Serotonin platelet-transporter measures in childhood attention-deficit/hyperactivity disorder (ADHD): clinical versus experimental measures of impulsivity

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Abstract:
Impulsivity in terms of aggression, suicide or poor cognitive control has been associated with low synaptic availability of serotonin (5-HT) in adults and children. However characteristics of the 5-HT transporter have not been studied in children with attention-deficit/hyperactivity disorder (ADHD: combined type) where impulsivity is a core symptom. Here, in 20 children with ADHD, we explore the relationship of the density (Bmax) and affinity (Kd) of the platelet 5-HT transporter measured with [^{3}H]paroxetine to both clinical ratings of impulsivity (Conners' Parent Questionnaire), and an experimental measure of impulsivity (the ability to withhold a prepotent response in the "stop-signal" paradigm). Decreases of affinity (increased Kd) correlated with a low probability of response inhibition, but not with the clinical ratings of impulsivity. However, ratings of distractibility and impulsivity correlated with the experimental measure of response-inhibition. In contrast, increased transporter affinity (low Kd) correlated modestly with higher ratings of aggressive and externalising behaviour. Bmax was not associated with any behavioural score. We conclude that the synaptic availability of 5-HT is under the control of the 5-HT transporter binding site affinity and that low affinity may be related to cognitive impulsivity (distractibility). Increased affinity of the transporter may also be related to conduct disturbance.

Key Words:
Childhood ADHD, distractibility, externalising behaviour, impulsivity, serotonin, transporter.

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Introduction:

Severe attention-deficit/hyperactivity disorder (ADHD) in children is usually treated with psychostimulant drugs, such as methylphenidate. Some improvement may be seen in up to 80% of patients (Swanson et al 1998). Clinical response is interpreted in terms of enhancing a hypoefficient dopamine system (Malone et al 1994), particularly in children with the hyperactive/impulsive subtype (DSM-IV). An alternative noradrenergic theory (Heilman et al 1991) may account for depressed activity in systems using nor-adrenaline as transmitter (Oades 1987) and be especially pertinent to the inattentive subtype (Lockwood et al 2001). But, interpretations here may be complicated by changes in other colocalized modulators like neuropeptide Y (Oades et al 1999). Past reviews considered

catecholaminergic alterations as primary, to the exclusion of serotonergic systems (Oades 1987, Zametkin and Rapaport 1987). This is surprising in view of the widely acknowledged attribution of changed serotonin (5-HT) transmission to some forms of impulsivity (Soubrie 1986), and the high heritability for ADHD-combined subtype for which impulsivity is a core feature (Rutter et al 1999). Further, in an animal model of ADHD (dopamine transporter knockout), hyperactive responsivity is modulated by the synaptic availability of serotonin (Gainetdinov et al 2000).

In a non-ADHD context (e.g. borderline personality disorder, suicide) impulsivity in terms of abrupt bursts of behaviour and aggression has been associated with reduced 5-HT activity (e.g. lower CSF levels of 5-HIAA: Kavoussi et al 1997). In aggressive children with ADHD there are also indications from provoked prolactin release for reduced 5-HT activity (Halperin et al 1997). Further, as in attempted suicide in adults (Engstrom et al 1999), in ADHD patients reduced HVA/5-HIAA ratios may be related to poor cognitive and inhibitory control (Oades 2002). Thus, one might predict that features leading to the reduced synaptic availability of 5-HT would relate to impulsive reactivity: but it will be noted that that low HVA/5-HIAA ratios would result from increased 5-HT metabolism. As the 5-HT transporter prominently controls the availability of 5-HT in the synapse, an exploration of the binding characteristics of platelet 5-HT transporters, a model for the central transporter receptor (Cheetham et al 1993) and their relationship to impulsivity in children with ADHD was conducted.

Children were recruited from those presenting to a newly advertised screening programme, from which the subgroup tested were those recommended to receive medication, for which a blood sample was necessary. The screening programme allowed clinical ratings of impulsivity as well as an experimental measure of the (in)ability to withhold a prepotent response at short notice (stop-paradigm: Slusarek et al. 2001) to be compared with 5-HT transporter measures.

