Title: The scheduling of adolescence with Netrin-1 and UNC5C

Authors

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Abstract

Dopamine axons are the only axons known to grow during adolescence. Here, using rodent models, we examined how two proteins, Netrin-1 and its receptor, UNC5C, guide dopamine axons towards the prefrontal cortex and shape behaviour. We demonstrate in mice (*Mus musculus*) that dopamine axons reach the cortex through a transient gradient of Netrin-1 expressing cells – disrupting this gradient reroutes axons away from their target. Using a

seasonal model (Siberian hamsters; *Phodopus sungorus*) we find that mesocortical dopamine development can be regulated by a natural environmental cue (daylength) in a sexually dimorphic manner – delayed in males, but advanced in females. The timings of dopamine axon growth and UNC5C expression are always phase-locked. Adolescence is an ill-defined, transitional period; we pinpoint neurodevelopmental markers underlying this period.

Introduction

Adolescence is a critical developmental period involving dramatic changes in behaviour and brain anatomy. The prefrontal cortex, the brain region responsible for our most complex cognitive functions, is still establishing connections during this time (Gogtay et al., 2004; Petanjek et al., 2011; Sowell et al., 2004). The trajectory of prefrontal cortex development in adolescence determines the vulnerability or resilience of individuals to adolescent-onset psychiatric diseases (Fuhrmann et al., 2015; Keshavan et al., 2014; Kessler et al., 2007, 2005; Lee et al., 2014). The age at which this adolescent development occurs therefore represents a critical window during which the brain is particularly susceptible to environmental influences. Traditionally, the onset of adolescence is thought to coincide with puberty (Hollenstein and Lougheed, 2013). In humans, the age of pubertal onset has been advancing throughout the 19th, 20th and 21st centuries, and environmental influences, such as nutrition, can pathologically alter the age of puberty (Wolf and Long, 2016). However, it remains entirely unknown whether the neural and cognitive maturational processes of adolescence can also be plastic. Here, for the first time, we examine how the timing of adolescence is programmed in the brain, and whether this timing can be plastic in response to a natural environmental cue, in parallel with pubertal plasticity.

45 Dopamine innervation to the prefrontal cortex increases substantially across adolescence and
46 psychopathologies of adolescent origin prominently feature dopamine dysfunction. Evidence
47 continues to emerge that protracted dopamine innervation is a key neural process underlying
48 the cognitive and behavioural changes that characterize adolescence (Larsen and Luna, 2018).
49 The mesocorticolimbic dopamine system – which includes the prefrontal cortex – is unique
50 because not only are connections being formed and lost during adolescence, but there is also
51 long-distance displacement of dopamine axons between brain regions. At the onset of
52 adolescence, both mesolimbic and mesocortical dopamine axons innervate the nucleus
53 accumbens in rodents, but the mesocortical axons leave the accumbens and grow towards the
54 prefrontal cortex during adolescence and early adulthood (Hoops et al., 2018; Reynolds et al.,
55 2018a). This is the only known case of axons growing from one brain region to another so late
56 during development (Hoops and Flores, 2017).

