A-to-I editing of the 5HT2C receptor and behaviour

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Abstract
Site-specific deamination of five adenosine residues in the pre-mRNA of the serotonin 2C receptor, 5HT2CR, alters the amino acid sequence of the encoded protein. Such RNA editing can produce 32 mRNA variants, encoding 24 protein isoforms that vary in biochemical and pharmacological properties. Because serotonin functions in the regulation of mood and behaviour, modulation of serotonin signalling by RNA editing may be relevant to such psychiatric disorders as anxiety and depression. Several recent human studies have reported changes in 5HT2CR editing in schizophrenia, major depression or suicide, but results are variable and not conclusive. Rodent studies have begun to examine effects of drug treatments and stress. Understanding the importance of 5HT2CR editing in mood and behaviour will be assisted by experiments designed to analyse multiple strains of mice, in different behavioural tests, with optimal evaluation of the time course of molecular changes.

Keywords: serotonin signalling; protein isoforms; RNA editing; mouse strain; psychiatric disorder

Adenosine-to-inosine (A-to-I) pre-mRNA editing deaminates specific adenosine (A) residues in specific pre-mRNAs to produce inosine (I) [1, 2]. When this occurs within a coding region, it has the potential to alter codon specificity because the ribosome reads inosine as guanosine and, as a result, amino acid sequence and protein function may be altered. When editing occurs in non-coding regions, i.e. in untranslated regions or introns, it may affect secondary structures and influence splicing, translational efficiency or other processes involving protein binding [3, 4]. The regulatory function of A-to-I editing is assumed to be critical, based in part on the importance of the protein coding targets, which include the glutamate receptor subunits, GluRB, C, D, 5 and 6, and the serotonin receptor 2C (5HT2CR).

Among the known substrates for A-to-I editing, 5HT2CR is the best studied and exhibits the most complexity. Each of the five adenosines within the sequence 5'-ATA CGT AAT CCT ATT-3' that encodes amino acids 156–160 can be edited (the A residues are designated, 5' to 3', as editing sites A, B, E, C and D, respectively) [5]. By considering all combinations, 32 different mRNA variants are possible. In the genomically encoded IRNPI, editing can change I to V or M, N to D, S or G, and I to V, resulting in 24 possible protein isoforms. The functional consequences of editing are not completely understood and not all variants have been analysed, however, the edited segment lies within the second intracellular loop believed to be involved in G-protein coupling. Furthermore, in vitro studies with a subset of isoforms have shown that they can differ in basal activity, affinity for serotonin, potency and G-protein specificity, as well as in responses to atypical antipsychotics and some recreational drugs [6–9]. The genomically encoded form, abbreviated INI, and the fully edited form, VGV, display the extremes of many biochemical and pharmacological properties, while partially edited forms display intermediate characteristics. Thus, INI exhibits the highest constitutive activity and the most efficient...
G-protein coupling, while VGV exhibits the least [6, 7]. Analysis of partially edited forms suggests an important role particularly for editing at the E and C sites in modulating receptor properties [9]. Changes in editing levels or patterns allow modulation of serotonin signalling in response to changes in environmental conditions.

In rodent and human brain, the 24 protein isoforms are not equally represented and proportions vary among brain regions. We have recently completed a study involving the sequencing of >700 5HT2CR RT–PCR cDNA clones from brains of eight mouse strains (Du and Gardiner, manuscript in preparation). In agreement with other reports, the partially edited form, VNV, alone accounted for ~40–50% of all forms in each of the strains, and together with VSV, VNI, INV and VSI and the unedited form, INI, accounted for a total of 85–90% of all forms. The fully edited VGV and 13 other forms were extremely rare in all strains, varying from 0–3%, while four forms, MGV/I and MDV/I, were never observed. In contrast, in human brain, Niswender et al. [10] reported that the predominant isoform, at 38%, is VSV, while VNV is present at only 8 and 16% in whole brain and hippocampus, respectively, and VGV is not particularly rare, at 8 and 10%, respectively. The importance of the rare isoforms should not be discounted, because they may be of higher frequency in specific regions or cell types, or associated with specific stimuli and/or may have novel functional attributes.