Methods:

Twenty children with a pervasive hyperkinetic disturbance (ICD-10: WHO), equivalent to the combined type (DSM-IV 314.01) were recruited. Biochemical data were obtained from 13 boys and 3 girls (mean age 10.4y, sd 2.4, range 7.2-14.5y) with a performance IQ of 87.4 (sd 14.0). Two had a simple hyperkinetic disorder (F90.0) and 14 hyperkinetic disorder of social behaviour (F90.1 that includes elements of DSM-IV 313.81); of these four had a secondary emotional disturbance (two with F93.8, one 94.1 and one 43.25). After receiving approval for the study from the faculty Ethics Committee, the children provided oral and the parents written informed consent to participation. The parents completed the Child Behaviour Checklist (CBCL: Achenbach and Edelbrock, 1983) and the Conners' Parent rating scale (Goyette et al. 1978). The sum score for Conners' ratings was 20.8 (sd 1.27). Exclusion criteria included the diagnosis of learning disorders, a score of <17 on the Conners' scale and a T score <60 on the CBCL attention scale. The present analysis was restricted to the CBCL scales for internalising (mean 68.9 sd 1.8), externalising (mean 71.8 sd 1.6) and aggressive behaviour (mean 75.9 sd 2.1), and to 4 of the ten items on the Conners' scale relevant to impulsivity (i.e. C1 activity [mean 2.3 sd 0.21], C2 impulsivity [mean 2.3 sd 0.18], C5 fidgeting [mean 2.1 sd 0.20], C6 distractibility [mean 2.4 sd 0.16]).

The stop-signal task was a forced-choice reaction time (RT) task. In the primary task children were asked to respond quickly and accurately with a left or right computer key when they saw an "X" appear to the left or right of a fixation point on the computer screen. After 20 trials to allow familiarisation with the task and 10 further trials for calculation of the individual mean RT, the secondary task was introduced. Here a 'Stop-Signal' was presented with an auditory 1 kHz, 500 ms tone on 25% of 192 trials after the target. The Stop-Signal was presented 50, 200, 350 or 500 ms before the expected response. The inter-stimulus interval was 7 sec. The stop-signal reaction time (SSRT) is the time between presentation of the stop-signal and the inhibition of response, estimated from the RT

distribution in the primary task (Logan 1994). Logan's probability of inhibition (p-inhibit) was also calculated for each of the different stop-signal delays. Data for the 50 and 500 ms intervals did not distinguish ADHD from healthy children due to floor and ceiling effects, respectively. Thus analysis here is restricted to the 200 and 350 ms intervals.

Platelets were prepared from platelet-rich plasma (25 ml EDTA blood) and stored as pellets at –80°C prior to analysis of 5-HT transporter binding features after the method of Maguire et al (1993). After thawing and homogenisation of the pellets in hypotonic lysis buffer, cells were washed in Tris-HCl (70 mmol/l) and solubilized in an assay buffer (Tris HCl 50 mmol/l; NaCl 120 mmol/l; KCl 5 mmol/l; pH 7.5). Binding was performed using 6 different concentrations of ^{3}H-paroxetine (0.2-5 nmol/l). Unspecific binding was assessed by 1 µmol/l fluoxetine in parallel experiments. The assay was carried out in a final volume of 250 µl incubating for 2 hours at 20°C. The reaction was stopped through rapid filtration (GF/F filter Whatman) and radioactivity measured in a Beckman scintillation counter.

Spearman correlation coefficients were calculated for comparisons of clinical and experimental behavioural indices with biochemical measures. Linear regression analyses with up to a maximum of 3 variables to predict impulsivity and the biochemical measures were used to seek support for the exploratory correlations.

Results:

The mean platelet count for the children was 266 x10^{3}/µl (sd 58.7, range 189-386). The mean values for Bmax and Kd were respectively 3682 fmol/mg protein (sd 2684.2) and 0.424 nmol/l (sd 0.356). The experimental indices on the stop-signal task (p-inhibit) were 0.33 and 0.54 (SSRT 468 and 488 ms) for the 200 and 350 ms intervals, respectively. Comparative values from healthy children for p-inhibit were 0.46 and 0.80 (SSRT 225 and 235 ms) at 200 and 350 ms, respectively. The clinical, experimental and biochemical measures were not associated with the age or the IQ of the children.

