INTRODUCTION

Chronic stress is a risk factor for many psycho-pathological conditions, including depression and anxiety disorders (Kendler et al. 1995, Sapolsky 1996, Shelton 2004). Chronic Variable Stress (CVS) has significant effects on the cellular integrity and function of certain brain areas, most especially the prefrontal cortex and hippocampus of rats (Heim et al. 1997, Joels et al. 2007). The medial prefrontal cortex (mPFC) of the rats is mainly subdivided into three cytoarchitectonic subregions: prefrontal (PL), infralimbic (IL), and anterior cingulate cortex (ACC) (Krettek and Price 1977, Uylings et al. 2003). mPFC is an essential neural substrate for coordinating the cognitive-affective information and modulating the hypothalamic–pituitary–adrenal axis response to emotional stress (Diorio et al. 1993, MacDonald et al. 2000). Previous studies reported the significant role of rats’ mPFC in stress. The study also demonstrated that chronic restraint stress changed the morphology of the neurons in the mPFC (Rajkowska et al. 1999, Akana et al. 2001). Furthermore, in clinical studies, structural brain modifications in depressed patients, such as decreased volume (Bremner et al. 2000) or neuronal density in hippocampus (Rajkowska et al. 1999), were similar to those found in...
Sertraline, curcumin and cortex in stress (Cotter et al. 2001, Joels et al. 2004). Previous studies also showed that chronic stress exposure resulted in decreased neurogenesis in the hippocampal formation (Gould and Tanapat 1999, Czeh et al. 2001). Moreover, it has been agreed that repeated stressful experiences have intense effects on neuronal plasticity in different brain areas, particularly in the hippocampal formation, the prefrontal cortex, and the amygdala. CVS has been widely used for studying clinical depression as well as evaluating the antidepressant effects of diverse drugs in animal models (Willner 2005). Therefore, the first aim of the present study is to demonstrate the structural changes of the mPFC after CVS. This study also aims to evaluate and compare the therapeutic effects of curcumin and sertraline on CVS.

Curcumin is the principal curcuminoid of the popular Indian spice, turmeric. Curcumin has been reported to have protective effects in animal models of diseases, such as Alzheimer’s, Parkinson’s, and epilepsy (Xu et al. 2005, Kulkarni et al. 2008). Previous studies reported that the individuals consuming curcumin in daily life had better brain function and higher cognitive abilities (Kulkarni et al. 2009). In another study, curcumin was shown to protect the dopamine producing cells of the substantia nigra area of the brain in a rat model of Parkinson’s disease (Kulkarni et al. 2009). Moreover, administration of curcumin completely reversed stress-induced serotonin and noradrenaline in the hippocampus and the frontal cortex (Xu et al. 2007).

Selective serotonin reuptake inhibitors, including sertraline, are primarily used to treat major depression in adult patients (Rush et al. 2006). The neuroprotective and antioxidant action of sertraline have also been reported in neurodegenerative diseases (Matar et al. 2006). In addition, various reports have suggested the beneficial effects of sertraline on animal model depression (Malberg et al. 2000, Santarelli et al. 2003).

The human studies have been reported that prefrontal cortex shows alterations in the cerebral structures in depressed patients (Lorenzetti et al. 2009). Our previous study on the animal model of stress showed that stress affected the behavioral tests, such as spatial learning and memory, anxiety, and anhedonia (Noorafshan et al. 2013a). The present study extends the earlier work to evaluate the histological parameters of the mPFC of the rats submitted to CVS using stereological methods. The possible protective effects of curcumin and sertraline on the structural parameters are evaluated, as well. Briefly, the study aims to find responses to the following questions:

How much does the volume of the mPFC and its subdivisions change after CVS? How many neurons and glial cells of the mPFC are lost after CVS? Do sertraline and curcumin protect the mPFC structure after CVS? Which drug is more effective?