Because serotonin signalling is involved in the regulation of mood and behaviours such as depression and anxiety, and because it is influenced by drugs such as fluoxetine (Prozac), a number of recent studies have looked for possible links between editing patterns of 5HT2CR and behavioural and pharmacological responses. These are summarized in Table 1.

Human studies have compared patterns of editing in patients with psychiatric disorders to those in normal controls. Niswender et al. [10] looked at editing at the A, C and D sites in patients with schizophrenia and major depression. No differences between either group and controls were found, but when suicides were compared either with all non-suicides or with non-suicides with schizophrenia or depression, significant increases in editing at the A site were observed, with a modest increase in editing also at the D site in the latter comparison. Not inconsistent with this are the results of Iwamoto and Kato [11] who examined A and D site editing in patients with bipolar affective disorder, schizophrenia or major depression. They reported a trend in increased D site editing in depression and A site editing in suicides. Sodhi et al. [12] also examined editing in schizophrenia. Decreases in B site editing and in the proportion of the INI form were reported but the depth of analysis, sequences of only 10 clones from each of five patients and five controls, was inadequate for definitive conclusions. Dracheva et al. [13] in a more detailed analysis of editing in schizophrenia found no differences from controls. In another study of editing in suicides, with a history of major depression, Gurevich et al. [14] observed significant increases in editing at the E site, a trend to increases at the C site and decreases at the D site. Although not completely consistent, the recurring observation in these studies is an increase in editing, in general shifting to less responsive receptor isoforms. In all these studies, however, there are two confounding factors. First, affected patients sometimes have a history of drug treatments associated with their illness. Based on studies in rodents, this will alter editing patterns (see next), blurring disease associations. Second, the controls show enormous variation in editing levels, e.g. from 29 to 82% and from 0 to 52% for the C and E sites, respectively in one study [13], and the range of values overlap with those of the affected individuals (Table 2). While this is reasonably attributable to genetic background and/or environmental factors, it raises the question of biological significance versus statistical significance in the differences reported between controls and patients with psychiatric disorders in some studies [10]. Certainly, altered editing is likely, at best, to be only one contributing factor in these disorders, and the biological significance of reports of human studies is worthy of further consideration and validation.

Studies in rodents should be helpful. Gurevich et al. [14] showed that, in 129Sv mice, fluoxetine caused a decrease in editing at the E site and an increase in editing at the D site. Consistent with these observations, they further showed [15] that stimulation with the 5HT2CR agonist DOI increased E and combined E and C editing, and that depletion of serotonin (by treatment with the tryptophan hydroxylase inhibitor, pCPA) decreased editing at the E and C sites. These studies suggest that editing patterns, particularly at the E and C sites,


