Seasonal variation in brain mu-opioid receptor availability

Lihua Sun[1*], Jing Tang[2], Heidi Liljenbäck[1,3], Aake Honkaniemi[1,4], Jenni Virta[1,3], Janne Isojärvi[1], Tomi Karjalainen[1], Tatu Kantonen[1,5], Pirjo Nuutila[1,6], Jarmo Hietala[1,7], Valtteri Kaasinen[1,5], Kari Kalliokoski[1], Jussi Hirvonen[8], Harry Scheinin[1], Semi Helin[1], Kim Eerola[9,10], Eriika Savontaus[9,10], Emrah Yatkin[11], Juha O. Rinne[1,5], Anne Roivainen[1,3], Lauri Nummenmaa[1,12]

1. Turku PET Centre, University of Turku, Turku, Finland
2. Research Program in Systems Oncology, Faculty of Medicine, University of Helsinki, Helsinki, Finland
3. Turku Center for Disease Modelling, University of Turku, Turku, Finland
4. Turku University Hospital, Turku, Finland
5. Clinical Neuroscience, Turku University Hospital, Finland
6. Department of Endocrinology, Turku University Hospital, Turku, Finland
7. Department of Psychiatry, Turku University Hospital, Turku, Finland.
8. Department of Radiology, University of Turku, Turku, Finland
9. Research Unit for Integrative Physiology and Pharmacology, Institute of Biomedicine, University of Turku, Turku, Finland
10. Unit of Clinical Pharmacology, Turku University Hospital, Turku, Finland
11. Central Animal Laboratory, University of Turku, Turku, Finland
12. Department of Psychology, University of Turku, Turku, Finland

*Correspondence to: lihua.sun@utu.fi

Turku PET Centre, University of Turku, Turku, Finland

Abstract

Seasonal rhythms influence mood and sociability. The brain μ-opioid receptor (MOR) system modulates a multitude of seasonally varying socioemotional functions, but its seasonal variation remains elusive with no previously reported *in vivo* evidence. Here, we studied the seasonal effects on brain MOR availability via analysing a dataset (n=204) of [^{11}C]carfentanil positron emission tomography (PET) scans of healthy volunteers. We found that seasonally varying daylength had an inverted U-shaped functional relationship with brain MOR availability. Brain regions sensitive to daylength spanned the socio-emotional brain circuits, where MOR availability formed a spring-like peak. Causal effect of daylength on brain MOR availability was further verified by a *post hoc* experiment with repeated PET imaging of rats (n=9) under seasonal photoperiodic simulation. Therefore, the *in vivo* brain MOR availability in normal humans shows significant seasonal variation, which aligns with expected seasonal variation in mood and suicidality.

Introduction

Seasonal rhythms profoundly impact mood. Negative affect including depression, anger, and hostility is at lowest during the summer [1], whereas seasonal affective disorder rates peak during the winter months [2]. These changes are mediated by slow phasic changes in different neuroreceptor systems. For instance, long daylength increases brain serotonin and norepinephrine levels in mice and reduces depression and anxiety behaviour, compared to short daylength [3]. Duration of daylight exposure is similarly associated with brain serotonin turnover in humans [4]. The intimate link between seasonal fluctuations in mood and the contribution of opioidergic neurotransmission in human emotions [5] suggests potential seasonal variation of *in vivo* μ-opioid receptor (MOR) signalling.

Several lines of evidence suggest that MOR availability could vary seasonally in humans. Opioids are among the most commonly used illicit drugs in the US, where 2 % of the population have had opioid use disorder during their lifetime [6]. Suicidal behaviour with prescription opioids follows a clear seasonal pattern, with suicide attempts peaking in the spring and fall [7]. Further, post-mortem studies have established that suicide victims have increased MOR densities [8,9], and serious self-injurious behaviour is associated with increased plasma opioid growth factor levels [10]. Although these studies have not directly assessed seasonal effects, it is well established that suicide rate peaks often in the spring regardless of the geographical location of the country [11]. Finally, MORs are potent modulators of feeding [12–14] due to their contribution of hedonic or "liking" responses in the brain [15], and human feeding patterns show seasonal variation with caloric intake of fats typically peaking in the fall or winter [16,17]. However, direct *in vivo* evidence on the causal seasonal effects on opioidergic neurotransmission is currently lacking.

