shell (Figure 3C; $F_{(2,29)} = 4.454$, $p = 0.021$), with WIS rats showing lower turnover compared to WKY ($p = 0.015$) but not SD rats ($p = 0.078$). Differences in 5-HT turnover

were also present within the core of the NAc (Figure 3C; $F_{(2,29)} = 5.440$, $p = 0.010$), with lower turnover found in WIS compared to both WKY ($p = 0.008$) and SD rats ($p = 0.028$).

In the monoamine cell body regions, WIS rats had lower 5-HT turnover than WIS or SD rats in most regions evaluated (Fig. 3D). Differences in 5-HT turnover between strains were detected in the dRN (Figure 3D; $F_{(2,29)} = 4.317$, $p = 0.023$) where WIS rats had lower 5-HT turnover when compared to WKY ($p = 0.017$) but not SD rats ($p = 0.072$). Within the medial raphe nucleus (mRN), an effect of strain on 5-HT turnover was also observed (Figure 3D; $F_{(2,29)} = 5.454$, $p = 0.010$), with lower 5-HT turnover found in WIS compared to SD ($p = 0.007$) but not WKY rats ($p = 0.081$). Similarly, significant differences in 5-HT turnover were also found between strains in the SNc (Figure 3D; $F_{(2,26)} = 14.022$, $p < 0.001$). In this case, WIS rats showed lower 5-HT turnover compared to both SD ($p = 0.001$) and WKY rats ($p < 0.001$). Similarly, in the LC (Figure 3D; $F_{(2,29)} = 8.227$, $p = 0.001$), WIS rats had lower 5-HT turnover when compared to SD ($p = 0.001$) and WKY rats ($p = 0.003$).

2.2 Concentrations of DA and DOPAC in Selected Brain Regions

In contrast to serotonergic measures, there were very few strain differences in DA or the DA metabolite dihydroxyphenylacetic acid (DOPAC) detected in the brain regions evaluated (Figures 4-6). Differences in DA levels were only observed in the cell body region of the VTA (Figure 4D; $F_{(2,25)} = 3.480$, $p = 0.046$), where WIS rats had higher DA levels compared to both SD ($p = 0.043$) and WKY rats ($p = 0.035$). The only effect of strain on the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) was found in the mPFC (Figure 5). Significant differences in DOPAC concentrations were observed in the mPFC between strains (Figure 5C; $F_{(2,28)} = 21.589$, $p < 0.001$), with lower DOPAC levels in WKY compared to SD ($p = 0.005$) and WIS rats ($p < 0.001$). Furthermore, WIS rats had higher DOPAC levels compared to SD rats ($p = 0.001$) in the mPFC (Figure 5C). In addition, there was a trend within the CeA towards significance between strains (Figure 5A; $F_{(2,23)} = 3.310$, $p = 0.055$).

As seen with other DA measures, there were very little effects of strain on DA turnover as estimated using the ratio of DOPAC to DA (Figure 6). Significant differences in DA turnover between strains were found only in the shell of the NAc (Figure 6C; $F_{(2,29)} = 3.362$, $p = 0.049$), with higher turnover found in WKY rats compared to WIS rats ($p = 0.044$) but not compared to SD rats ($p = 0.529$). There was a trend towards a significant effect of strain on DA turnover in the core of the NAc (Figure 6C; $p = 0.065$), but no significant differences were found in any other region in which DA and DOPAC were detected (Figure 6).

2.3 Concentrations of NE in Selected Brain Regions

There were a number of strain differences in NE concentrations within the brain regions sampled, with some of the effects related to differences in WKY rats compared to the other two strains (Figure 7). Differences in NE levels between strains were found in the vHipp (Figure 7A; $F_{(2,28)} = 4.149$, $p = 0.026$), with WKY rats showing lower concentrations of NE compared to SD ($p = 0.024$) but not WIS rats ($p = 0.066$). Within the LH, there was also an effect of strain on NE levels (Figure 7B; $F_{(2,28)} = 5.827$, $p = 0.008$), but in this case WKY rats were lower when compared to WIS ($p = 0.006$) but not SD rats ($p = 0.056$).

