

1 **Validity and reliability of extrastriatal [¹¹C]raclopride**
2 **binding quantification in the living human brain**

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33

34 Abstract

35

36 [¹¹C]raclopride is a well established PET tracer for the quantification of dopamine 2/3
37 receptors (D_{2/3}R) in the striatum. Outside of the striatum the receptor density is up to two
38 orders of magnitude lower. In contrast to striatal binding, the characteristics of
39 extrastriatal [¹¹C]raclopride binding quantification has not been thoroughly described.
40 Still, binding data for e.g., neocortex is frequently reported in the scientific literature.
41 Here we evaluate the validity and reliability of extrastriatal [¹¹C]raclopride binding
42 quantification. Two sets of healthy control subjects were examined with HRRT and
43 [¹¹C]raclopride: i) To assess the validity of extrastriatal [¹¹C]raclopride binding estimates,
44 eleven subjects were examined at baseline and after dosing with quetiapine, a D_{2/3}R
45 antagonist. ii) To assess test-retest repeatability, nine subjects were examined twice. Non
46 displaceable binding potential (*BP_{ND}*) was quantified using the simplified reference tissue
47 model. Quetiapine dosing was associated with decrease in [¹¹C]raclopride *BP_{ND}* in
48 temporal cortex (18±17% occupancy) and thalamus (20±17%), but not in frontal cortex.
49 Extrastriatal occupancy was lower than in putamen (51±4%). The mean absolute
50 variation was 4-7% in the striatal regions, 17% in thalamus, and 13-59% in cortical
51 regions. Our data indicate that [¹¹C]raclopride PET is not a suitable tool for D_{2/3}R binding
52 quantification in extrastriatal regions.

53 Keywords: dopamine, extrastriatal, positron emission tomography,
54 raclopride, reference region

55

56

57 **Introduction**

58

59 The dopamine (DA) system is of key interest both in normal brain function and in the
60 pathophysiology of neurological¹, and psychiatric^{2,3} disorders. Striatum is the brain
61 region with the highest concentration of dopamine receptors⁴ and also the most studied
62 using positron emission tomography (PET). In recent years, quantification of dopamine
63 receptors in extrastriatal regions has received more interest^{2,5}. Specifically, striatal and
64 extrastriatal availability of the dopamine D2 receptor family has been of particular
65 interest in psychiatry research as drugs targeting D_{2/3} receptors (D_{2/3}R) is an established
66 treatment of psychosis and mood disorders⁶.

67

68 The dopamine D2/3R radioligand [¹¹C]raclopride was developed in the 80's⁷ and is one
69 of the most frequently used PET radioligands to date. Due to its relatively low affinity to
70 D_{2/3}R (Kd = 1.3 nM) [¹¹C]raclopride has primarily been used to study receptor
71 availability in striatal regions. Extrastriatally, the concentration of D_{2/3}R is up to two
72 orders of magnitude lower than in striatum⁸. To study regions with low levels of D_{2/3}R,
73 high affinity radioligands have been developed, e.g. [¹¹C]FLB-457 (Kd = 0.02 nM) and
74 [¹⁸F]fallypride (Kd=0.03 nM)^{9,10}. These tracers are, however, not ideally suited to
75 quantify D_{2/3}R in striatum. If [¹¹C]raclopride binding to extrastriatal D_{2/3}R could be
76 shown to be validly and reliably quantifiable, fewer PET-examinations would be required
77 for studies where D_{2/3}R in the whole brain is of interest. Although there is some
78 indication of reliable quantification of the extrastriatal [¹¹C]raclopride signal^{11,12} (i.e.,
79 adequate test-retest properties), there is a lack of data supporting quantifiable specific

80 binding in these regions. In spite of this, several PET-laboratories, including our own,
81 have applied [¹¹C]raclopride to measure extrastriatal D_{2/3}R availability in thalamus^{13–15},
82 and in cortical regions^{16–18}.