**METHODS**

**Animals**

In this study, 42 adult male Sprague-Dawley rats (260±20 g) were obtained from the laboratory animal center of Shiraz University of Medical Sciences, Shiraz, Iran. The Ethics Committee of the University approved the animal experiment (Approval No. 91-6124). The male rats were randomly assigned to experimental and control groups. Each group included 6 rats that were housed under standard conditions, room temperature (22–24°C), and a 12:12 h light-dark schedule and had free access to water and food. The animals were divided into seven groups: (I) stress +...
distilled water group daily receiving stress and distilled water, (II) stress + olive oil group daily receiving stress and olive oil, (III) stress + curcumin group daily receiving stress and curcumin (100 mg/kg/day) solved in olive oil (Noorafshan et al. 2013a,b), (IV) stress + sertraline group daily receiving stress and sertraline (10 mg/kg/day) solved in distilled water (Yildirim et al. 2012), (V) control group, (VI) curcumin group receiving curcumin (100 mg/kg/day) solved in olive oil (Noorafshan et al. 2013a,b), and (VII) sertraline group receiving sertraline (10 mg/kg/day) solved in distilled water (Yildirim et al. 2012).

**Stress model**

The animals were submitted to a CVS regime over a 56-day period or remained in their home cages without stress manipulation (Tagliari et al. 2010). The induction of CVS is described in Table I. The following stressors were applied: (1) 24 hours of water procription, (2) 1 to 3 hours of restraint, as described below, (3) 1.5 to 2 hours of restraint at 4°C, (4) flashing light during 120 to 210 minutes, (5) isolation (2 to 3 days), (6) tilting of the home cages at a 45° angle for 4–6 hours, and (7) moist bedding (pouring 300 ml water onto the bedding during 1.5 to 2 hours). Restraint was applied by placing the animals in a 25×7 cm plastic cylinder with the holes for breathing. In addition, exposure to flashing light was completed by placing the rats in a 50 cm-high, 40×60 cm open field made of brown floor with a frontal glass wall. A 40 W lamp was flashed at a frequency of 60 flashes per minute (Tagliari et al. 2010).

**Tissue preparation**

The rats were deeply anesthetized and decapitated. Then, their brains were exposed by an incision along the midline of the skull. A small amount of fixative was poured on the exposed brain immediately. The brains were removed and mPFC was identified according to atlas of Paxinos and Watson (Paxinos and Watson 2007) (Fig. 1). The right prefrontal cortex was immersed in neutral formaldehyde for one week and then embedded in paraffin block. Then, complete serial coronal sections (26 µm thick) were made at 4.70–2.70 mm ventral and 4.70–2.70 mm dorsal to the bregma using a microtome (Akana et al. 2001). Every 10 section was sampled starting with a random number between 1 and 10. After all, about 8–10 sections in each animal were selected in a systematic random manner. The sections were stained with cresyl violet (0.1% in distilled water) in order to estimate the volume of the region as well as the total number of the neurons and glial cells of the mPFC. It should be mentioned that the glial cells were distinguished from neurons by their smaller size and lack of a nucleolus and stained cytoplasm.

**Estimation of the volume of mPFC and its subdivisions (PL, IL, ACC)**

Using a projecting microscope, the live image of each brain section was evaluated according to the rat brain atlas (Gundersen et al. 1988a,b). The boundaries of the mPFC were considered from the most anterior section where the underlying white matter appeared and continued on every mounted section up to the appearance of the genu of the corpus callosum where decussating of the fibers could be observed. Following this procedure, 8–10 sections were obtained and analyzed for each mPFC. The volumes were calculated using the Cavalieri method (Gundersen et al. 1988a,b). Besides, the image of each section was evaluated according to the rat brain atlas at the final magnification of 25× (Paxinos and Watson 2007). Using the stereological software designed at Histomorphometry and Stereology...
Research Center (Shiraz University of Medical Sciences), a grid of points was superimposed on the images (Fig. 1). The volume of the mPFC was estimated by the following formula:

\[ V(mPFC) = \sum P \times \left( \frac{a}{p} \right) \times d, \]

where “\( \sum P \)” was the total points hitting the mPFC sections (here 450–500 points per animal), “\( a/p \)” was the area associated with each point (here was 0.04 mm\(^2\)), and “\( d \)” was the distance between the sampled sections.