Animal studies also suggest that there is seasonal variation in MOR-dependent signalling. The

61 endogenous opioid signalling is crucial for the photoperiodic control of the seasonal reproductive
62 cycle in mammals [18]. Endogenous opioids inhibit the release of gonadotropin and sex hormones,
63 and this inhibitory effect is enhanced under short daylength compared to long daylength [19]. Also,
64 MOR expression in hamster testes is increased during short days, with increased inhibitory control
65 for testosterone secretion [20]. In Siberian hamsters, brain expression of dynorphin A, an endogenous
66 peptide agonist for opioid receptors, is increased under relatively longer daylength compared to
67 shorter daylength [21], suggesting a potential impact of daylength on opioidergic signalling in the
68 brain. Furthermore, effects of morphine on feeding behaviour of ground squirrels vary in accord
69 to the hibernation state [22], indirectly reflecting a seasonal variation of MOR signalling.
70 Here, we tested whether seasonal variation in daylength, a noise-free cue for seasonal rhythms,
71 influences MOR availability in the brain. MOR availability was quantified using *in vivo* positron
72 emission tomography (PET) with MOR-sensitive agonist ligand [^{11}C]carfentanil. We first
73 conducted a cross-sectional study with previously acquired human PET imaging data to test
74 whether there was seasonal difference in MOR availability. We then investigated experimentally
75 whether seasonal variation in daylength causally influences brain MOR availability in rats. While
76 rats underwent daylength cycle simulating seasonal changes, repeated [^{11}C]carfentanil PET
77 imaging was conducted (Supplementary Fig. 1). Our data suggest that there is seasonal variation
78 in brain MOR availability in both humans and rats, with MOR availability peaking in seasons with
79 intermediate daylengths.

80 **Results**

81 ***Daylength associated brain MOR availability in Humans***

82 Figure 1A shows the mean MOR distribution in the human brain. We examined the association
83 between natural variation in daylength (Fig. 1B) and MOR availability in humans. Brain full

volume analysis revealed a statistically significant quadratic polynomial effects in a large brain cluster (39000 voxels; Fig. 1C-D), spanning the cingulate cortex, the superior frontal, medial frontal, middle temporal and superior temporal gyrus, insula and orbitofrontal cortex. Brain MOR availability in the brain cluster peaked at 15-19 hours (Fig. 1D).