83

84 In a statistical context, reliability is the repeatability or consistency of a measurement. In
85 PET research, the reliability of a binding measurement is typically assessed in a test-
86 retest design, where PET-experiments are performed twice in a group of individuals, and
87 the between- and within-individual variability of the measurements are evaluated¹⁹.

88 Validity is the degree to which a measurement corresponds to what it is supposed to
89 measure. A common approach to assess validity, i.e., determine whether, and the extent
90 by which, the radioligand binds to the target of interest, is to perform a pharmacological
91 challenge where PET measurements are conducted before and after administration of a
92 competitor from a different chemical class.

93 Several such studies have been published for [¹¹C]raclopride and striatum^{20–}
94 ²². Extrastriatally, however, the data is sparse. Using haloperidol as a competitor
95 Mawlawi (2001) showed that while achieving an occupancy of ~90% in striatum, only
96 half of the purported specific binding in thalamus was displaced²¹. To our knowledge no
97 competition experiments assessing [¹¹C]raclopride binding in cortex have been published.

98

99 The aim of the present study was to explore both the validity and reliability of
100 [¹¹C]raclopride binding in extrastriatal regions. We performed a competition study in
101 healthy controls attempting to replicate the results from Mawlawi (2001) for thalamus,
102 but also to assess [¹¹C]raclopride binding in cortex. This part of the study will from here

103 on be referred to as COMP. In the second part, from here on referred to as TRT, we
104 evaluated the reliability of [¹¹C]raclopride binding in extrastriatal regions using a test-
105 retest design in a separate sample of healthy controls.

106

107 **Material and Methods**

108 Study design

109 Two independent datasets were used for the competition and the test-retest design. In
110 COMP eleven healthy male subjects (21 - 29 (25±2.5) years) participated in a previously
111 published occupancy study of quetiapine²³, clinical trial registration number:
112 NCT00832221 (<http://www.clinicaltrials.gov/>). Quetiapine is a multimodal drug with
113 D_{2/3}R antagonist properties (K_i = 245 nM)²⁴. Extended release (XR) or immediate release
114 (IR) quetiapine was given once-daily during 12 days. After 4 days of dose titration of
115 quetiapine XR from 50 mg to 300 mg, each subject received 300 mg quetiapine XR for 4
116 days. Treatment was then directly switched to 300 mg quetiapine IR for 4 days. The
117 subjects participated in five PET measurements with [¹¹C]raclopride: at baseline and at
118 time for expected peak (T_{max}) and trough (T_{min}) plasma concentration for both drug
119 formulations. The PET-experiments at T_{max} were performed on the fourth day of
120 administration of XR and IR respectively and the T_{min} examination the morning after the
121 last dose of each formulation. See the original publication for details²³.

122

123 TRT consist of data from nine (six females) healthy subjects (37 - 71 (53±12) years) not
124 previously published. The subjects participated in two PET measurements with
125 [¹¹C]raclopride. Time between measurements was 14 to 27 days (20±5, mean±SD).

126

127 All subjects in both studies were healthy according to a clinical interview of medical
128 history; physical examination; psychiatric interview; blood and urine chemistry; and
129 magnetic resonance imaging (MRI) of the brain. The procedures in both studies were
130 approved by the Research Ethics Committee in Stockholm, Sweden, and the Radiation
131 Safety Committee at Karolinska University Hospital, Stockholm, and were performed in
132 accordance with the 2004 revision of the Declaration of Helsinki. All subjects gave their
133 written informed consent before participation.