Table 1: Changes in 5HT2CR editing patterns, human and rodent studies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species/strain (Brain region) method</th>
<th>Behaviour, (No of patients per group)</th>
<th>Drug</th>
<th>Comments</th>
<th>Editing changes site; amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niswender et al. [10]</td>
<td>Human Cortex Brodmann 8,9 Primer extension</td>
<td>13 Schizophrenia ± antipsychotics or antidepressants</td>
<td>Assayed A, C and D sites only</td>
<td>↑ A suicides vs non-suicides ~↑ D</td>
<td></td>
</tr>
<tr>
<td>Sodhi et al. [12]</td>
<td>Human Cortex Brodmann 46 10 clones</td>
<td>5 Schizophrenia</td>
<td></td>
<td>↓ B</td>
<td></td>
</tr>
<tr>
<td>Dracheva et al. [13]</td>
<td>Human Brodmann 46 &gt;30 clones</td>
<td>15 Schizophrenia ± neuroleptics</td>
<td></td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Gurevich et al. [14]</td>
<td>Human Cortex Brodmann 9 &gt;40 clones</td>
<td>± suicide 6 Suicide + mood disorder ± antidepressants</td>
<td></td>
<td>↑ E, ~C</td>
<td></td>
</tr>
<tr>
<td>Gurevich et al. [14]</td>
<td>Mouse 129Sv Cortex &gt;50 clones</td>
<td>+ fluoxetine 1 injection × 3 days, ×28 days</td>
<td></td>
<td>↓ E, ~C</td>
<td></td>
</tr>
<tr>
<td>Gurevich et al. [15]</td>
<td>Mouse 129Sv Cortex &gt;50 clones</td>
<td>+ pCPA Decreases + DOI</td>
<td>SHT levels SHT2CR agonist</td>
<td>↑ VNV ↑ EC</td>
<td></td>
</tr>
<tr>
<td>Sodhi et al. [16]</td>
<td>Sprague–Dawley rat Hippocampus Pyrosequencing</td>
<td>haloperidol 1 × 14 days, risperidone 1 × 14 days clozapine 1 × 14 days, cloropromazine 1 × 14 days</td>
<td></td>
<td>↓ D, VNV ↑ VNI</td>
<td></td>
</tr>
<tr>
<td>Iwamoto et al. [18]</td>
<td>Sprague–Dawley rat Cortex 50 clones</td>
<td>± inescapable shock (LIH) ± lithium ± fluoxetine ± imipramine</td>
<td></td>
<td>↑ E</td>
<td></td>
</tr>
<tr>
<td>Englander et al. [17]</td>
<td>Mouse BalbC/129Sv/EvTac C57BL/6j Cortex 50 clones</td>
<td>± forced swim test (FST) ± fluoxetine (FLX) 6 min</td>
<td></td>
<td>↑ E, INI, VSV</td>
<td></td>
</tr>
<tr>
<td>Gardiner and Du, in preparation</td>
<td>Mouse C57BL/6j Brain without cerebellum Morris Water Maze Hippocampus Cortex Primer extension</td>
<td>± swim ± forced swim test ± 4 blocks of 4 trials, ± 1 min swim</td>
<td></td>
<td>↑ C, E, ↓ D</td>
<td></td>
</tr>
</tbody>
</table>

*Editing patterns were evaluated in brain regions from human, rat or mouse, using either primer extension, cloning plus sequencing (the number of clones sequenced per individual is given) or pyrosequencing. For human studies: behaviour, the number of patients with each diagnosis is given; ± suicide, a subset in each group committed suicide; drug, a subset of each group had been treated with the indicated class. For rodent studies: details of learned helplessness (LH) and the forced swim test (FST) can be found in the references; comments, summary of treatment protocols, e.g.‘1 × 14 days’, drug was injected once daily for 14 days. Editing changes: statistically significant increases, ↓, and decreases, ↑, at the individual sites A–E, and of specific protein isoforms are indicated; ~, indicates that the change shows a trend towards significance. NC, no change.

are modulated in response to external conditions to maintain constant signalling strength.

Sodhi et al. [16] recently looked at hippocampus in rats after two weeks of treatments with four different drugs. Haloperidol caused significant decrease in D site editing and in the level of the associated VNV form, and an increase in VNI. Presumably this is an indirect effect because haloperidol is not known to have an affinity for 5HT2CR. Lesser, and somewhat opposite, effects were observed with risperidone, where only levels of VNV were altered. An important feature of this study was the use of pyrosequencing to ascertain editing patterns. This technique has the advantage of
being faster and cheaper than standard sequencing, and provides complete isoform information rather than the site-specific data generated by primer extension assays.

Additional studies have looked at the effects of stress on editing and the consequences of stress plus pharmacological treatment. Englander et al. [17] first compared editing levels in naive mice from three inbred strains, BALBc, 129Sv/JvTAC and C57BL/6J. While naive 129Sv and C57BL/6J had very similar editing patterns at all sites, BALBc had significantly lower levels. When C57BL/6J were exposed to the forced swim test (FST) or fluoxetine treatment or both, editing patterns were unaffected. In contrast, when BALBc were exposed to the FST, editing at the C and D sites increased, and with fluoxetine, editing at all sites except the E site increased. Paradoxically, exposure to the FST after chronic treatment with fluoxetine abolished all editing changes.