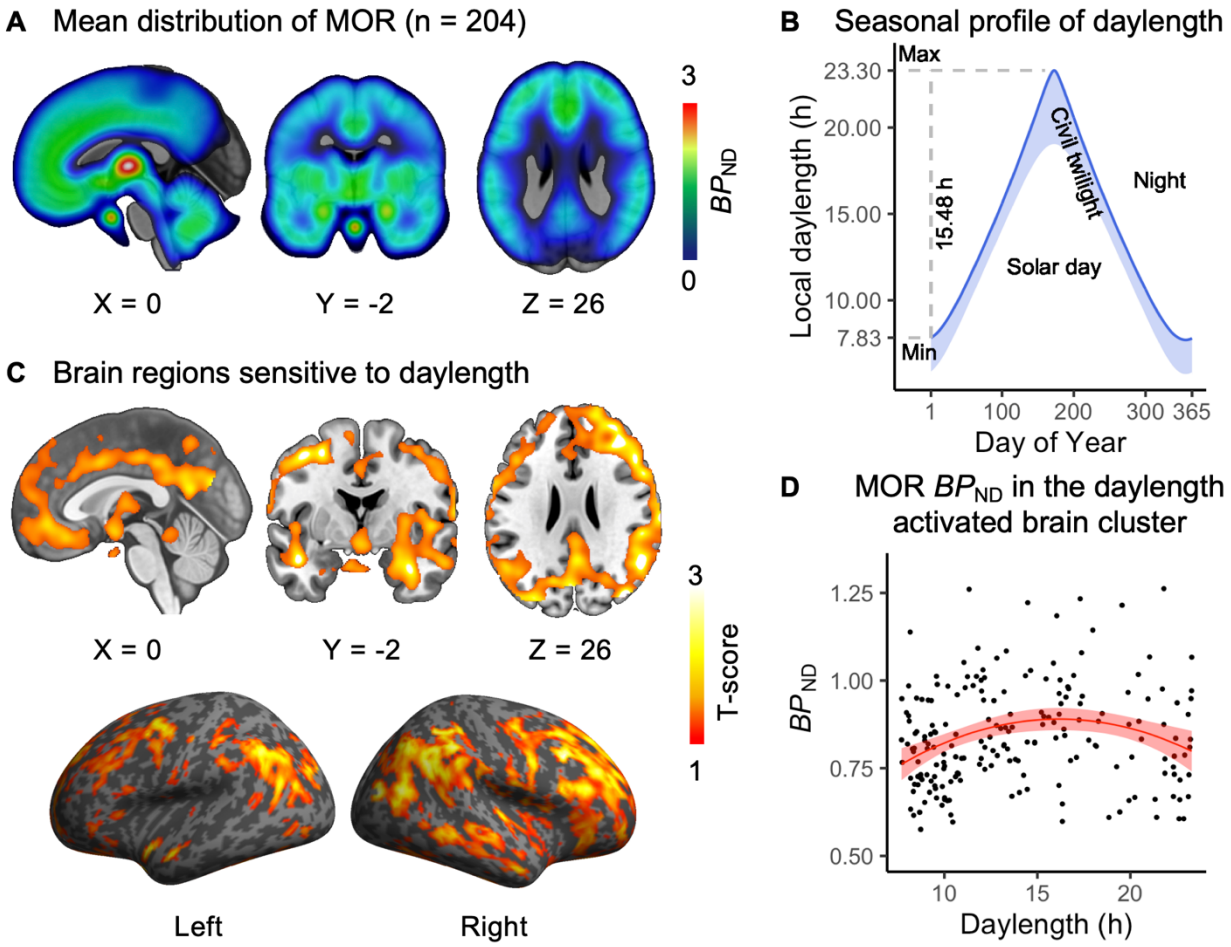

Figure 1. Human brain MOR availability and natural variation in daylength. **A.** Mean distribution of MOR availability in the subjects. **B.** Local seasonal distribution of daylength (day + civil twilight). **C.** The brain cluster sensitive to the seasonal daylength changes in quadratic polynomial regression model. $p < 0.05$, FDR-corrected. **D.** [^{11}C]carfentanil BP_{ND} in the brain cluster as a function of daylength. Red line shows polynomial LS regression curve (y ~ x + x^2) predicting BP_{ND}, and shaded area shows 95% CI.

95 In addition, we did brain region of interest (ROI) analysis by involving thirty structurally
96 determined brain ROIs (see Method). While involving all ROIs in the statistical analysis, both
97 daylength (β = 0.044, 95% CI [0.008, 0.08]) and squared daylength (β = -0.0014, 95% CI [-0.0026,
98 -0.00021]) were significant predictors for [^{11}C]carfentanil BP_{ND}, indicating an inverted-U shape
99 functional relationship. No interaction effects between daylength and squared daylength with brain
100 hemisphere (left versus right) or ROIs were found. Effect of daylength on individual ROIs is shown
101 in Supplementary Table 1.

102 ***Meteorological seasons and brain MOR availability in Humans***

103 Traditionally defined seasons, according to the northern meteorological definition, including
104 spring (March, April, May), summer (June, July, August), fall (September, October, November),
105 and winter (December, January, February) were used as predictors for MOR BP_{ND} in the brain
106 cluster (Fig. 1C-D). Each season is dummy-coded in a linear regression model, and we found that
107 spring predicted higher brain MOR availability (β = 0.08, 95% CI [0.02, 1.14]), Fig. 2A. No other
108 seasons had significant effects on MOR availability. In the local region, spring is with daylength
109 of 16.39 ± 2.78 h (Fig. 2B). Therefore, MOR availability peaking at daylength 15-19 hours (Fig.
110 1D) suggests a spring-like peak.