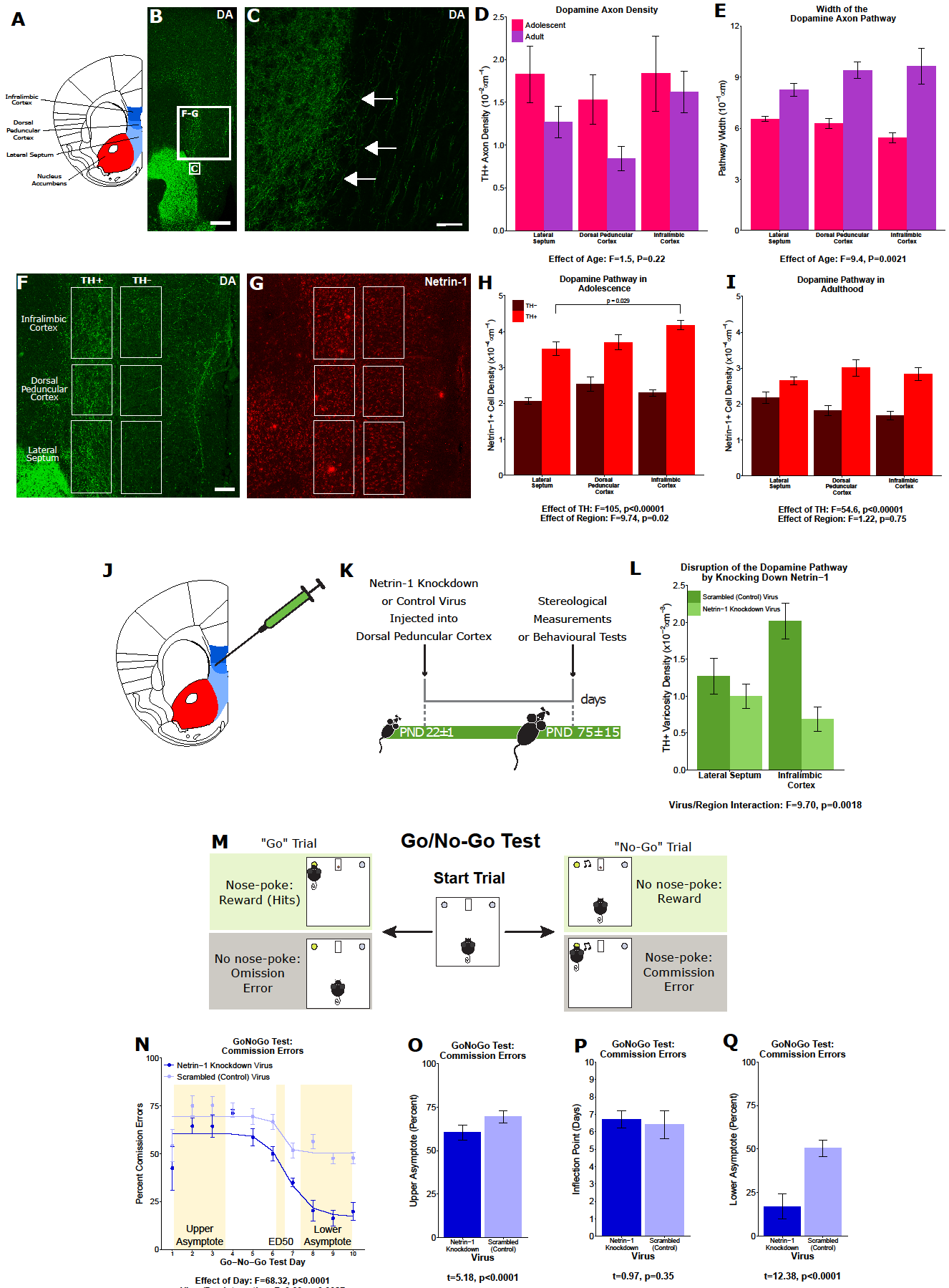
57 The prolonged growth trajectory renders mesocortical dopamine axons particularly vulnerable
58 to disruption. Environmental insults during adolescence (e.g. drug abuse) alter the extent and
59 organization of dopamine innervation in the prefrontal cortex, leading to behavioural and
60 cognitive changes in mice throughout adulthood (Drzewiecki and Juraska, 2020; Hoops and
61 Flores, 2017; Reynolds and Flores, 2021). These changes often involve cognitive control, a
62 prefrontal function that develops in parallel with dopamine innervation to the cortex in
63 adolescence (Luna et al., 2015). Disruption of dopamine innervation frequently seems to result
64 in “immature” cognitive control persisting through adulthood (Larsen and Luna, 2018).

65 Here, we examine the guidance of growing dopamine axons to the prefrontal cortex, and its
66 timing. The guidance cue molecule Netrin-1, upon interacting with its receptor DCC, determines

67 *which* dopamine axons establish connections in the nucleus accumbens and which ones leave
68 this region to grow to the prefrontal cortex (Hoops and Flores, 2017; Reynolds and Flores,
69 2021). We hypothesized that the answers to *how* and *when* this extraordinary developmental
70 feat is achieved may also lie in the Netrin-1 signalling system.