There were no significant correlations for the Bmax of the 5-HT transporter with either the clinical ratings or the experimental measures (p-inhibit/SSRT). However, figure 1 shows that on the stop-signal task decreasing p-inhibit correlated with increasing Kd values (i.e. decreasing affinity). Similar associations were found for the SSRT (figure 1). Kd was not associated with any of the Conners' clinical ratings. However, the experimental p-inhibit measures at 350 ms on the stop-signal task showed a modest relationship to the scales of impulsivity ($r = -0.51$, $p = 0.05$) and distractibility ($r = -0.46$, $p = 0.08$).

Linear regressions confirmed that the experimental measures (p-inhibit at 200 and 350 ms) tended to predict Kd for the 5-HT transporter (p-inhibit-350, partial correlation –0.50, $p = 0.06$, R^2 48.9) but Conners' and CBCL ratings made no significant contribution. Indeed the biochemical measures (Kd, Bmax, $F[2, 12] = 4.2$, $p = 0.04$) predicted p-inhibit-350 on the stop -signal task (Kd, partial correlation –0.63, $p = 0.014$, R^2 3.7), and Conners' ratings (fidgeting, impulsivity, distractibility; $F[3,11] = 4.0$ $p = 0.038$) contributed similarly to an explanation of the p-inhibit-350 measure, (distractibility: partial correlation –0.57, $p = 0.038$, R^2 37.4).
Further, we noted a modest correlation for increasing externalising and aggressive behaviour with lower Kd values for the 5-HT transporter: increasing affinity correlated similarly with both ratings: $r = -0.4$, $p = 0.04$).

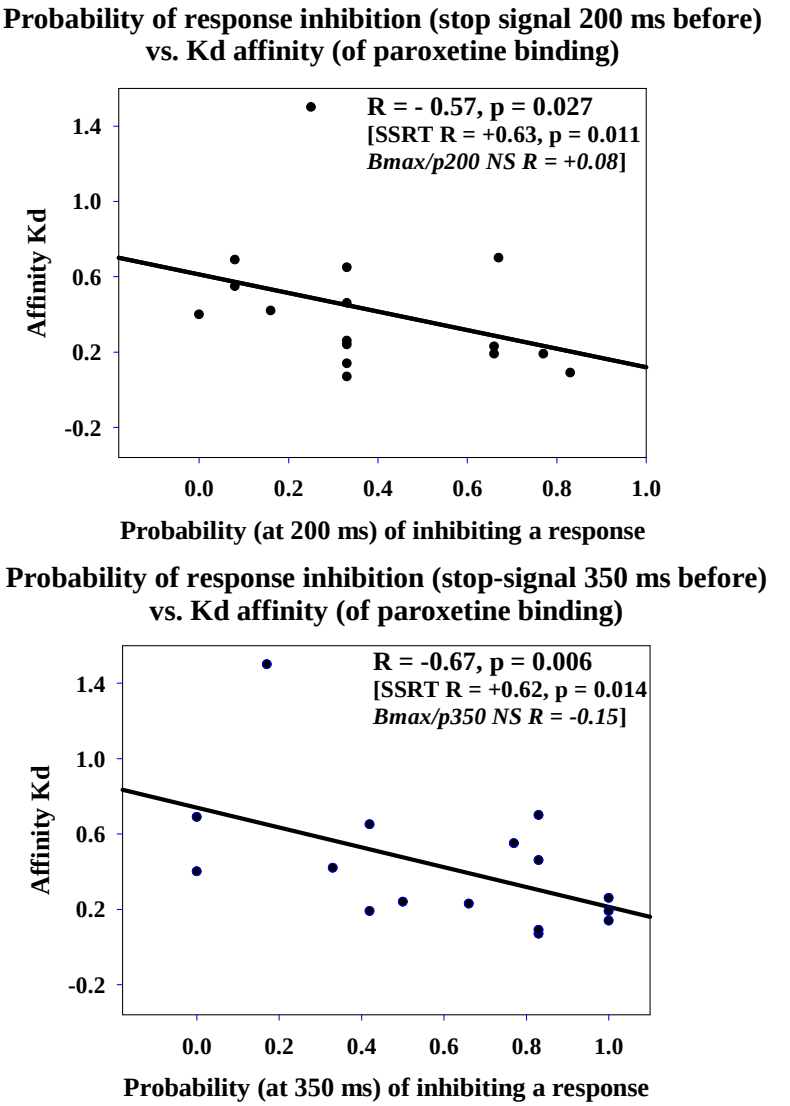

Figure 1:

The affinity (Kd) of the 5-HT transporter binding site (nmol/l) is plotted against the probability of inhibiting a response in the "stop-signal" task , where stop signal intervals of 200 ms and 350 ms before the expected mean response time for 16 ADHD children are shown in the top and bottom diagram, respectively. (The single high Kd value is attributable to a 10y old girl with a mild comorbid disturbance of bonding.)