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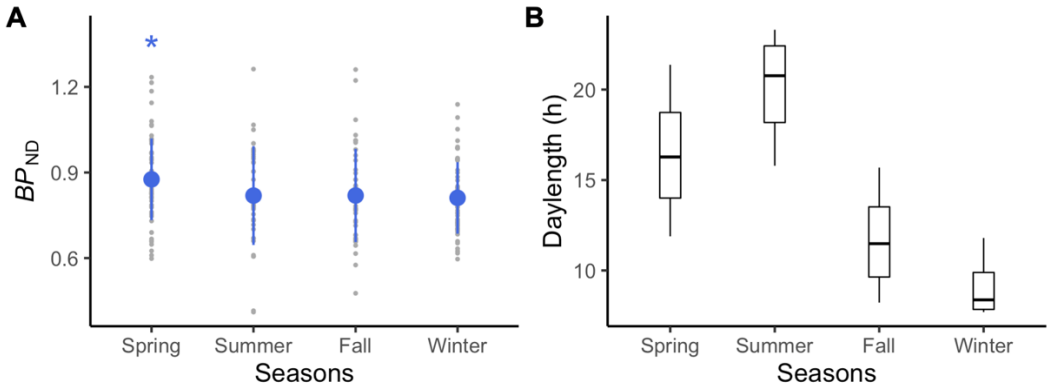
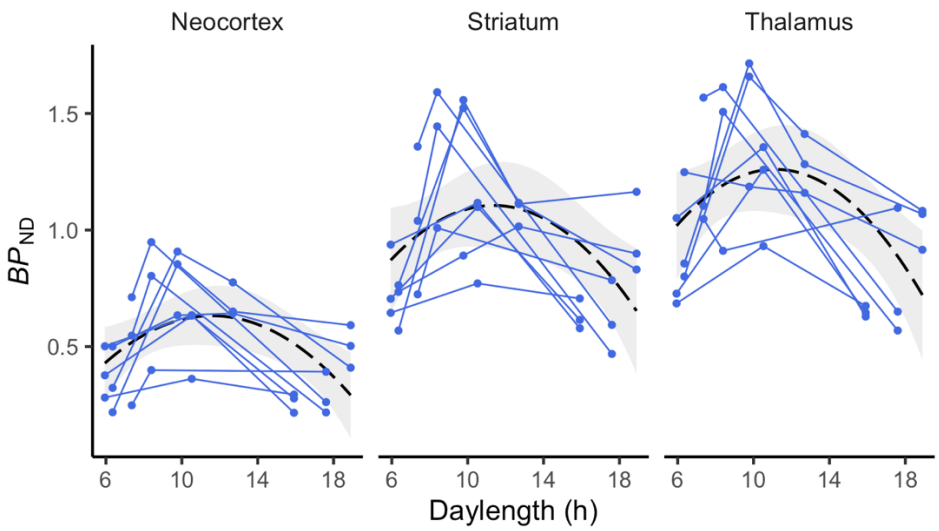


Figure 2. Spring is associated with highest brain MOR BP_{ND} in humans. **A.** MOR availability in the daylength activated brain cluster is plotted to seasons. Blue dots stand for means and error bars for standard deviations. **B.** Local daylength distribution in different seasons. * = $p < 0.05$.

Causal effect of daylength on brain MOR availability in Rats

In addition to analysis of the human database, we set up a rat model PET imaging experiment to verify potential causal effect of daylength on brain MOR availability. This is also a crucial complement considering the quasi-experimental design nature of human data analysis which could not differentiate the effect of daylength from other highly correlated seasonal factors such as temperature. The rat model study consisted of experimental groups (n = 9) where daylength simulated the local daylength changes (no twilight) and control groups (n = 3) where daylength were kept consistent (12h light ON). When both experimental and control groups were included in the statistical model, both daylength ($\beta = 0.16$, 95% CI [0.035, 0.28]) and squared daylength ($\beta = -0.0067$, 95% CI [-0.012, -0.0017]) were significant predictors; the MOR availability and daylength had an inverted U-shaped relationship. Age, sex or group did not influence MOR availability, and no interaction effects were found. Analysis excluding the control group gave similar results (see Supplementary data).