Iwamoto et al. [18] examined editing in a learned helplessness (LH) model of depression. In this paradigm, LH rats were identified as those failing to escape electric shocks when escape was possible, a behaviour caused by prior exposure to inescapable shocks. The LH rats showed increased editing at the E site when compared with controls. Treatment of LH rats with fluoxetine or imipramine, both abolished the LH behaviour (the rats now escaped the foot shocks when they had the opportunity) and returned E site editing levels to normal.

The recurring theme in these results is the dynamic editing at the E and C sites in response to drugs and environmental stimuli. This editing alters the genomic N to D, G or S, and, among those forms that have been examined, all have functional properties intermediate between the highly responsive unedited INI and the less responsive fully edited VGV.

The above studies looked at editing changes not coupled specifically to transcription changes. Cavallaro et al. [19] carried out microarray analysis on mRNA isolated from rat hippocampus after training in the Morris water maze (MWM). The 5HT2C receptor was described as the one of a number of ‘learning and memory genes’ because significant changes in transcript levels were observed during the 24h after exposure to this test of spatial learning, and these changes were different from those that occurred after exposure to swimming alone. To ask how editing is affected, we conducted similar MWM experiments on C57BL/6J mice. Our protocol involved several blocks of one minute swimming times, sacrificing mice at 1, 6 and 24h after testing, and assaying 5HT2C editing patterns by primer extension. Details of these experiments will be published elsewhere and we summarize results here. When the whole brain with cerebellum removed was assayed, there were significant increases in editing at the C and E sites and decreases in the D site, at 1 and 6h after the last exposure. Changes were similar in swimming controls and learning mice, suggesting they were largely a response to the stress of swimming, although this protocol involved less time swimming than typically used in LH paradigms (i.e. 1 min versus 6 min [17]). When experiments were repeated to examine hippocampus and cortex separately, as regions relevant to learning and stress, respectively, two observations were unexpected. First, in a set of 13 naive mice, two mice showed surprisingly low levels of editing in hippocampus. While other mice were consistently edited to 78% at the A site, these two mice showed only 25% editing. The combination of editing at the A+B sites and at the B without the A site, as well as subsets of C and E site editing combinations were also decreased. These low levels were seen only in the hippocampus of these mice; levels in cortex were normal. Because these mice are genetically identical, this argues for variation caused by some environmental or technical effect, although none is obvious, i.e. there were no differences in the time of day, housing or RNA quality among mice tested. The second unexpected observation was that the specific editing changes that were seen in whole

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>E</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niswender et al. [10] Controls</td>
<td>77–85%</td>
<td>ND</td>
<td>50–66%</td>
<td>60–75%</td>
<td>ND</td>
</tr>
<tr>
<td>Major depression</td>
<td>78–90%</td>
<td>ND</td>
<td>55–70%</td>
<td>56–77%</td>
<td>ND</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>74–88%</td>
<td>ND</td>
<td>50–64%</td>
<td>54–70%</td>
<td>ND</td>
</tr>
<tr>
<td>Dracheva et al. [13] Control</td>
<td>67–100%</td>
<td>0–75%</td>
<td>9–28%</td>
<td>35–100%</td>
<td>ND</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>49–94%</td>
<td>37–69%</td>
<td>3–23%</td>
<td>17–64%</td>
<td>47–74%</td>
</tr>
<tr>
<td>Gurevich et al. [14] Control</td>
<td>59–97%</td>
<td>17–45%</td>
<td>23–46%</td>
<td>35–78%</td>
<td>33–63%</td>
</tr>
<tr>
<td>Suicide</td>
<td>80–90%</td>
<td>13–42%</td>
<td>54–83%</td>
<td>56–89%</td>
<td>13–38%</td>
</tr>
</tbody>
</table>