71 **Part 1: Netrin-1 "paves the way" for dopamine axons in adolescence**

72 To identify the route by which dopamine axons grow from the nucleus accumbens to the
73 medial prefrontal cortex we visualized dopamine axons in the adult mouse forebrain. We
74 observed that dopamine axons medial to the nucleus accumbens form a distinct bundle
75 oriented dorsally towards the cortex (Figure 1A,B). Individual fibres can be seen crossing the
76 boundary between the nucleus accumbens shell and this bundle (Figure 1C). We hypothesized
77 that these are the fibres that grow to the prefrontal cortex during adolescence. If this is correct,
78 the number of dopamine axons in the bundle should continue to increase until adulthood. To
79 test this, we used a modified unbiased stereological approach (Kim et al., 2011) where axons
80 are counted only if they crossed the upper and lower bounds of a counting probe. We also
81 measured the average width of the dopamine axon bundle. We found, in both male and female
82 mice, that the density of dopamine axons does not change between adolescence (21 days old)
83 and adulthood (75 days old; Figure 1D). However, the width of the dopamine axon bundle does
84 change, increasing between adolescence and adulthood (Figure 1E). These results indicate that
85 the total number of dopamine axons passing through this bundle increases over adolescence
86 and that dopamine axons grow to the medial prefrontal cortex via this route.


A
Infralimbic Cortex
Dorsal Peduncular Cortex
Lateral Septum
Nucleus Accumbens
B
DA
F-G
C
C
DA
D
Dopamine Axon Density
Adolescent
Adult
TH+ Axon Density (10^-2 cm^-1)
Lateral Septum
Dorsal Peduncular Cortex
Infralimbic Cortex
Effect of Age: F=1.5, P=0.22
E
Width of the Dopamine Axon Pathway
Pathway Width (10^-1 cm)
Effect of Age: F=9.4, P=0.0021
F
TH+
TH-
DA
Infralimbic Cortex
Dorsal Peduncular Cortex
Lateral Septum
G
Netrin-1
H
Dopamine Pathway in Adolescence
TH-
TH+
p = 0.029
Netrin-1+ Cell Density (x10^-4 cm^-1)
Effect of TH: F=105, p<0.00001
Effect of Region: F=9.74, p=0.02
I
Dopamine Pathway in Adulthood
Netrin-1+ Cell Density (x10^-4 cm^-1)
Effect of TH: F=54.6, p<0.00001
Effect of Region: F=1.22, p=0.75
J
K
Netrin-1 Knockdown or Control Virus Injected into Dorsal Peduncular Cortex
Stereological Measurements or Behavioural Tests
days
PND 22±1
PND 75±15
L
Disruption of the Dopamine Pathway by Knocking Down Netrin-1
Scrambled (Control) Virus
Netrin-1 Knockdown Virus
TH+ Varicosity Density (x10^-3 cm^-3)
Lateral Septum
Infralimbic Cortex
Virus/Region Interaction: F=9.70, p=0.0018
M
Go/No-Go Test
"Go" Trial
Nose-poke: Reward (Hits)
No nose-poke: Omission Error
Start Trial
"No-Go" Trial
No nose-poke: Reward
Nose-poke: Commission Error
N
GoNoGo Test: Commission Errors
Netrin-1 Knockdown Virus
Scrambled (Control) Virus
Percent Comission Errors
Upper Asymptote
ED50
Lower Asymptote
Go-No-Go Test Day
Effect of Day: F=68.32, p<0.0001
Virus/Day Interaction: F=9.00, p=0.0027
O
GoNoGo Test: Commission Errors
Upper Asymptote (Percent)
Netrin-1 Knockdown
Scrambled (Control)
Virus
t=5.18, p<0.0001
P
GoNoGo Test: Commission Errors
Inflection Point (Days)
Virus
t=0.97, p=0.35
Q
GoNoGo Test: Commission Errors
Lower Asymptote (Percent)
Virus
t=12.38, p<0.0001