130 We also modelled regional BP_{ND} separately for the experimental group rats (within-animal design),
131 using fixed-effect factors daylength and squared daylength and varying intercepts for each rat.
132 Daylength and squared daylength were significant predictors for [^{11}C]carfentanil BP_{ND} in all
133 regions (Fig 3; Supplementary Table 2). Including age and sex in the models did not improve the
134 models, as evidenced by higher Akaike Information Criterion (AIC) values (Supplementary results
135 & Supplementary Table 3). In addition, rats in the experimental versus control group showed
136 slower weight gain (Supplementary Fig. 3) and had a higher level of blood corticosterone (β =
137 44.93, 95% CI [12.37, 77.48]).



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139 **Figure 3.** Within-animal changes in brain MOR BP_{ND} as a function of daylength. Individual
140 animals are shown as separate lines. Black dashed line shows the LS-regression curve for the
141 polynomial model (y ~ x + x^2) predicting BP_{ND} across the whole sample. Shaded area shows 95%
142 CI for the LS curve.

143 **Discussion**

144 The current study reveals that seasonally varying daylength influences in vivo brain MOR
145 availability in healthy humans. Increasing daylength modulated brain MOR availability following

146 an inverted-U shape, with highest MOR bindings observed in intermediate daylengths. In rats,
147 daylength causally influences MOR availability paralleling the quasi-experimental study in
148 humans using a large database of historical PET imaging data. Most profound seasonal variation
149 of MOR availability was found in large brain clusters including the amygdala, striatum, thalamus,
150 cingulate cortex, orbitofrontal cortex, superior frontal and temporal cortex. Previous studies have
151 established the role of seasonal variation in serotonin signalling on brain affective functions and
152 pointed towards the role of daylight duration on its seasonal rhythm [3,4]. The present data show that
153 also MOR system undergoes significant seasonal variation, which might be linked with seasonal
154 variation in mood and sociability.
155 The human data revealed salient seasonal variation of MOR availability in brain regions linked
156 with emotional and social processing, such as amygdala, cingulate cortex and posterior superior
157 temporal cortices [5]. To our knowledge, this is the first *in vivo* evidence indicative of seasonal
158 variation in MOR system in humans, and it fits with other types of evidence implying seasonal
159 effects on MOR. In high-latitude regions daylight changes influence sleep quality, mood, and
160 social behaviour [23–26]. Prevalence of seasonal affective disorders or subsyndromes (time-locked to
161 short daylength) in the local region is remarkably high [27]. The spring-like peak of MOR availability
162 (Fig. 2A) parallels with seasonal variation in opioid-modulated emotional changes such as mood
163 [1], suicidality [11], particularly with prescription opioids [7] that peak particularly in the spring.
164 Considering the impact of photoperiod on seasonal affective disorders [28] as well as the intimate
165 linkage between MOR signalling and social and affective functions [5,29], the current study suggests
166 that seasonal variation in MOR availability could be a potential brain mechanism under seasonal
167 affective changes.

168 The rat experiment finding demonstrates that daylength causally influences brain MOR availability,
169 verifying the human data findings, and goes in line with previously reported impact of photoperiod
170 on MOR dependent signalling in mammals [19–22]. Exact mechanism on the daylength-mediated
171 alterations in brain MORs is however unclear. In mammals, neural responses to daylength are
172 largely mediated by the superchiasmatic nucleus (SCN). The SCN generates endogenous neural
173 signal, and adjusts the duration of this signal in accord to changes of the solar day length as is
174 detected via the retinohypothalamic tract [30,31]. While MORs are most widely expressed in the
175 subcortical and limbic brain regions, it is possible that daylength affects MOR signalling via the
176 retinohypothalamic tract, calling for in-depth studies.
177 In rats, we also found that seasonally alternating daylength slowed weight gain and increased blood
178 corticosterone levels in adult rats (in comparison with rats kept in a fixed 12-hour light cycle), in
179 line with previous findings [32,33]. Daylength-dependent changes in corticosterone levels may be due
180 to elevated stress caused by extremely long daylength, as supported by a positive effect of
181 daylength on blood corticosterone levels in rats (Supplementary results). In humans, exposure to
182 extremely long daylength in summer also increases human blood cortisol levels [34]. Blood
183 corticosterone levels have been linked with social stress and activity of MOR signalling in
184 mammals [35], where increased MOR avidity is along with lower cortisol level under stress. This is
185 similarly supported by our finding that brain MOR availability in rats is negatively associated with
186 blood corticosterone levels (Supplementary Fig. 4).
187 While the current study cannot disentangle the relation between corticosterone levels and MOR
188 signalling in humans, the findings suggest that social and physiological stress could be linked with
189 MOR availability. Regardless of the euphoric effect of longer daylength [1,28], cerebral MOR
190 availability did not increase linearly with daylength but demonstrated an inverted-U shape,