134

135 MRI

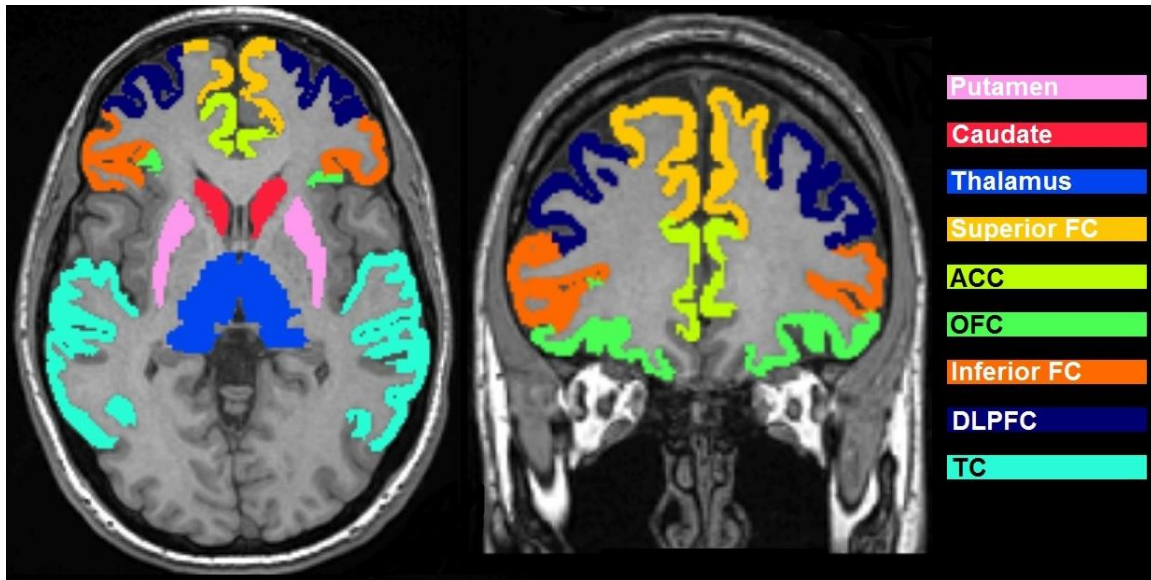
136 T1-weighted MRI images were acquired using a 1.5 T (COMP) or a 3 T (TRT) GE Signa
137 system (GE Medical Systems, USA).

138

139 Regions of interest

140 FreeSurfer (version 6.0, <http://surfer.nmr.mgh.harvard.edu/>)²⁵ was used to define ten
141 regions of interest (ROIs) on the T1-weighted MRIs of all subjects (Figure 1). ROIs were
142 chosen based on their relevance for both neurological and psychiatric disorders, as well
143 as for comparison with previous test-retest studies on extrastriatal [¹¹C]raclopride
144 binding^{11,12}.

145



146

147 **FIGURE 1.** MRI for one subject from the COMP data with regions of interest overlaid.
148 Nucleus accumbens not visible. ACC, Anterior cingulate cortex; DLPFC, dorsolateral
149 prefrontal cortex; FC, frontal cortex; OFC, orbitofrontal cortex; TC, temporal cortex.

150

151 Radiochemistry

152 [¹¹C]raclopride was prepared as described previously²⁶. The injected radioactivity in
153 COMP ranged between 227-235 MBq (232±2) for the baseline examination; 207-236
154 (225±10) for Tmax XR (p=0.11); and 223-236 (231±5) for Tmax IR (p=0.67). The
155 specific radioactivity was 336±264 GBq/μmol for the baseline examination;
156 342±280 GBq/μmol for Tmax XR (p=0.96); and 198±89 for Tmax IR (p=0.17). Injected
157 mass was 0.32±0.17 μg for the baseline examination; 0.36±0.29 μg for Tmax XR
158 (p=0.67); and 0.49±0.26 for Tmax IR (p=0.12). In TRT the injected radioactivity ranged
159 between 296-524 MBq (397±98) for PET1 and 156-561 (411±135) for PET2 (p=0.80).
160 The specific radioactivity was 148±49 GBq/μmol for PET1 and 206±75 GBq/μmol for
161 PET2 (p=0.07) corresponding to an injected mass of 1.08±0.64 μg for PET1 and
162 0.82±0.52 μg for PET2 (p=0.37).