88 **Figure 1.** A “pathway” of Netrin-1 expressing cells “paves the way” for dopamine (DA) axons growing from the
89 nucleus accumbens to the medial prefrontal cortex during adolescence. **A**, The brain regions containing the bundle
90 of dopamine fibres passing to the medial prefrontal cortex are highlighted in a line drawing of a coronal mouse
91 brain section derived from Paxinos and Franklin (Paxinos and Franklin, 2013). **B**, An image of a coronal section
92 through the forebrain of an adult mouse at low magnification (4x). Green fluorescence indicates immunostaining
93 for tyrosine hydroxylase (TH), a marker for dopamine. The smaller and larger white squares indicate the regions
94 enlarged in panel C and panels F & G, respectively. Scale bar = 500 µm. **C**, The nucleus accumbens (left) is densely
95 packed with dopamine axons (in green). Some of these axons can be observed extending from the nucleus
96 accumbens medially into a bundle of dopamine fibres (white arrows). Scale bar = 10 µm. **D**, Modified stereological
97 quantification revealed no significant difference in dopamine axon density in the fibre bundle between
98 adolescence (21 days old) and adulthood (75 days old). Mixed-effects ANOVA, effect of age: $F=1.53$, $p=0.22$; region
99 by age interaction: $F=1.44$, $p=0.49$. **E**, The average width of the fibre bundle increased significantly from
100 adolescence to adulthood, revealing that there is an increase in the total number of fibres passing to the medial
101 prefrontal cortex during this period. Mixed-effects ANOVA, effect of age: $F=9.45$, $p=0.0021$; region by age
102 interaction: $F=5.74$, $p=0.057$. **F**, In order to quantify the Netrin-1 positive cells along the dopamine fibre bundle,
103 the bundle was contoured in each region, and a contour of equal area was placed medial to the fibre bundle as a
104 negative control. Scale bar = 200 µm. **G**, Using quantitative stereology, Netrin-1 positive cell density was
105 determined along and adjacent to the fibre bundle for each region. Red fluorescence indicates immunostaining for
106 Netrin-1. **H**, In adolescent mice there are more Netrin-1 positive cells along the dopamine fibre bundle (“TH+”)
107 than medial to it (“TH-“). This is what we refer to as the “Netrin-1 pathway”. Along the pathway, there is a
108 significant increase in Netrin-1 positive cell density in regions closer to the medial prefrontal cortex, the
109 innervation target. Mixed-effects ANOVA, effect of TH: $F=105$, $p<0.0001$. Effect of region: $F=9.74$, $p=0.021$. A post-
110 hoc Tukey Test revealed a difference ($p = 0.029$) between the densities of the lateral septum and infralimbic
111 cortex, but only within the dopamine fibre bundle. **I**, In adult mice the Netrin-1 pathway is maintained, however
112 there is no longer an increasing density of Netrin-1 positive cells towards the medial prefrontal cortex. Mixed-
113 effects ANOVA, effect of TH: $F=54.56$, $p<0.0001$. Effect of region: $F=1.22$, $p=0.75$. **J,** The virus injection location
114 within the mouse brain. A Netrin-1 knockdown virus, or a control virus, was injected into the dopamine fibre