There were also several differences in NE levels between SD, WKY and WIS rats within the striatal and accumbal regions (Figure 7C). In the NAc shell (Figure 7C; $F_{(2,27)} = 4.058$, $p = 0.029$), SD rats had higher NE concentrations when compared to WKY ($p = 0.033$) but not WIS rats ($p = 0.055$). There were also differences between strains in NE levels within the core of the NAc (Figure 7C; $F_{(2,28)} = 3.835$, $p = 0.034$). In this brain region, NE

concentrations in WIS rats were higher compared to SD ($p = 0.020$) and WKY rats ($p = 0.050$). Within the striatum, strain effects on NE levels were observed in the LStr (Figure 7C; $F_{(2,27)} = 4.089$, $p = 0.028$), with NE concentrations higher in WIS rats compared to WKY ($p = 0.020$) but not SD rats ($p = 0.274$). The same pattern was detected within the medial striatum (MStr) (Figure 7C; $F_{(2,28)} = 4.421$, $p = 0.021$), with WIS rats exhibiting higher NE levels compared to WKY ($p = 0.016$) but not SD rats ($p = 0.067$).

Strain differences in NE concentrations were also found within monoamine cell body regions (Figure 7C). Within the dRN, strains had significantly different NE levels (Figure 7D; $F_{(2,29)} = 4.059$, $p = 0.028$), with lower levels found in WKY compared to SD ($p = 0.023$) but not WIS rats ($p = 0.103$). A strain effect was also observed in the NE cell body region, the LC (Figure 7D; $F_{(2,26)} = 9.384$, $p < 0.001$), with higher NE concentrations found in WIS rats compared to SD ($p = 0.004$) and WKY rats ($p < 0.001$). There was no effect of strain on NE levels within any other cell body or terminal region sampled (Figure 7; $p > 0.05$).

3. Discussion

The current results point to several significant alterations in central monoamine levels and monoaminergic turnover between the WKY rat strain and its two commonly utilized outbred controls, the SD and WIS rat strains. The majority of the monoaminergic differences across strains were in serotonergic and norepinephrine systems, with few differences in dopaminergic systems. Moreover, noteworthy differences in monoaminergic levels or turnover were observed between the two control strains in specific brain regions. Given that the technique used to measure concentrations of monoamines in each brain region cannot distinguish between intracellular and extracellular levels, caution should be used in interpreting turnover (metabolite/monoamine ratio) data as a measure of monoaminergic activity. However, the approach used in this study provides an estimate of steady-state monoaminergic activity (Moore and Johnson, 1982; Shannon et al., 1986), and has the distinct advantage of being able to measure multiple monoamines in multiple brain regions. Therefore, the observed findings will be able to direct future work employing *in vivo* techniques to sample individual monoamines in brain regions identified by this study to differ between strains. Such studies may ultimately elucidate the neurotransmitter differences between strains of rats as they relate to behavioral phenotype.

Our results show that in many limbic and brainstem regions, WKY rats consistently had lower basal concentrations of 5-HT compared to either or both SD and WIS strains. The current study indicates lower 5-HT concentrations in the NAc shell in WKY compared to WIS. De La Garza and Mahoney (2004) reported no difference between these strains in this region in the non-stressed condition but did show lower 5-HT levels in the NAc shell of stressed WKY compared to stressed WIS rats (De La Garza and Mahoney, 2004). In the current study, WKY were also found to have lower 5-HT levels in the NAc shell, the basolateral amygdala, and subregions of the hypothalamus when compared to SD rats. Therefore, lower 5-HT in the NAc shell (whether basal or stress-induced) appears to be a characteristic of WKY rats across studies and control strains used.