163

164 PET experimental procedure

165 In each PET-experiment a saline solution containing [¹¹C]raclopride was injected into a
166 antecubital vein as a bolus (<10s). The cannula was then immediately flushed with 10 mL
167 saline.

168 All subjects were examined using a high-resolution research tomograph (HRRT;
169 Siemens Molecular Imaging, USA) with a maximum spatial resolution of ~2mm full-
170 width-half-maximum²⁷. Transmission scans were performed prior to each PET
171 measurement in order to correct for signal attenuation.

172 Brain radioactivity was measured continuously, in COMP for 63 minutes and in
173 TRT for 51 minutes. The radioactivity was reconstructed in consecutive time frames, in
174 COMP, four 15 s, four 30 s, six 1 min, six 3 min and six 6 min frames. In TRT the initial
175 frame sequence was identical to COMP whereas the number of 6 min frames at end of
176 data acquisition was reduced to four.

177

178 Quantitative analysis

179 PET images were corrected for head motion using a frame-to-first-minute realignment
180 procedure²⁸. Using SPM5 (Wellcome Department of Cognitive Neurology, University
181 College, London, UK), the T1-weighted MR-images were co-registered to a summed
182 PET-image. To obtain regional time-activity curves, the ROIs were projected onto the
183 realigned dynamic PET-image.

184

185 From the time-activity curves, BP_{ND} was estimated using the simplified reference
186 tissue model (SRTM)²⁹. Cerebellum, a region where specific binding has been considered
187 negligible⁸, was used as reference. The cerebellar cortex volume was first defined using
188 FreeSurfer, then trimmed in an automated process to include only voxels above lowest
189 plane of pons; behind and below the posterior tip of the 4th ventricle. Only voxels located
190 laterally of the left- and rightmost point of the 4th ventricle was included. The outer layer
191 of the resulting mask was then eroded by one voxel (Supplementary Figure S1).

192

193 Calculations and statistics

194 Statistical analyses and data visualization were performed using R (version 3.3.3).

195 Occupancy (%) of quetiapine was calculated according to the equation:

196

$$197 \text{ Occupancy} = \frac{(BP_{ND}^{\text{baseline}} - BP_{ND}^{\text{drug}})}{BP_{ND}^{\text{baseline}}} \times 100 \quad (1)$$

198

199 The validity of extrastriatal [¹¹C]raclopride BP_{ND} was tested comparing the baseline
200 examination with examinations after pretreatment with quetiapine XR and IR
201 respectively. Specific binding was defined as present when a significant ($p < 0.05$)
202 decrease was showed using paired one sided t test.

203

204 Test-retest reproducibility for the TRT data was assessed using the following metrics:

205

206 *Absolute variability (VAR):*

207

208
$$VAR = \frac{|BP_{ND}^{PET1} - BP_{ND}^{PET2}|}{\frac{1}{2}(BP_{ND}^{PET1} + BP_{ND}^{PET2})} \times 100 \quad (2)$$

209

210 VAR is a measure of the absolute reliability of a measurement expressed as a percentage
211 of the average BP_{ND} value. PET1 refers to the first PET measurement, and PET2 refers to
212 the second PET measurement. The reported value is the average VAR for all subjects.

213

214 *Intraclass correlation coefficient (ICC):*

215

216
$$ICC = \frac{MS_B - MS_W}{MS_B + MS_W} \quad (3)$$

217

218 where MS_B denote the between subjects mean sum of squared variance and MS_W the
219 within subject mean sum of squared variance. ICC normalizes the measurement error to
220 the between-subject variance and will give information on how well a test can distinguish
221 between individuals. The score can vary between -1 and 1, values closer to 1 indicate that
222 most of the variance is due to between-subject rather than within-subject variation³⁰.

223

224 *Standard error of measurement (SEM):*

225

226
$$SEM = SD\sqrt{(1 - ICC)} \quad (4)$$

227

228 SEM is expressed in the same unit as the outcome (in this study BP_{ND}). It is an estimate
229 of the standard deviation of the measurement error and can be viewed as the uncertainty

230 surrounding the outcome in a single examination³⁰. Notably, though similarly named, the
231 standard error of the *mean* and standard error of *measurement* are diverse statistical
232 concepts.