115 bundle at the level of the dorsal peduncular cortex. **K,** Our experimental timeline: at the onset of adolescence a
116 Netrin-1 knockdown virus, or a control virus, was injected, and then in adulthood the mice were sacrificed and
117 stereological measurements taken. **L,** Dopamine varicosity density was quantified in the region below the injection
118 site, in the lateral septum, and in the region above the injection site, in the infralimbic cortex. There was a
119 significant decrease in dopamine varicosity density only in the infralimbic cortex. Mixed-effects ANOVA, virus by
120 region interaction: $F=16.41$, $p<0.0001$. **M,** The experimental set-up of the final (test) stage of the Go/No-Go
121 experimental protocol. A mouse that has previously learned to nose-poke for a reward in response to a visual cue
122 (illuminated nose-poke hole), must now inhibit this behaviour when the visual cue is paired with an auditory cue
123 (acoustic tone). **N,** Mice injected with the Netrin-1 knockdown virus show improved action impulsivity than
124 controls because they incur significantly fewer commission errors across the Go/No-go task. Mixed-effects ANOVA,
125 effect of day: $F=68.32$, $p<0.0001$. Day by virus interaction: $F=9.00$, $p=0.0027$. A sigmoidal curve is fit to each group
126 of mice to determine how the two groups differ. Points indicate group means and error bars show standard error
127 means. **O**, During the first days of Go/No-Go testing, both groups incur commission errors with high frequency,
128 but, the Netrin-1 knockdown group has fewer errors than the control group (t-test, $t=5.18$, $p<0.0001$. **P,** The ED50
129 – the inflection point in each sigmoidal curve – are not different between groups, indicating that all mice improve
130 their ability to inhibit their behavior at around the same time (T-test, $t=0.97$, $p=0.35$). **Q,** Mice microinfused with
131 the Netrin-1 knockdown virus incur substantially fewer commission errors in the last days of the Go/No-Go task
132 compared to mice injected with the control virus (T-test, $t=12.38$, $p<0.0001$). For all barplots, bars indicate group
133 means and error bars show standard error means.

134

135 Next, we focussed on Netrin-1, a secreted protein that acts as a guidance cue to growing axons
136 and is important for dopamine axon targeting in the nucleus accumbens in adolescence (Cuesta
137 et al., 2020). Using unbiased stereology, we quantified the number of Netrin-1 expressing cell
138 bodies along the dopamine axon route, and in an adjacent medial region as a control (Figure 1
139 F, G). We found that in adolescence there are more Netrin-1 positive neurons within the

140 dopamine axon route than adjacent to it. Furthermore, along the axon route the density of
141 Netrin-1 positive cells increases towards the medial prefrontal cortex, forming a dorsoventral
142 gradient (Figure 1H). In adulthood, there remains a higher density of Netrin-1 positive cells
143 along the dopamine route compared to the adjacent region, however the dorsoventral gradient
144 is no longer present (Figure 1I).

145 To determine if Netrin-1 along the dopamine axon route is necessary for axon navigation, we
146 silenced Netrin-1 expression in the dorsal peduncular cortex, the transition region between the
147 septum and the medial prefrontal cortex, at the onset of adolescence (Figure 1J,K). In
148 adulthood we quantified the number of dopamine axon terminals in the regions below and
149 above the injection site. Silencing Netrin-1 did not alter dopamine terminal density below the
150 injection, in the lateral septum (Figure 1L). In the infralimbic cortex, which is the first prefrontal
151 cortical region the axons reach after the injection site, terminal density was reduced in the
152 Netrin-1 knock-down group compared to controls (Figure 1L). The knock-down appears to erase
153 the Netrin-1 path to the prefrontal cortex, resulting in dopamine axons losing their way and
154 failing to reach their correct innervation target. We conclude that Netrin-1 expressing cells
155 "pave the way" for the mesocortical dopamine axons.

156 We next examined how the Netrin-1 path may be important for behaviour. The dopamine input
157 to the prefrontal cortex is a key factor in the transition from juvenile to adult behaviours that
158 occurs in adolescence. We hypothesized that cognitive processes involving mesocortical
159 dopamine function would be altered when these axons are misrouted in adolescence. To test
160 our hypothesis we used the Go/No-Go behavioural paradigm. This test quantifies inhibitory
161 control, which matures in parallel with the innervation of dopamine axons to the prefrontal

162 cortex in adolescence (Casey et al., 2008; Klune et al., 2021; Luna et al., 2015; Paus, 2005;
163 Reynolds and Flores, 2021; Spear, 2000) and it is impaired in adolescent-onset disorders like
164 depression and schizophrenia (Catts et al., 2013; Clementz et al., 2016; McTeague et al., 2016;
165 Millan et al., 2012).