Table 2: Site-specific editing levels in human studies

Dracheva et al. [13] carried out microarray analysis on mRNA isolated from rat hippocampus after training in the Morris water maze (MWM). The 5HT2C receptor was described as the one of a number of ‘learning and memory genes’ because significant changes in transcript levels were observed during the 24h after exposure to this test of spatial learning, and these changes were different from those that occurred after exposure to swimming alone. To ask how editing is affected, we conducted similar MWM experiments on C57BL/6J mice. Our protocol involved several blocks of one minute swimming times, sacrificing mice at 1, 6 and 24h after testing, and assaying 5HT2C editing patterns by primer extension. Details of these experiments will be published elsewhere and we summarize results here. When the whole brain with cerebellum removed was assayed, there were significant increases in editing at the C and E sites and decreases in the D site, at 1 and 6h after the last exposure. Changes were similar in swimming controls and learning mice, suggesting they were largely a response to the stress of swimming, although this protocol involved less time swimming than typically used in LH paradigms (i.e. 1 min versus 6 min [17]). When experiments were repeated to examine hippocampus and cortex separately, as regions relevant to learning and stress, respectively, two observations were unexpected. First, in a set of 13 naive mice, two mice showed surprisingly low levels of editing in hippocampus. While other mice were consistently edited to 78% at the A site, these two mice showed only 25% editing. The combination of editing at the A+B sites and at the B without the A site, as well as subsets of C and E site editing combinations were also decreased. These low levels were seen only in the hippocampus of these mice; levels in cortex were normal. Because these mice are genetically identical, this argues for variation caused by some environmental or technical effect, although none is obvious, i.e. there were no differences in the time of day, housing or RNA quality among mice tested. The second unexpected observation was that the specific editing changes that were seen in whole
brain were not observed in either cortex or hippocampus, suggesting that other brain regions were involved.

As with human studies, experiments with rodents have confounding factors, in these cases arising from variations in experimental design. A first consideration is the choice of assay system: primer extension, which is cheap and fast but limited to site-specific information cloning plus sequencing, which is expensive but provides complete isoform information [9] or cloning plus pyrosequencing, which combines the best of both alternatives [15]. Additional important considerations include the strain and substrain of mice used, and details of the drug regimen and behavioural protocols. For example, Bouwknecht et al. [20] have classified the 129S6/SvEvTac strain as high anxiety and C57BL/6J as low anxiety. Yet naive mice from the two strains were reported to have the same editing patterns [17]. In addition, while 129Sv (substrain information was not provided) showed altered editing patterns in response to fluoxetine in one study [14], in a subsequent study [17], 129Sv/SvEvTac was omitted from the fluoxetine experiments and FSTs. Presumably, it would have provided results intermediate between those seen in BALBc and C57BL/6J, which might have been informative. Bouwknecht and Paylor [21] have also described multiple assays for anxiety/stress, including the light–dark, open field and hyperthermic response tests. Importantly, they note that responses to different tests do not correlate within strains. Thus, editing changes may be greater or lesser among strains depending upon the behavioural assay. Lastly, the time of sacrifice relative to the last drug treatment or to the behavioural test is important to consider. After the MWM, the most significant changes in editing patterns occurred within 1–6 h, and by 24 h, most editing levels had returned to normal. In the FST [16], assays were done at 24 h and therefore, may have missed significant changes, particularly in the C57BL/6J. Both immediate and longer term molecular phenotypes may be critical to evaluating eventual behavioural responses.

Unravelling the relevance, if any, of 5HT2CR editing to human psychiatric disorders may be informed by larger scale behavioural analyses that include multiple strains of mice, multiple testing paradigms and examination of a broader time frame.

Key Points

- Proportions of the 32 mRNA variants vary among brain regions and between human and rodents.
- Interpretations of results of human studies are confounded by wide inter-individual variation in editing patterns and possible influences of drug treatments.
- Ideal mouse studies will compare the temporal effects on different inbred strains of several stress and anxiety tests and pharmacological treatments.

References