233

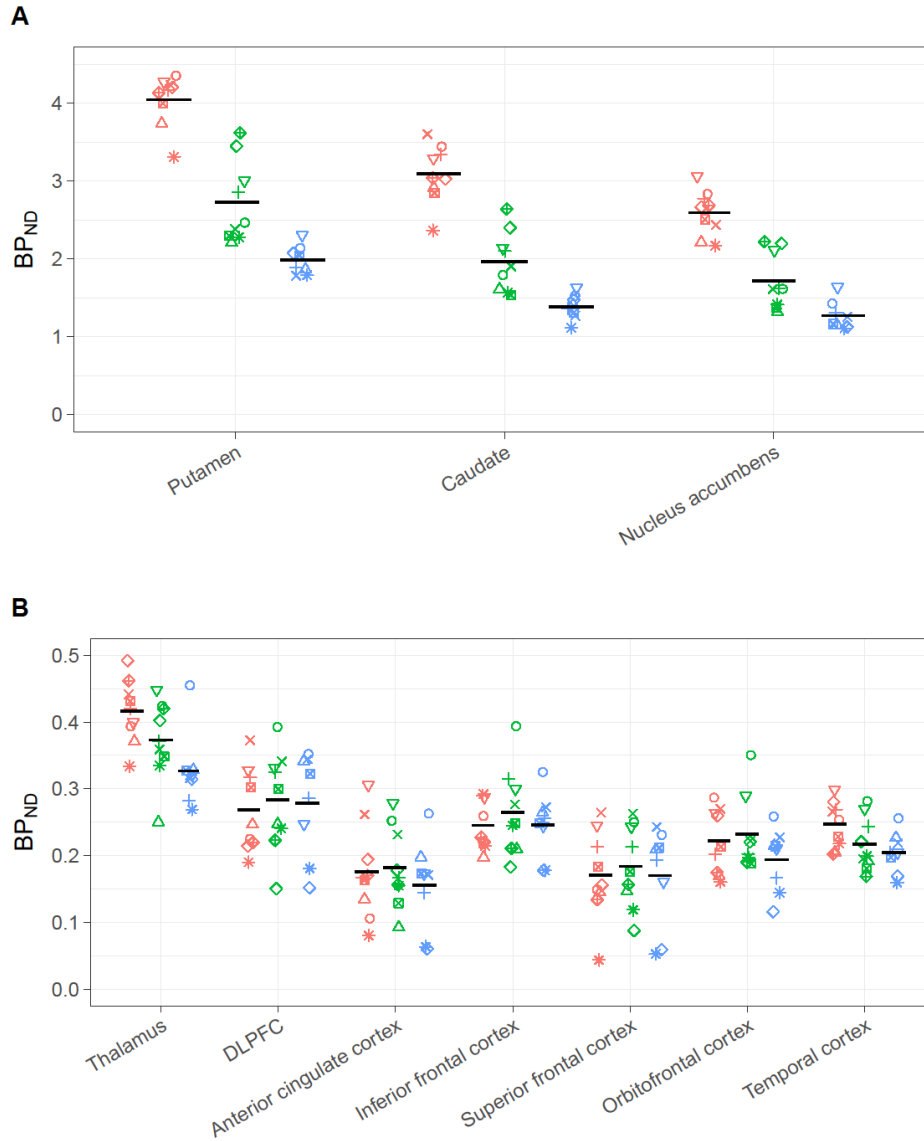
234 **Results**

235

236 In the COMP dataset, two subjects were excluded before image analysis due to excessive
237 head movement during the baseline measurement. Excessive head movement was defined
238 as more than 3 mm displacement from the reference position in more than 10% of the
239 frames as seen in the realignment plot. In addition, PET acquisition data from the
240 quetiapine IR measurement for one subject was excluded due to a delay of the
241 examination of two hours beyond expected T_{\max} for the plasma concentration of the drug.
242 Nine subjects were included in the final analysis of baseline and quetiapine XR data. For
243 the quetiapine IR data eight subjects were analyzed. In the TRT dataset SRTM failed in
244 the anterior cingulate cortex in one individual producing a negative BP_{ND} value. This
245 value was excluded from further analysis.