166 At the onset of adolescence, we injected the Netrin-1 silencing, or a scrambled control virus,
167 bilaterally into the dorsal peduncular cortex; in adulthood we tested the mice in the Go/No-Go
168 task. This paradigm first involves discrimination learning and reaction time training
169 (Supplementary Figure 1), followed by a Go/No-Go test consisting of "Go" trials where mice
170 respond to a cue as previously trained and "No-Go" trials where mice must abstain from
171 responding to the cue (Figure 1M). Correct responses to both trial types are reinforced with a
172 food reward. We quantified the percent of "No-Go" trials where the mice incorrectly responded
173 to the cue ("Commission Errors") and the percent of "Go" trials where the mice correctly
174 responded ("Rewards" or "Hits"; Supplementary Figure 2). The ability of mice to respond
175 correctly overall to both trial types is quantified as the Correct Response Rate (Supplementary
176 Figure 3) (Cuesta et al., 2019; Reynolds et al., 2018a, 2018b; Vassilev et al., 2021).

177 Mice injected with the Netrin-1 silencing virus differed from controls in their performance
178 during "No-Go" trials. As the mice learn to withhold their responses over the course of the test,
179 the number of commission errors they made in No-Go trials decreased in a sigmoidal fashion
180 (Figure 1N). The upper and lower asymptotes of the sigmoidal curve quantify the number of
181 commission errors committed during early and late test days, respectively, while the inflection
182 point (ED50) indicates when mice start improving their ability to inhibit their behavior. Already
183 at the start of the Go/No part of the task, the Netrin-1 silencing group make slightly fewer

184 commission errors (Figure 1O) than control groups, although both groups begin to improve in
185 the No-Go task at around the same time. However, the Netrin-1 silencing group achieved a
186 substantially higher level of behavioural inhibition, quantified as a lower percentage of
187 commission errors in the last test days (Figure 1Q), indicating an improved ability to withhold
188 their behaviour on cue. These behavioural results demonstrate that the maturation of action
189 impulsivity is sensitive to the organization of the ventro-dorsal Netrin-1 path that guides
190 mesocortical dopamine axon growth. Deviations in this route lead to striking changes in the
191 cognitive development that is characteristic of adolescence.

192

193 **Part 2: UNC5C expression coincides with the onset of adolescence**

194 When axons leave the nucleus accumbens during adolescence, they follow a Netrin-1 "path"
195 through intermediate brain regions to reach their intended innervation target. However, only a
196 small subset of the dopamine axons that have reached the nucleus accumbens by early
197 adolescence leave; the vast majority stay and form connections in the accumbens throughout
198 life (Reynolds et al., 2018a). The "decision making" process of whether to "stay" (in the
199 accumbens) or "go" (to the cortex via the Netrin-1 path) happens during a narrow
200 developmental window at the onset of adolescence (Reynolds et al., 2018b). It remains
201 unknown how the timing of this process is determined.