Previous work has shown that the depressive and anxiety-like behaviors of WKY rats are resistant to SSRIs when compared to SD rats (Pollier et al., 2000; Lopez-Rubalcava and Lucki 2000; Pare et al., 2001; Tejani-Butt et al., 2003). Given that the therapeutic effects of SSRIs are imparted, in part, by the long-term neuroadaptations that result from increasing extracellular 5-HT via reuptake blockade in the dRN (e.g. Castro et al., 2008), findings that WKY rats show lower 5-HT or related measures in a number of limbic brain regions raises the possibility that the blunted response to SSRIs could be due to reduced 5-HT in key

regions such as the dRN within the WKY rats. Thus, lower 5-HT concentrations in these regions within WKY rats could translate to reduced extracellular 5-HT to provide less pharmacological substrate for reuptake inhibition to effectively promote SSRI-induced neuroadaptations. Furthermore, lower 5-HT levels in WKY rats within key serotonergic regions may be a result of reduced intracellular 5-HT reflecting a reduction in 5-HTT sites, providing a further mechanism for insensitivity to SSRIs. Pollier et al (2000) suggest that WKY behavioral insensitivity to SSRIs is independent of 5-HTT mechanisms when the comparison group is an inbred control (spontaneously hypertensive rats), but there is a paucity of information related to 5-HTT sites in WKY and SD (or WIS) rats in the absence of stressors or SSRI treatment.

Serotonin levels in the lateral and dorsal hypothalamus measured in WKY rats were lower compared to both SD and WIS rats. Of relevance, WKY rats have increased 5-HT_{1A} receptors in the hypothalamus compared to SD rats following a stressor (Pare and Tejani-Butt, 1996). This may reflect increased postsynaptic sensitivity to 5-HT as an adaptation for reduced functional 5-HT in this area. Such a possibility warrants further testing.

Interestingly, SD and WIS did not differ from one another in 5-HT levels within any of the brain regions studied, yet 5-HIAA levels and 5-HT turnover were different between these two control strains. In fact, WIS rats had lower levels of 5-HT turnover compared to the SD strain (and often when compared to WKY rats as well) in various amygdaloid, hypothalamic and NAc subregions and in a majority of brainstem regions. When 5-HT turnover was previously assessed between non-stressed WKY and WIS rats, no differences were observed between strains in the regions (mPFC, LStr, NAc shell and BLA) measured (De La Garza and Mahoney, 2004). Overall, the choice of the comparison outbred strain appears to determine whether WKY exhibit altered 5-HT levels or activity. However, behavior in open field or forced swim testing does not appear to differ between SD and WIS rats (Tejani-Butt et al., 2003), implying that the striking differences in 5-HT turnover or metabolism between these two strains does not have functional consequences for emotive-based behaviors.

WKY rats appear to exhibit altered dopaminergic markers in a variety of brain regions when compared to outbred SD and WIS controls (Jiao et al., 2003; De La Garza and Mahoney, 2004; Novick et al., 2008). In particular, differences in DAT between the three strains have been reported in the sub-regions of the amygdala, hippocampus, hypothalamus, striatum, NAc and brainstem (Jiao et al., 2003). However, we find few differences across strains in DA or DOPAC levels or DA turnover in the same brain regions. Of these few differences, the finding that DA turnover within the shell of the NAc is higher in WKY compared to WIS rats may be related to decreased DAT expression in this region of WKY rats (Jiao et al., 2003), since reduced re-uptake could increase extracellular DA and thus DA turnover. While this finding replicates that of De La Garza and Mahoney (2004) in non-stressed WKY and WIS rats, our study also shows that DA turnover did not differ between SD and WKY rats in the NAc shell, suggesting that the difference observed between WKY and WIS rats is control-strain specific. In contrast, DOPAC levels in the mPFC were lower in WKY rats compared to both SD and WIS rats, a result that is also consistent with the work of De La Garza and Mahoney (2004), who demonstrated the same pattern in non-stressed rats when WKY were compared to WIS only. Interestingly, partial DA lesion of the mPFC increased extracellular DA levels released in the NAc as elicited by reinforcing stimuli (Mitchell and Gratton, 1992). Based on tissue concentrations, the present study also found an inverse relationship between DA activity in the mPFC and NAc in WKY rats.