191 potentially due to the increased physiological or psychological stress under extremely long
192 daylength. This link between MOR signalling and stress is corroborated by the fact that opioid
193 agonists and partial agonist buprenorphine reduce symptoms of anxiety and depression in rats [36]
194 as well as alleviate the effects of psychological stressors such as separation distress [37]. In line with
195 this, human molecular and functional imaging studies have found that high opioidergic tone
196 suppresses hemodynamic brain responses to viewing distressing videos [38,39].
197 While PET being the optimal non-invasive approach to quantify *in vivo* brain MOR availability,
198 drawbacks exist in the current study. The human study was based on historical data, where each
199 subject was imaged only once, and quasi-experimental design (natural changes in daylight), as
200 longitudinal multi-scan studies would yield a significant radiation load. Contribution of other
201 seasonal factors (such as temperature) cannot however be ruled out in the human data, whereas we
202 verified the causal effect of daylength on brain MOR availability using animal experiments.
203 Furthermore, daylength was estimated using astronomical data without considering day-to-day
204 (e.g. cloud cover) and individual variation (e.g. time spent outside) in light exposure. Nevertheless,
205 we had a large sample and the results paralleled those from the experiment done in rats, albeit with
206 smaller effect size. The human data were also sampled from different projects and scanners, which
207 were corrected for potential scanner-related biases in the analyses. Technically, while
208 [^{11}C]carfentanil BP_{ND} in baseline condition is proportional to MOR density, the exact contributions
209 of MOR density, receptor affinity, and baseline occupancy by endogenous opioids cannot be
210 assessed in a single measurement [40,41], and these components cannot be differentiated in a single
211 scan. [^{11}C]carfentanil is an agonist tracer preferably binding MORs in the high-affinity state [41] and
212 has low test-retest variability in PET imaging [42]. Although endogenous opioids compete for
213 binding sites with [^{11}C]carfentanil [43,44], the basal opioid concentrations are often very low at least

214 in rats [45]. Accordingly, [^{11}C]carfentanil BP_{ND} in baseline likely reflects density of MORs. Finally,
215 current findings based on human data should be interpreted while keeping in mind the large
216 magnitude of local photoperiodic variation, which should be cautiously interpolated to regions
217 with lower latitudes.

218 Taken together, we conclude that seasonal variation in daylength influences brain MOR
219 availability, following an inverted U-shaped curve and peaking at 15-19 hours in humans. Given
220 the intimate links between MOR signalling and socioemotional behaviour [29,46], these results
221 suggest that the MOR system might underlie seasonal variation in human mood and social
222 behaviour [1,2], and imply that MOR might be a feasible target for treating seasonal affective
223 disorders.

224 **Methods**

225 ***Study with human PET image database***

226 **Subjects**

227 Human study was performed using retrospective analysis of historical subjects scanned with PET
228 using radioligand [^{11}C]carfentanil at different times of the year (Supplementary Fig. 1). The data
229 were retrieved from the AIVO database (http://aivo.utu.fi) of *in vivo* molecular images hosted by
230 Turku PET Centre. We identified all the baseline scans from individuals with no neurologic or
231 psychiatric disorders who had been scanned between 2003 and 2018. The resulting data frame of
232 204 individuals (132 males, 72 females; age 32.4 ± 10.8 y) consists of scans from 11 research
233 projects and five PET scanners, with details of the subjects described in [47]. No subjects abused
234 alcohol or illicit drugs or used medications affecting the central nervous system.