Discussion

This preliminary study of the potential contribution of the 5-HT transporter binding site to measures of impulsivity in children with ADHD throws up three points of interest. First, the relevant features of the transporter in the platelet model are not the number or density of binding sites (Bmax) but its affinity (Kd). Increasing difficulty with inhibiting response in the stop-signal task was correlated with *decreasing* receptor affinity. Second, while clinical ratings of impulsivity were not related to the transporter (Kd), they and, to a greater extent, ratings of distractibility were associated with the experimental likelihood of withholding a response. Third there was a modest correlation between *increasing* affinity of the transporter receptor and increasing disruptive behaviour. This latter seems to support reports that indices of lower 5-HT activity coincide with the expression of violent behaviour (see introduction).

This preliminary investigation did not attempt to compare diagnosis-dependent differences for 5-HT transporter measures. However, the

Bmax values were comparable with reports from impulsive and non-impulsive adult suicide attempters (Verkes et al 1998), normal 11-12 year-old male and female children (Sigurdh et al 1999) and adolescents with conduct disorder (Unis et al 1997). The Kd values were also comparable, except that the median value cited by Sigurdh et al was somewhat lower, probably reflecting minor methodological differences (pers. comm. Oct. 2001). We are unable to say if the higher values in the present study and that of Unis et al reflect the related disorders of ADHD (here) and conduct disorder (Unis et al)

How should differences of Kd be interpreted? Reductions of binding site affinity should normally be off-set by increased receptor capacity. It may be assumed that where this does not occur that more 5-HT remains available in the synapse. If this increase is associated with impulsivity then it stands in contrast to studies in adult subjects (see introduction).

For an alternative explanation the question then shifts to whether the association with a decreased likelihood of response inhibition reflects a relationship to impulsivity? It seems intuitive to describe a reduced ability to inhibit a response tendency as one that reflects an impulsive disposition, one that is not planned, poorly controlled, inaccurate or maladaptive (Solanto et al 2001). But, this description could apply to rapid responses to an irrelevant distracter that would normally be inhibited. Thus, it would also be possible to describe unusually fast reactions to the stop-signal as reflecting an impulsive disposition of the subjects. It is instructive that the clinical ratings that predicted p-inhibit closest were those of 'distractibility', albeit that the relationship was negative. Thus, the least 'distractible' children were most likely to inhibit a response. Those who were less likely to inhibit responses (the more distractible) showed a lower 5-HT transporter affinity with putatively greater synaptic availability of 5-HT. This is consistent with a report of an association of poorer signal detection indices on sustained attention tasks with 5-HT metabolism (Oades 2000). Clearly a new and larger sample of children should be examined to test this proposal with a contrast of 5-HT measures in those rated high or low on distractibility and impulsivity ratings, backed up with continuous performance test measures of commission errors (impulsive cognition).

Thus an apparent conflict with the literature on 5-HT and impulsivity may be resolved in terms of the information processing involved in the experimental measures. The apparent increased affinity (lower synaptic 5-HT availability) in those with more externalising and aggressive behaviour is concordant with the literature. Children with a stable diagnosis of ADHD, especially those with the combined subtype are likely to show marked conduct disturbance (August et al 1998; Faraone et al 1998). But both hyperactive and non-hyperactive children with conduct disturbance are likely to show impulsive behaviour (Taylor 1998). Thus, our evidence of differential associations of types of impulsive cognition and impulsive aggression with features controlling the availability of 5-HT warn against premature use of adjunctive serotonergic medication without detailed consideration of the individual's symptoms. While it may seem likely that antagonists (of the uptake or the postsynaptic site) could be beneficial in some circumstances (disruptive behaviour), inappropriate use could exacerbate the patient's tendencies to poor stimulus-response control with potential long-term consequences for risk-taking behaviour and substance misuse.

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