246

247 Results from COMP are shown in Figure 2. In extrastriatal regions a significant decrease
248 of BP_{ND} was seen only in thalamus and temporal cortex (TC) after treatment with XR as
249 well as IR formulations of quetiapine (Table 1). In putamen the occupancy was $33\pm 11\%$
250 and $51\pm 4\%$ (mean \pm SD) in the quetiapine XR and IR measurements respectively.
251 Occupancy was lower in extrastriatal regions: $10\pm 14\%$ and $20\pm 17\%$ in thalamus and
252 $12\pm 11\%$ and $18\pm 17\%$ in TC (Table 1).



253

254 **FIGURE 2.** COMP [¹¹C]raclopride binding data. A) Striatal ROIs (for reference). Each
255 ROI represents three PET examinations, from left to right: Baseline (red); at T_{max} post
256 quetiapine XR (green); at T_{max} post quetiapine IR (blue). Horizontal bars represent mean
257 BP_{ND}. B) Extrastriatal ROIs, same order of PET examinations as in A. DLPFC,
258 dorsolateral prefrontal cortex.

259

260

261

262

TABLE 1. Quetiapine occupancy data

Region	Baseline (n = 9)		Quetiapine XR (n = 9)		Quetiapine IR (n = 8)		
	Mean ± SD (BP _{ND})	Mean ± SD (BP _{ND})	test vs baseline (p)	occ (%)	Mean ± SD (BP _{ND})	test vs baseline (p)	occ (%)
Putamen	4.05±0.33	2.73±0.53	<0.001	32.58	1.99±0.19	<0.001	50.62
Caudate	3.1±0.37	1.97±0.39	<0.001	36.14	1.39±0.16	<0.001	54.96
Nucleus Accumbens	2.6±0.29	1.72±0.36	<0.001	33.86	1.27±0.18	<0.001	50.75
Thalamus	0.42±0.05	0.37±0.06	0.029	9.93	0.33±0.06	0.007	19.53
DLPFC	0.27±0.06	0.28±0.07	0.747	-7.49	0.28±0.08	0.556	-3.19
Anterior cingulate	0.18±0.07	0.18±0.06	0.616	-15.82	0.16±0.07	0.287	-2.48
Inferior frontal cortex	0.25±0.04	0.26±0.07	0.865	-7.7	0.25±0.05	0.465	-0.44
Superior frontal cortex	0.17±0.07	0.18±0.06	0.782	-23.26	0.17±0.07	0.418	-2.67
OFC	0.22±0.05	0.23±0.06	0.602	-2.37	0.19±0.05	0.059	12.63
Temporal cortex	0.25±0.03	0.22±0.04	0.007	12.07	0.2±0.03	0.01	17.75

P-values calculated using one sided paired t tests. BP_{ND}, binding potential using simplified reference tissue model; DLPFC, dorsolateral prefrontal cortex; occ, occupancy; OFC, orbitofrontal cortex; SD, standard deviation

263

264 TRT was completed in nine control subjects. ICC values were higher and VAR values

265 were lower in striatal ROIs, compared to extrastriatal regions (Table 2).