**Estimation of the number of neurons and glia**

The total number of neurons and glia cells was determined using the optical disector method at the final magnification of 2800× (Gundersen et al. 1988a,b). The position of the microscopic fields was selected by systematic uniform random sampling, with moving the stage in equal distances in x- and y-directions. An oil immersion objective lens (60×, numerical aperture: 1.4) was used, as well. An unbiased counting frame with inclusion and exclusion borders was superimposed on the images (Fig. 1). This frame avoids the “edge effect” and biased counting of the cells. The focal plane was moved downwards in z-direction. Then, a microcator was attached to the stage of the microscope to measure the z-axis traveling (depth). The upper and lower guard zones were used to avoid cutting artifacts that occurred at the upper and lower surfaces of the tissue sections. The height of the disector was defined as the section thickness excluding the 4 µm thick guard zone at the top and bottom of each section. Any nucleus coming into maximal focus within the next focal sampling plane was selected if it lay completely or partly inside the counting frame and did not touch the exclusion line (Fig. 1).

The suitable guard zone was determined after estimating the percent of nuclei in the ten columns of the z-axis thickness. Each column presented 10 percent of the section thickness. According to the histogram, the upper and lower 20% was considered as the guard zone. The remaining columns were considered as the height of the disector (Fig. 2). The numerical density (\( N_v \)) was estimated using the following formula (Dorph-Petersen et al. 2001):

\[
N_v \left( \frac{Cells}{mPFC} \right) = \left[ \frac{\sum Q^-}{\sum P \times \left( \frac{a}{f} \right) \times h \times BA} \right],
\]

where “\( \sum Q^- \)” was the number of the nuclei coming into focus and counted (on the average, 340–450 neurons and 120–260 glial cells were counted per mPFC), “\( \sum P \)” was the total number of the counting frames in all fields, “\( a/f \)” was the area per frame (35×35 µm\(^2\)), “\( h \)” was the height of the disector, “\( t \)” was the real section thickness measured using the microcator when the Q was counted (here ~20 µm on the average), and “BA” was the block advance of the microtome which was set at 26 µm. The total number of the neurons was estimated by multiplying the numerical density (\( N_v \)) by the \( V \) (mPFC) (Gundersen et al. 1988a,b).

**Estimation of the coefficient of error (CE)**

The CE for the estimate of the volume; i.e., CE(V), was calculated by the following formula:

\[ CE(V) = \left( \frac{\sum P}{1} \right) \times \left[ \frac{1}{200} \times \left( \frac{3 \times \sum Pi Pi + \sum Pi Pi - 4 \sum Pi^{1-2}}{\left[ \sum Pi \right]^3} \right) + 0.0724 \times \frac{B}{A} \times \left( \frac{n^2}{\sum Pi} \right)^{1/2} \],
\]

where “\( B \)” and “\( A \)” represented the mean section boundary length and mean sectional area, respectively. The CE for the estimate of the total neuron and glial cell number, CE(N), was derived from CE(V) and CE(Nv) using the following formula (Gundersen and Jensen 1987, Braendgaard et al. 1990, Gundersen et al. 1999):

\[ CE(N) = \sqrt{CE^2(Nv) + CE^2(V)} \]

\[ CE(Nv) = \left( \frac{n}{n-1} \right) \times \left[ \frac{\sum Q^-}{\sum Q \times \sum Q} + \frac{\sum P + 2 \sum Q \times P}{\sum Q \times \sum P} \right]^{1/2}. \]

**Statistical analysis**

The data were analyzed using Kruskall-Wallis, and Mann-Whitney U-test with adjusted alpha level. \( P \leq 0.05 \) was considered as statistically significant.
Table I

The protocol for induction of the chronic variable stress (CVS) in 56 days for the rat model