235 **PET image analysis**

236 PET data were analysed and modelled using Magia toolbox (https://github.com/tkkarjal/magia)
237 [48], an automated processing pipeline developed at the Turku PET Centre running on MATLAB
238 (The MathWorks, Inc., Natick, Massachusetts, United States). Pre-processing consisted of
239 framewise realignment and co-registration of the PET and magnetic resonance images. Tracer
240 binding was quantified using BP_{ND} [49], which is the ratio of specific binding to non-displaceable
241 binding in tissue. BP_{ND} was estimated using simplified reference tissue model with occipital cortex
242 as the reference region [50,51]. Parametric BP_{ND} images were also calculated for full-volume analysis.
243 They were spatially normalized to MNI-space via segmentation of T1-weighted MRIs and
244 smoothed with an 8-mm Gaussian kernel. Regions of interest, including the reference region, were
245 parcellated for each subject using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/).

246 **Full-volume human data analysis**

247 For each subject, daylength was calculated as the daytime plus civil twilight on the day when the
248 PET image was acquired. Civil twilight involves morning civil twilight that begins when the
249 geometric centre of the sun is 6° below the horizon and ends at sunrise and evening civil twilight
250 that begins at sunset and ends when the geometric centre of the sun reaches 6° below the horizon.
251 Calculation was done using R package "suncalc", where calculations were based on geographic
252 location of the Turku PET Centre (Turku, Finland; latitude = 60.4518, longitude = -22.2666) and
253 formulas provided by Astronomy Answers articles about position of the sun and the planets
254 (https://www.aa.quae.nl/en/reken/zonpositie.html).

255 We first modelled the effect of daylength on MOR availability using multiple regression, as
256 implemented in SPM12 (http://www.fil.ion.ucl.ac. uk/spm/). Polynomial expansion of daylength
257 to the second order and linear component of daylength were used as regressors. Subject age, sex,
258 scanner and body mass index (BMI) were used as nuisance covariates. MOR BP_{ND} values in the

259 daylength-sensitive brain cluster were also estimated using the MarsBaR toolbox [52], and plotted as
260 a function of daylength and seasons for visualization.

261 **Region of interest analysis**

262 We also analysed the regional MOR availability in 15 brain ROIs (based on subject-wise
263 FreeSurfer delineations) for both hemispheres (in total 30 ROIs), as listed in Supplementary Table
264 1. Pooled ROI values were analysed using linear mixed-effects model with varying intercepts for
265 each subject. Fixed effect factors included daylength, squared daylength, age, sex, BMI, scanner
266 type, and their interaction effects with ROI and brain hemisphere (right vs. right). In the single
267 ROI data analysis, linear regression model was used with factors including daylength, squared
268 daylength, age, sex, BMI, and scanner type. Regional MOR BP_{ND} was log-transformed in the
269 statistical analysis as previously [47]. R statistical software (version 3.6.0) using lme4 package were
270 used in analysing the human ROI data.

271 ***Rat model PET imaging study***

272 **Animal handling and seasonal simulation**

273 The National Animal Experiment Board of Finland approved all the procedures and protocols
274 (license number ESAVI/8355/2019) in accordance with the EU Directive 2010/63/EU on the
275 protection of animals used for scientific purposes. Eighteen adult (age > 90 days) Sprague-Dawley
276 rats (twelve of them were PET imaged as described in the next section; 11 males, 7 females;
277 Central Animal Laboratory, University of Turku, Turku, Finland) were housed under controlled
278 laboratory conditions in open top cages with free access to CRM-E diet (SDS, UK) and water.
279 Rats were caged in groups of two or three same-sex rats. No rats were removed before finishing
280 the last PET imaging to keep the social environment stable and to avoid additional stress [53]. For
281 the rats in the experimental group (9 males, 5 females), in-house light with variable ON/OFF

282 duration was programmed, simulating the local seasonal change of daylength with a speeded cycle
283 completed in three months (Supplementary Fig. 1). Rats in the control group (2 males, 2 females)
284 were kept in a different room with constant daylength cycle (12h ON/ 12h OFF, no twilight), with
285 all other conditions the same with the experimental group. The same type of LED lighting was
286 used for control groups and the experimental groups.