TABLE 2. [¹¹C]raclopride test-retest data; BP_{ND} values and statistics

Region	Scan 1 (BP _{ND})		Scan 2 (BP _{ND})		Difference				
	n	Mean ± SD	n	Mean ± SD	Range	SD	VAR (%)	ICC	SEM
Putamen	9	3.59±0.35	9	3.66±0.33	-0.17 to 0.36	0.16	3.71	0.88	0.12
Caudate	9	2.61±0.3	9	2.62±0.19	-0.28 to 0.28	0.19	6.15	0.74	0.12
Nucleus Accumbens	9	2.21±0.27	9	2.22±0.22	-0.35 to 0.43	0.24	7.42	0.57	0.15
Thalamus	9	0.42±0.08	9	0.45±0.06	-0.1 to 0.13	0.08	16.33	0.27	0.06
DLPFC	9	0.16±0.07	9	0.17±0.05	-0.03 to 0.08	0.03	25.96	0.81	0.03
Anterior cingulate	8	0.17±0.09	8	0.21±0.05	-0.03 to 0.15	0.06	35.43	0.57	0.05
Inferior frontal cortex	9	0.22±0.06	9	0.22±0.05	-0.05 to 0.06	0.04	12.91	0.82	0.02
Superior frontal cortex	9	0.13±0.08	9	0.15±0.04	-0.04 to 0.09	0.04	58.88	0.81	0.03
OFC	9	0.14±0.07	9	0.13±0.04	-0.13 to 0.04	0.05	21.63	0.64	0.03
Temporal cortex	9	0.21±0.05	9	0.21±0.03	-0.04 to 0.05	0.03	13.83	0.70	0.02

BP_{ND}, binding potential using simplified reference tissue model; DLPFC, dorsolateral prefrontal cortex; ICC, interclass correlation coefficient; OFC, orbitofrontal cortex; SD, standard deviation; SEM, standard error of measurement; VAR, absolute variability

266

267 **Discussion**

268

269 We have examined the validity and reliability of the extrastriatal binding characteristics
270 of [¹¹C]raclopride. No specific raclopride binding could be detected in most examined
271 cortical areas, as determined using a pharmacological competition analysis. In the
272 thalamus and TC ROIs we observed some indication of specific binding, although
273 occupancy was lower than in striatum. Further, the test-retest repeatability of extrastriatal
274 BP_{ND} was low in our data.

275

276 Our results have implications when interpreting and planning clinical studies. Similar to
277 Mawlawi (2001) the COMP data indicate that only half of the calculated BP_{ND} in
278 thalamus reflect specific binding. Estimations of effect sizes need to be adjusted
279 accordingly. Assuming that 10% difference in the density of D_{2/3}R in thalamus is
280 considered a relevant clinical finding in a cross-sectional study, the corresponding
281 apparent effect size would be ~0.8, requiring 25 subjects per group for 80% power. Our
282 results however indicate that the actual effect size would be ~0.3, translating into 175
283 subjects per group. Importantly, a non-significant finding in an extrastriatal region in a
284 [¹¹C]raclopride study powered for striatal regions will give very little information on
285 whether an effect is present or not.

286

287 We investigated the test-retest repeatability of [¹¹C]raclopride binding in a sample with a
288 clinically relevant age- and gender diversity. We observed a VAR of 3.7-7.4% and ICC
289 between 0.57-0.88 in striatal regions. The results are similar to a previous test-retest study

290 of [¹¹C]raclopride in a bolus-constant infusion protocol and a high resolution PET
291 system¹¹, and numerically superior to previously published lower resolution PET
292 data^{31,32}. The reliability of our data was lower in extrastriatal regions compared to
293 striatum (Table 2). We were not able to replicate the VAR values of 3.7-13.1% or ICC of
294 0.64-0.92 in the extrastriatal regions reported by Alakurtti et al (2015). Further, it should
295 be noted that before validity is proven it is difficult to interpret ICC and VAR, or rather:
296 poor values still indicate a problem even if the validity is good, but before accepting a
297 high ICC or low VAR as indicative of reliable *specific* binding, validity need to be
298 established.