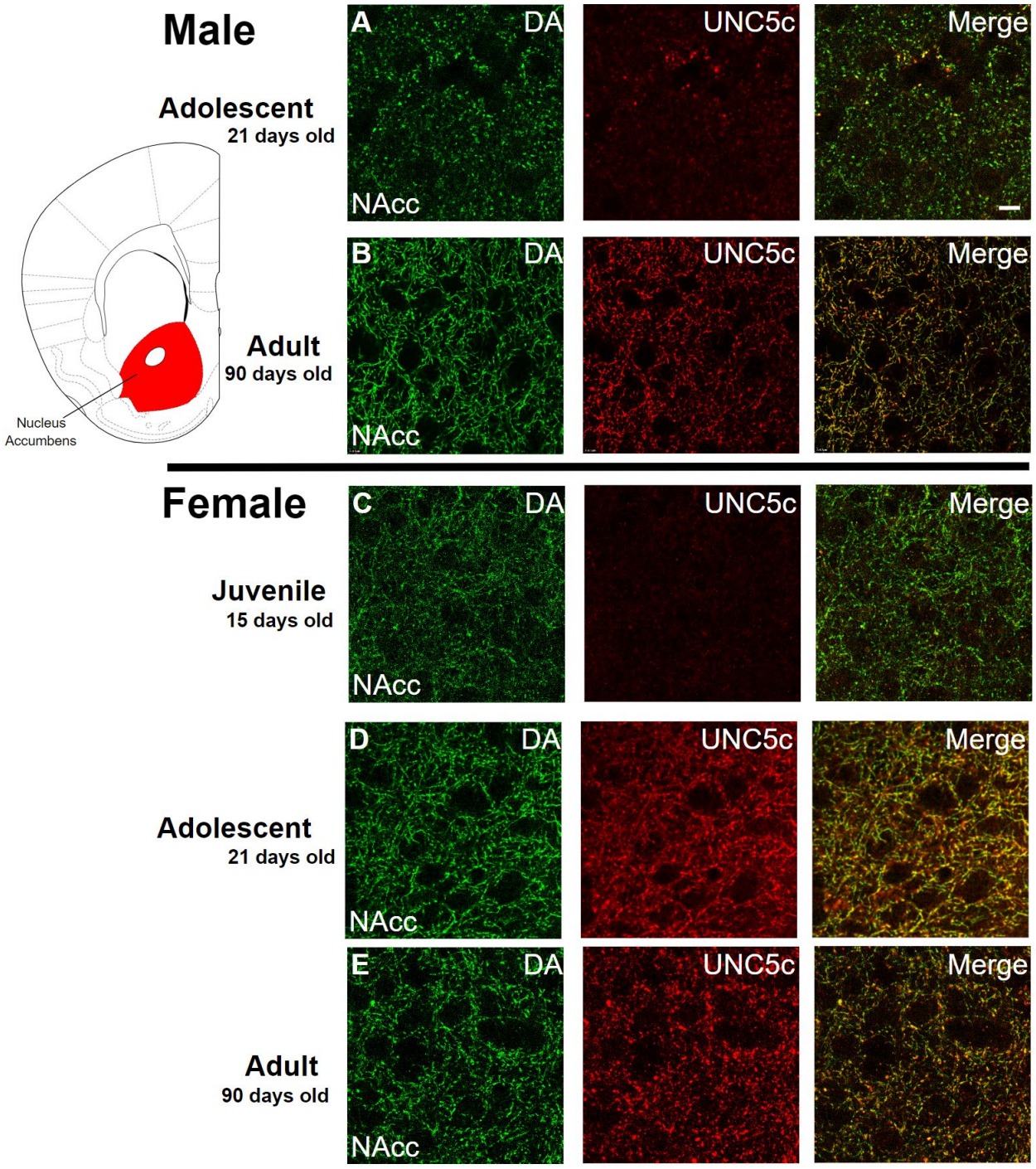


Figure 2 The age of onset of UNC5C expression by dopamine axons in the nucleus accumbens of mice is sexually dimorphic. Images are representative of observed immunofluorescence patterns in the nucleus accumbens core (approx. location highlighted in the coronal mouse brain section Plate 19, modified from Paxinos & Franklin, 2013). For each row, six individuals were sampled. In males (**A-B**), UNC5C expression on dopamine fibres in the nucleus

207 accumbens appears during adolescence. **A,** At the onset of adolescence (21 days old) dopamine fibres do not
208 express UNC5C. Scale bar = 10 µm. **B,** By adulthood (90 days old), dopamine fibres express UNC5C. In females (**C-**
209 **E**), UNC5C expression on dopamine fibres in the nucleus accumbens appears prior to adolescence. **C,** In juvenile
210 (15 day old) mice, there is no UNC5C expression on dopamine fibres. **D,** By adolescence, dopamine fibres express
211 UNC5C. **E,** In adulthood, dopamine fibres continue to express UNC5C.