287 **PET imaging and processing**

288 Twelve out of eighteen rats (experimental group: 6 males, 3 females; control group: 2 males, 1
289 female) were studied with dynamic [^{11}C]carfentanil PET imaging for 3-4 times under isoflurane
290 anaesthesia. Radiotracers were divided by three rats at each scanning day (2 males, 1 female), and
291 via using two different scanners to maximize the data collection. Due to larger body size, Inveon
292 Multimodality PET/CT (Siemens Medical Solutions, Knoxville, TN, USA) was used for imaging
293 the male rats (two rats each time), whereas Molecubes PET/CT (Molecubes, Gent, Belgium) was
294 used for the female rats. In total, there were 42 PET/CT scans. Rats were weighed on the scanning
295 day. For the Inveon Multimodality PET/CT scanner, with relatively lower resolution and
296 sensitivity, the aimed dosage of radiotracer was 5 MBq, while for Molecubes PET/CT scanner rats
297 were scanned with aimed dosage of 1 MBq. Accordingly, sex effects cannot be separated from
298 scanner type and dosage dependent effects.

299 Dynamic PET images were analysed using Carimas software (version 2.10.3.0) developed at the
300 Turku PET Centre, Finland. The PET data sets were reconstructed into 20 time frames using
301 OSEM3D algorithm: 6 × 0.5 minutes, 3 × 1 minutes, 4 × 3 minutes, and 7 × 6 minutes. We first
302 created a template of structural CT image and brain atlas. In the template, a rat's skull CT image
303 was aligned with the Waxholm Space Atlas of the Sprague Dawley rat brain [54] to be used as the
304 reference for the normalization of the brain scans. We first visually checked that the PET and CT

305 images were in alignment. Next, PET and CT images were locked regarding their positions using
306 Carimas. Individual skull images were then manually realigned and resized to the template CT
307 image. Once the skull image was scaled with the CT image, the CT and the PET images were
308 rigidly co-registered with the CT template (Supplementary Fig. 2).
309 Simplified reference tissue model was used for estimating of non-displaceable MOR binding
310 potential (BP_{ND}) in the neocortex, striatum and thalamus [49]. Cerebellum was used as the reference
311 region because it is devoid of MOR in rats (see Supplementary Fig. 5 for *ex vivo* biodistribution
312 results). We excluded the first five minutes from model fitting to rule out confounding perfusion
313 effects. Time-activity curves (TACs) from brain regions of interest (thalamus, striatum and
314 neocortex) defined by the Waxholm atlas were then extracted for data analysis.

315 **Serum sampling and measurement of corticosterone levels**

316 Blood samples were collected by lateral tail vein puncture at 9:00-11:00 am. For experimental
317 group rats, samples included collections conducted one day prior to the PET imaging (30 samples
318 out of 33). For the control group, sample collections were randomly conducted. Altogether, 51
319 blood samples were obtained (18 for the control group). The samples were centrifuged at 7000
320 rpm for 1.5 minutes and the serum was stored at -20°C. Levels of serum corticosterone were
321 measured using an ELISA kit (Enzo Life Sciences, Ann Arbor, MI, USA).

322 **Statistical analysis**

323 Data were analysed with R (version 3.6.0) using lme4 package [55]. To estimate the effects of
324 daylength on MOR availability, we used a linear mixed-effects model with varying intercepts for
325 each rat. Fixed effect factors included daylength, squared daylength, age, sex, group, and the
326 interaction effects between regions of interests (ROI) and all fixed effect factors were included.

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473

474 **Conflict of interest**

475 The authors declare no conflict of interest.

476 **Author contribution**

477 LS, JT, EY, AR designed the rat imaging experiment. LS analysed the data under supervision of
478 JT, AR, and LN. All authors wrote the manuscript. HL, AH, JV, SH conducted the rat imaging
479 experiments under supervision of EY and AR. KE and ES measured the corticosterone levels. JI,
480 TKarjalainen, TKantonen, PN, JH, VK, KK, JH, HS, JOR, LN contributed to construction of the
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