Additionally, we found a strain difference in DA levels within the VTA, an area where Jiao et al. (2003) reported that WKY rats had lower DAT expression compared to SD but not WIS rats. However, we found higher concentrations of DA within the VTA of WIS rats

compared to both the WKY and SD strains suggesting, in this case, that DAT expression across strains may not be related to strain differences in DA concentrations. The lack of strain difference in VTA concentrations of DOPAC or in DA turnover also supports this argument, but this will need confirmation with direct measurement of DA release and reuptake in this region.

Our results show several strain differences in NE within multiple brain regions of interest. Many of the strain differences were actually due to one of the control strains (SD or WIS) having different levels of NE, which emphasizes the need for using multiple control strains in these types of studies. This is highlighted by previous findings where comparisons between non-stressed WKY and one control strain (WIS) did not reveal any differences in NE levels in the regions measured (mPFC, LStr, NAc shell and BLA; De La Garza and Mahoney, 2004), whereas the current study shows that WIS rats have higher levels of NE in the LStr when compared to SD and WKY rats. Previously, increased expression of genes encoding for NE synthesis and metabolism in the LC were observed in WKY rats compared to SD rats (Pearson et al., 2006). The current results suggest that this previously observed increase in NE gene expression in the LC is not reflective of increased NE concentrations in the LC of WKY rats, either due a lack of continuity between gene expression and actual neurotransmitter levels, or a result of increased NE metabolism in this area balancing increased synthesis in WKY rats. Furthermore, the current results suggest that the previously observed increase in genes related to NE synthesis and metabolism in the LC of WKY rats (Pearson et al., 2006) may not translate into increased terminal NE levels, since WKY rats showed no difference or lower NE levels within specific terminal sites (such as the vHipp, LH and dRN). Lower NE levels in these terminal sites of WKY rats most likely reflects local reductions in NE synthesis, rather than global changes to NE systems derived from the LC, since WKY rats did not differ from control strains in NE levels within many other sites innervated by the LC. Interestingly, lower NE levels in the dRN of WKY rats may relate to the lower 5-HT concentration in the dRN of these rats, since reduced NE levels in the dRN results in a concurrent decrease in 5-HT levels in this region (Pudovkina et al., 2002).

One finding that is relevant to the neurobiology of depression is that the WKY rats have lower NE levels in the vHipp but not the dHipp. Experimental evidence suggests that the vHipp plays an important role in mediating emotive states which can be dissociated from dHipp mediation (Bannerman et al., 2002; 2003). Furthermore, NE is known to be necessary for cell proliferation and new neuronal survival that underlies adult neurogenesis in the hippocampus (Hiramoto et al., 2006; Rizk et al., 2006), and decreased adult hippocampal neurogenesis is associated with depressive and anxiety-like behaviors in rodents (Duman, 2004; Bergami et al., 2008; Revest et al., 2009). Thus, reduced NE levels in the vHipp of WKY rats may lead to reduced neurogenesis in this region. This might represent an underlying neurobiological factor for the depressive and anxiety-like behaviors evident in this strain. Whether adult hippocampal neurogenesis is blunted in WKY rats compared to control strains could represent a future research direction to assess a genetic component to the link between neurogenesis and depressive or anxiety-like behaviors.

Overall, the results show that while WKY rats have discrete differences in neural monoaminergic concentration when compared to outbred control strains, the differences between these control strains are also apparent. Thus, our findings highlight the importance of future work in this field utilizing both SD and WIS strains as controls. Furthermore, the current study has identified brain regions for future pharmacological, microdialysis or neurogenesis studies in WKY rats to further the understanding of the neurobiology that underlies depressive or anxiety-like behaviors. Most consistently and globally, the WKY rat exhibited lower 5-HT levels in multiple limbic regions compared to control strains. Our findings, combined with others, suggest the WKY strain as an appropriate rat model of

depressive-like behavior with 5-HT dysfunction and SSRI insensitivity. This has important implications for the testing of novel antidepressant agents to ameliorate depression that is resistant to SSRI treatment, given that approximately 50% of patients do not respond to SSRI therapy (Koenig and Thase, 2009).