<table>
<thead>
<tr>
<th>Day</th>
<th>Stressor applied</th>
<th>Day</th>
<th>Stressor applied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cold restraint (1.5 h)</td>
<td>29</td>
<td>Damp bedding (2 h)</td>
</tr>
<tr>
<td>2</td>
<td>Inclination of home cages (4 h)</td>
<td>30</td>
<td>No stressor applied</td>
</tr>
<tr>
<td>3</td>
<td>Flashing light (2 h)</td>
<td>31</td>
<td>Water deprivation (24 h)</td>
</tr>
<tr>
<td>4</td>
<td>Restraint (2 h)</td>
<td>32</td>
<td>Inclination of home cages (6 h)</td>
</tr>
<tr>
<td>5</td>
<td>Isolation</td>
<td>33</td>
<td>Flashing light (2 h)</td>
</tr>
<tr>
<td>6</td>
<td>Isolation</td>
<td>34</td>
<td>Cold restraint (2 h)</td>
</tr>
<tr>
<td>7</td>
<td>Isolation</td>
<td>35</td>
<td>Isolation</td>
</tr>
<tr>
<td>8</td>
<td>Damp bedding (2 h)</td>
<td>36</td>
<td>Isolation</td>
</tr>
<tr>
<td>9</td>
<td>Inclination of home cages (6 h)</td>
<td>37</td>
<td>Isolation</td>
</tr>
<tr>
<td>10</td>
<td>No stressor applied</td>
<td>38</td>
<td>Flashing light (3 h)</td>
</tr>
<tr>
<td>11</td>
<td>Flashing light (2 h)</td>
<td>39</td>
<td>Damp bedding (2 h)</td>
</tr>
<tr>
<td>12</td>
<td>Water deprivation (24 h)</td>
<td>40</td>
<td>Restraint (3 h)</td>
</tr>
<tr>
<td>13</td>
<td>Restraint (3 h)</td>
<td>41</td>
<td>Cold restraint (1.5 h)</td>
</tr>
<tr>
<td>14</td>
<td>Damp bedding (3 h)</td>
<td>42</td>
<td>Inclination of home cages (4 h)</td>
</tr>
<tr>
<td>15</td>
<td>Inclination of home cages (4 h)</td>
<td>43</td>
<td>Flashing light (2 h)</td>
</tr>
<tr>
<td>16</td>
<td>Cold restraint (2 h)</td>
<td>44</td>
<td>Restraint (2 h)</td>
</tr>
<tr>
<td>17</td>
<td>Flashing light (3 h)</td>
<td>45</td>
<td>Isolation</td>
</tr>
<tr>
<td>18</td>
<td>Restraint (2.5 h)</td>
<td>46</td>
<td>Isolation</td>
</tr>
<tr>
<td>19</td>
<td>Damp bedding (3 h)</td>
<td>47</td>
<td>Isolation</td>
</tr>
<tr>
<td>20</td>
<td>Isolation</td>
<td>48</td>
<td>Damp bedding (2 h)</td>
</tr>
<tr>
<td>21</td>
<td>Isolation</td>
<td>49</td>
<td>Inclination of home cages (6 h)</td>
</tr>
<tr>
<td>22</td>
<td>Isolation</td>
<td>50</td>
<td>No stressor applied</td>
</tr>
<tr>
<td>23</td>
<td>Cold restraint (1.5 h)</td>
<td>51</td>
<td>Flashing light (2 h)</td>
</tr>
<tr>
<td>24</td>
<td>Water deprivation (24 h)</td>
<td>52</td>
<td>Water deprivation (24 h)</td>
</tr>
<tr>
<td>25</td>
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<td>53</td>
<td>Restraint (3 h)</td>
</tr>
<tr>
<td>26</td>
<td>Restraint (3 h)</td>
<td>54</td>
<td>Damp bedding (3 h)</td>
</tr>
<tr>
<td>27</td>
<td>Flashing light (3 h)</td>
<td>55</td>
<td>Inclination of home cages (4 h)</td>
</tr>
<tr>
<td>28</td>
<td>Restraint (1 h)</td>
<td>56</td>
<td>Cold restraint (2 h)</td>
</tr>
</tbody>
</table>

Table II

Coefficients of error (CE) for total volume, neuronal and glial numbers as well as neuronal and glial densities in the medial prefrontal cortex