299

300 Our data indicate that the greater part of [¹¹C]raclopride BP_{ND} measured in neocortex
301 does not reflect specific binding. However, since we consistently measure higher
302 [¹¹C]raclopride signal in, e.g., frontal cortex, compared to cerebellum the question arises
303 to what this difference should be ascribed if not to specific binding? The explanation
304 suggested by Mawlawi (2001) is a systematically lower non-displaceable compartment
305 (V_{ND}) in cerebellum compared to cerebral target regions²¹, a V_{ND} -bias. This interpretation
306 is in line with our observations of lower occupancy in regions with lower densities of
307 $D_{2/3}R$ (see Figure S2 for an explanation on how V_{ND} -bias propagates to occupancy
308 values). The presence of a discrete difference in V_{ND} between target and reference will
309 not matter much in receptor rich regions (i.e. striatum) but will become a serious validity
310 issue in low-binding regions. If, for example, V_{ND} is 10% lower in the reference region
311 then the “true” BP_{ND} in the target region will be falsely increased with 0.1 and 10%³³. In,
312 e.g., frontal cortex where we might have a “true” [¹¹C]raclopride BP_{ND} of 0.05 or less,

313 even a small V_{ND} -bias would thus be highly problematic. However, since the protocol did
314 not include arterial blood sampling, a more detailed analysis of V_{ND} in different ROIs was
315 not possible.

316

317 There are other possible explanations for the observed differences in quetiapine
318 occupancy between high and low density $D_{2/3}R$ regions: (i) quetiapine could have
319 different occupancy in different brain regions. In the time span between baseline- and
320 post drug examinations quetiapine could (ii) cause the extrastriatal expression of $D_{2/3}R$ to
321 increase, or (iii) cause the concentration of endogenous dopamine to decrease. However,
322 several previous occupancy studies of quetiapine at steady-state using high affinity
323 radioligands have shown similar or higher occupancy of $D_{2/3}R$ in cortex compared to
324 striatum^{34,35} and no study has, to our knowledge, shown lower occupancy. This makes i-
325 iii unlikely explanations to our findings.

326

327 There are some limitations to this study. The standardized uptake value (SUV) in
328 cerebellum was lower in the examinations performed after pretreatment with quetiapine
329 compared to baseline (supplement, Figure S3 and Table S1). This may be explained by (i)
330 presence of specific [¹¹C]raclopride binding to $D_{2/3}R$ in cerebellum; (ii) that quetiapine
331 displaces non-specific binding of raclopride, or (iii) that quetiapine decreases
332 [¹¹C]raclopride brain uptake. (i) will result in an underestimation of occupancy equally in
333 low- and high binding regions and would thus not alter the conclusions of our results^{33,36}.
334 The same is true for (ii) given that the displacement of non-specific binding is equal in all
335 regions. Additionally, we observed that centrum semiovale (Figure S1), a region

336 containing only white matter, showed similar decrease of SUV (Figure S3) which lends
337 support to explanation (ii) and (iii). The explanation we find most probable, (iii), would
338 also likely not affect our results since the decrease of measured radioactivity would be
339 proportional in target and reference regions.

340 Regarding the test-retest dataset, a caveat that should be highlighted is the fact
341 that time between examinations was 20 ± 5 days. Most commonly, PET test-retest
342 examinations are performed within 1-2 days. This protocol was chosen to mimic that of
343 typical clinical studies where patients are examined repeatedly under an extended period
344 of time, an established test-retest design for evaluation of clinical applicability^{32,37}.

345

346 **Conclusions**

347

348 In most brain regions outside striatum, we could not find proof of valid [¹¹C]raclopride
349 binding quantification, as little or no decrease in BP_{ND} was seen after administration of a
350 competitor. Further, we found extrastriatal test-retest repeatability to be poor. While
351 confirming the validity and reliability of [¹¹C]raclopride binding quantification in
352 striatum, our findings indicate that [¹¹C]raclopride PET not is a suitable tool for D_{2/3}R
353 binding quantification in extrastriatal regions. Before validity is proven strong caution is
354 warranted when interpreting studies applying [¹¹C]raclopride for measuring of D_{2/3}R
355 availability in extrastriatal regions.

356

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