4. Experimental Procedure

4.1 Animals

Adult male rats of three strains were used for comparison. Sprague Dawley rats (n=12, age 3-4 months; from Harlan (Indianapolis, IN), Wistar rats (n=8, age 3-4 months; Charles River Laboratories (Kingston, NY) and Wistar-Kyoto rats (n=12, age 3-4 months; from Charles River Laboratories (Kingston, NY). They were handled according with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 and with Institutional Animal Care and Use Review Committee's approval. Animals were individually housed at 22°C and placed on a 12-hr light/dark cycle. Food and water were available ad libitum. Rats were kept in home cages under controlled settings until use.

4.2 Collection of brains and monoamine analysis

Rats were removed from their home cages at ~ 1000 hours and decapitated. Brains were collected and frozen at -80°C until sectioned. Frozen brains were serially sliced at 300 µm in the coronal plane at -12°C, and sections were stored at -80°C until microdissection. The following regions were identified using Paxinos and Watson (1998): limbic regions, including central nucleus of the amygdala (CeA), medial amygdala (MeA) basolateral amygdala (BLA), dorsal hippocampus (dHipp), ventral hippocampus (vHipp), and the lateral, ventral and dorsal hypothalamus (LH, VH and DH respectively); striatal, accumbal and cortical regions, including shell and core of the nucleus accumbens (NAc shell and core), lateral striatum (LStr), medial striatum (MStr) and the medial prefrontal cortex (mPFC); cell body regions, including ventral tegmental area (VTA), dorsal and medial raphe nuclei (dRN, mRN), substantia nigra pars compacta (SNc), and the locus coeruleus (LC). According to guides, regions were all microdissected using either a 300 µm id cannula or a 500 µm id cannula using a dissecting microscope and a Physitemp freezing stage (North Central Instruments, Inc., Clifton, NJ). Microdissected tissue was expelled into 60 µL of a sodium acetate buffer (pH 5.0) that contained 9.42 pg/µL of an internal standard (dihydroxybenzylamine, DHBA); cells were lysed by a cycle of freeze-thawing.

All microdissected brain regions were analyzed for serotonin (5-HT), its metabolite 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) and norepinephrine (NE) using high-pressure liquid chromatography. Details of this assay are published elsewhere (Renner et al., 1987; Watt et al., 2009). Briefly, brain regions were thawed at room temperature and 2 µL of a 1 mg/mL ascorbate oxidase solution (2mg/10mL dH₂O; Sigma-Aldrich, St. Louis, MO) and centrifuged at 17,000 rpm for 5 minutes. The supernatant was removed and 45 µL was injected into a chromatographic system (Waters Associates, Inc., Milford, MA, USA) and analyzed electrochemically with a LC-4B potentiostat (BioAnalytical Systems, West Lafayette, IN). The electrode potential was set at +0.6 V with respect to an Ag/AgCl reference electrode. Mobile phase consisted of 14 g citric acid, 8.6 g sodium acetate, 110 mg 1-octanesulfonic acid, 150 mg EDTA disodium salt, and 100 mL of methanol in 1L of deionized water (Sigma-Aldrich, St. Louis MO). Flow rate was maintained at 1.4 mL/min.

The remaining tissue pellet was dissolved in 110 µL of 0.4 M NaOH, and the protein content was assayed using the Bradford method (Bradford, 1976). Concentrations of monoamines in

samples were determined by calculating peak height compared to known standards with internal standard, and monoamines were corrected for recovery using CSW32 v1.4 Chromatography Station for Windows (DataApex, Prague, Czech Republic). Neurotransmitter and metabolite levels are all expressed as pg amine/ug protein. Ratios of metabolite/amine were also calculated as an index of turnover for DA and 5-HT.

4.3 Data Analysis

Levels in basal monoamine levels and metabolites were compared between all three strains of rat using separate one-way ANOVAs for each neurotransmitter and brain region. Significant effects were further analyzed using Student Newman-Keuls (SNK) post-hoc test for multiple pairwise comparisons. All analyses were performed using SigmaStat (version 3.5; ©2006, Systat Software, Inc., Richmond, CA), with the alpha level set at 0.05.