<table>
<thead>
<tr>
<th>Groups</th>
<th>Volume</th>
<th>Neuron density</th>
<th>Neuron number</th>
<th>Glia density</th>
<th>Glia number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>0.03</td>
<td>0.05</td>
<td>0.05</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>Olive Oil</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Curcumin</td>
<td>0.04</td>
<td>0.04</td>
<td>0.06</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Sertraline</td>
<td>0.03</td>
<td>0.03</td>
<td>0.05</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Stress + curcumin</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Stress + Sertraline</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Control</td>
<td>0.04</td>
<td>0.04</td>
<td>0.06</td>
<td>0.04</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Fig. 3. Dot plots showing the total volume of the mPFC and its subdivisions, including prelimbic (PL), infralimbic (IL), and anterior cingulate cortex (ACC). Each dot represents an animal in stressed or non-stressed group with or without curcumin or sertraline treatment.

Fig. 4. Dot plots showing the total number of the neurons and the total number of the glial cell in the mPFC. Each dot represents an animal in stressed or non-stressed group with or without curcumin or sertraline treatment.
RESULTS

Volume of the mPFC

The study results revealed ~8% decrease in the volume of the mPFC in the stressed groups (+ distilled water or + olive oil) in comparison to the non-stressed rats (Fig. 3). However, the total volume of the mPFC remained unchanged in the stressed rats treated with sertraline or curcumin ($P<0.01$).

Volume of the PL

The study findings showed ~8% reduction in the volume of the prelimbic region in the stressed groups (+ distilled water or + olive oil) in comparison to the non-stressed rats (Fig. 3). Nonetheless, the volume of the prelimbic region remained unchanged in the stressed rats treated with sertraline or curcumin ($P<0.01$).

Volume of the IL

The study results revealed ~24% decrease in the volume of the infralimbic region in the stressed groups (+ distilled water or + olive oil) in comparison to the non-stressed rats (Fig. 3). Nonetheless, the volume of the infralimbic region remained unchanged in the stressed rats treated with sertraline or curcumin ($P<0.01$).

Volume of the ACC

According to the findings of the current study, the volume of the anterior cingulated cortex remained unchanged in the stressed groups (+ distilled water or + olive oil) in comparison to the non-stressed rats (Fig. 3).

Total number of the neurons

The results showed that the total number of the neurons in the mPFC was significantly reduced by 11% in the stress + olive oil group in comparison to the stress + curcumin group. Further analysis also revealed 5% decrease in the stress + distilled water group in comparison to the stress + sertraline group ($P<0.01$) (Fig. 4).

Total number of the glial cells

The results showed that the total number of the cells in the mPFC was significantly reduced by 5% in the stress + olive oil group in comparison to the stress + curcumin group. Further analysis also revealed 5% decrease in the stress + distilled water group in comparison to the stress + sertraline group ($P<0.01$) (Fig. 4).

DISCUSSION

The present study investigated the changes in the total volume and total number of the neurons and glial cells in mPFC resulting from CVS using stereological methods. The advantage of using stereological studies is obtaining unbiased and accurate estimations. The volume of the mPFC and different regions of the PFC was evaluated in advance. The volume reduction which was found in this study is in accordance with other studies that explained the structural changes followed by stress. Holmes and Wellman (2009) explained that exposure to even short periods of severe stress was sufficient to cause major structural remodeling of the main projection neurons within the rodents’ PFC. In addition, Garrett and Wellman (2009) showed that stress could decrease the apical dendritic branch number and length in male rats, but could increase the apical dendritic length in female rats. According to these preclinical findings, chronic stress reduced the grey matter volume in the mPFC (Frodl et al. 2008). This study demonstrated that chronic exposure to stress reduced the volume of the mPFC as well as the PFC sub-regions (PL and IL), whereas the volume of the ACC region remained unchanged. Therefore, there are functional and anatomical differences among these three PFC sub-regions. It has been stated that in comparison to the ACC region, neurons in the prelimbic and infralimbic regions of the mPFC are more susceptible to stress (Helmeke et al. 2008). The present study also indicated that chronic exposure to stress reduced the number of the neurons in the mPFC, which is in agreement with the results of another study conducted on the issue (Bachis et al. 2008). These researchers found an increase in caspase-3 positive neurons in the cerebral cortex, suggesting apoptosis as a result of chronic mild stress. Furthermore, in clinical studies, structural brain modifications, such as decreased neuronal density in mPFC of the depressed patients, were
Sertraline, curcumin and cortex in stress