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Abbreviations

5-HIAA	5-hydroxyindoleacetic acid
BLA	basolateral amygdala
CeA	central nucleus of the amygdala
DOPAC	3,4-dihydroxyphenylacetic acid
DA	dopamine
DAT	dopamine transporter
dHipp	dorsal hippocampus
DH	dorsal hypothalamus
dRN	dorsal raphe nucleus
LH	lateral hypothalamus
LStr	lateral striatum
LC	locus ceruleus
MeA	medial amygdala
mPFC	medial prefrontal cortex
mRN	medial raphe nucleus
MStr	medial striatum
NE	norepinephrine
NET	norepinephrine transporter
NAc	nucleus accumbens
SSRI	selective serotonin reuptake inhibitor
5-HT	serotonin
5-HTT	serotonin transporter
SD	Sprague-Dawley

SNK	Student Newman-Keuls
SNc	substantia nigra pars compacta
vHipp	ventral hippocampus
VH	ventral hypothalamus
VTa	ventral tegmental area
WIS	Wistar
WKY	Wistar-Kyoto

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Figure 1. Neural Serotonin Concentrations

Serotonin concentrations in (A) amygdalar and hippocampal subregions (B) hypothalamus subregions (C) striatal, accumbal and cortical regions and (D) cell body regions for SD, WKY and WIS rats. #represents differences between SD and WKY rats, and &represents differences between WIS and WKY rats. Data represent mean $\pm$ SEM.

Figure 2. Neural 5-HIAA Concentrations

5-HIAA concentrations in (A) amygdalar and hippocampal subregions (B) hypothalamus subregions (C) striatal, accumbal and cortical regions and (D) cell body regions for SD, WKY and WIS rats. #represents differences between SD and WKY rats, *represents differences between SD and WIS rats and &represents differences between WIS and WKY rats. Data represent mean $\pm$ SEM.

Figure 3. Serotonin Turnover

Serotonin turnover in (A) amygdalar and hippocampal subregions (B) hypothalamus subregions (C) striatal, accumbal and cortical regions and (D) cell body regions for SD, WKY and WIS rats. *represents differences between SD and WIS rats and & represents differences between WIS and WKY rats. Data represent mean $\pm$ SEM.

Figure 4. Neural Dopamine Concentrations

Dopamine concentrations in (A) amygdalar and hippocampal subregions (B) hypothalamus subregions (C) striatal, accumbal and cortical regions and (D) cell body regions for SD, WKY and WIS rats. *represents differences between SD and WIS rats and &represents differences between WIS and WKY rats. Data represent mean $\pm$ SEM. DA concentrations in the LC were not above detection limits.

Figure 5. Neural DOPAC Concentrations

DOPAC concentrations in (A) amygdalar and hippocampal subregions (B) hypothalamus subregions (C) striatal, accumbal and cortical regions and (D) cell body regions for SD, WKY and WIS rats. #represents differences between SD and WKY rats, *represents differences between SD and WIS rats and &represents differences between WIS and WKY rats. Data represent mean $\pm$ SEM. Missing data indicates that DOPAC levels were not above detection limits.

Figure 6. Dopamine Turnover

Dopamine turnover in (A) amygdalar and hippocampal subregions (B) hypothalamus subregions (C) striatal, accumbal and cortical regions and (D) cell body regions for SD, WKY and WIS rats. *represents differences between WIS and WKY rats. Data represent mean $\pm$ SEM. DA turnover was only calculated for those regions in which both DA and DOPAC were detected.

Figure 7. Neural NE Concentrations

Norepinephrine concentrations in (A) amygdalar and hippocampal subregions (B) hypothalamus subregions (C) striatal, accumbal and cortical regions and (D) cell body regions for SD, WKY and WIS rats. #represents differences between SD and WKY rats, *represents differences between SD and WIS rats and &represents differences between WIS and WKY rats. Data represent mean $\pm$ SEM.