similar to those found in the animal models of the present study (Rajkowska et al. 1999). The previous works reporting a decrease in cytogenesis in the rat mPFC following chronic stress (Czeh et al. 2007, 2008). Czeh and coworkers (2007) showed that chronic stress repressed cell proliferation both in the cerebral hemispheres of adult rats. They also showed that the survival rate of the newborn cells was also inhibited by chronic stress in both hemispheres. In the present study, the number of glial cells in the mPFC was also quantified using stereological techniques and the results showed reductions in the number of the glial cells in the mPFC after chronic stress. Other studies have also indicated a significant reduction in the number of the glial cells in the mPFC in major depressive disorder (Bowley et al. 2002). Ongur and colleagues (1998) and Cotter and others (2001) also reported a significant decrease in the glial cell number in the mPFC after major depressive disorder.

The findings of animal studies have suggested that many of these changes can be reversed by antidepressant treatment (Sapolsky 2000, Henn and Vollmayr 2004, Joels et al. 2004). In rats, antidepressants reverse hippocampus and prefrontal cortex atrophy, compartmental changes, and spatial memory dysfunction (Rocher et al. 2004). In this study, we evaluated the protective effect of chronic administration of a natural compound (curcumin) and a synthetic compound (sertraline) on the histological parameters altered by stress. Our results demonstrated that both curcumin and sertraline administered in stress model were able to prevent the reduction in the number of the neurons and glial cells and the total volume of the mPFC with no preferences. In general, sertraline is used to treat major depression and many other neurodegenerative disorders (Matar et al. 2006, Rush et al. 2006). Selective serotonin reuptake inhibitors are the most widely prescribed antidepressant class today and exert their antidepressant-like effects by inhibiting the neuronal reuptake of serotonin and increasing the synaptic concentrations of serotonin (Matar et al. 2006, Rush et al. 2006). Studies have reported that antidepressants administration prevented both hippocampal atrophy and altered neurogenesis in dentate gyrus induced by social stress (Gould and Tanapat 1999). Czeh and coworkers (2007) also showed that concomitant fluoxetine treatment counteracted the stress-induced effect on cytogenesis.

Curcumin can be a future drug for treatment of various neurological disorders, such as major depression, tardive dyskinesia, and other related neurodegenerative disorders (Kulkarni et al. 2008). Various studies have explained different mechanisms for curcumin. Curcumin possesses antidepressant activity and modulates the levels of nor-epinephrine, dopamine, and serotonin in the brain (Kulkarni et al. 2009). There are also some evidence suggesting curcumin to have the ability to increase the levels of brain derived neurotrophic factor (Kulkarni et al. 2009). Moreover, curcumin is an inhibitor of monoamine oxidase enzyme. By inhibiting the activity of monoamine oxidases enzyme, curcumin increases the concentration of these neurotransmitters in the synapse (Kulkarni et al. 2009). Furthermore, there is evidence that curcumin may work better than other antidepressants for some subtypes of depression because its side effects are much less pronounced (Kulkarni et al. 2008, 2009).

CONCLUSION

In conclusion, our result indicated that treatment of rats with curcumin and sertraline prevented the reduction of the number of neurons and glial cells and the volume of the mPFC induced by stress.

ACKNOWLEDGMENTS

This work was performed at Histomorphometry and Stereology Research Centre, Shiraz University of Medical Sciences, Shiraz, Iran. This study is a part of the thesis written by Mohammad-Amin Abdollahifar, PhD student of Anatomy. The work was financially supported by the Research Vice-chancellor of Shiraz University of Medical Sciences (Grant No. 91-6124). The authors would like to appreciate Rouz Darou Pharmaceutical Company for their kind provision of sertraline. They are also grateful for Ms. A. Keivanshekouh at the Research Improvement Center of Shiraz University of Medical Sciences for improving the use of English in the manuscript.

REFERENCES


Sapolsky RM (2000) Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 57: 925–935.