212

213 In adolescence, dopamine neurons begin to express the repulsive Netrin-1 receptor UNC5C, and
214 reduction in UNC5C expression appears to cause growth of mesolimbic dopamine axons to the
215 prefrontal cortex (Auger et al., 2013; Manitt et al., 2010; Pokinko et al., 2015). Using
216 immunohistochemistry, we assessed the expression of UNC5C on nucleus accumbens dopamine
217 axons across development. In male mice, we found little expression of UNC5C on dopamine
218 axons at the onset of adolescence (Figure 2A), while we did find UNC5C expression on
219 dopamine axons in adults (Figure 2B). Remarkably, when we assessed this in females, we found
220 dopamine axons already expressing UNC5C in the nucleus accumbens at the onset of
221 adolescence (Figure 2D), similar to adult females (figure 2E), indicating that the onset of UNC5C
222 expression on dopamine axons in the nucleus accumbens is sexually dimorphic, with an earlier
223 emergence in females. We examined the nucleus accumbens in pre-adolescent female mice
224 and indeed found little UNC5C expression on dopamine axons (Figure 2C). The onset of UNC5C
225 expression in mesocorticolimbic dopamine axons is therefore peri-adolescent but occurs earlier
226 in females than in males, consistent with the earlier emergence of adolescence in female
227 rodents and the earlier onset of adolescence and puberty in humans (Wolf and Long, 2016).

228 **Part 3: Environmental control of the timing of adolescence**

229 We hypothesize that at the emergence of adolescence, UNC5C expression by dopamine axons
230 in the nucleus accumbens signals the initiation of the growth of dopamine axons to the
231 prefrontal cortex. We therefore examined whether the developmental timings of UNC5C
232 expression and dopamine innervation of the prefrontal cortex are similarly affected by an
233 environmental cue known to delay pubertal development in seasonal species.

234 Siberian hamsters (*Phodopus sungorus*) regulate many aspects of their behavior and physiology
235 to meet the changing environmental demands of seasonality (Paul et al., 2008; Stevenson et al.,
236 2017). In winter, they increase the thickness of their fur, exchange their brown summer coats
237 for white winter ones, and undergo a daily torpor to conserve energy (Scherbarth and
238 Steinlechner, 2010). In addition, adults suppress reproduction and juveniles delay puberty
239 (Pevet, 1988; Yellon and Goldman, 1984), including developmental changes in gonadotropin
240 releasing hormone neurons in the hypothalamus (Buchanan and Yellon, 1991; Heywood and
241 Yellon, 1997). This seasonal plasticity is regulated by long or short periods of daylight
242 (Heldmaier and Steinlechner, 1981; Hoffmann, 1978) and raises the possibility that aspects of
243 adolescent development are sensitive to these environmental cues. To our knowledge,
244 adaptive variation in the timing of adolescent neural development has never been recorded in
245 any animal.

246 Here, we tested whether day length regulates *when* dopamine axons grow to the cortex, and
247 whether the timing of UNC5C expression in the nucleus accumbens and adolescent changes in
248 exploratory behavior are similarly affected.

249

250 **3.i The seasonality of adolescence**

251 Male hamsters were examined at three ages: 15 days old (±1), 80 days old (±10), and 215 days
252 old (±20). We compared the density of the dopamine innervation to the medial prefrontal
253 cortex in male hamsters housed under lighting conditions that replicate summer daylengths
254 (long days, short nights) or winter daylengths (short days, long nights) (Figure 3A,B). We will
255 refer to these two groups as "summer hamsters" and "winter hamsters" to emphasize the
256 natural stimulus we are replicating in the laboratory environment. We confirmed that puberty
257 is delayed in male winter hamsters compared to summer hamsters in the present experiment
258 by measuring their gonadal weights across ages (Supplementary Figure 4).

259 In male summer hamsters, dopamine input density to the prefrontal cortex increases during
260 adolescence, after 15 days old and before 80 days old (Figure 3C), consistent with dopamine
261 axon growth in mice (Manitt et al., 2013, 2011; Reynolds et al., 2018a). Prefrontal cortex
262 dopamine innervation in summer hamsters continues to increase after 80 days old (Figure 3C).

263 In male winter hamsters, dopamine innervation to the prefrontal cortex is delayed until after 80
264 days, which coincides with their delayed pubertal onset (Figure 3D, Supplementary Figure 2B).
265 This is the first demonstration of an environmental cue shaping adolescent brain development.

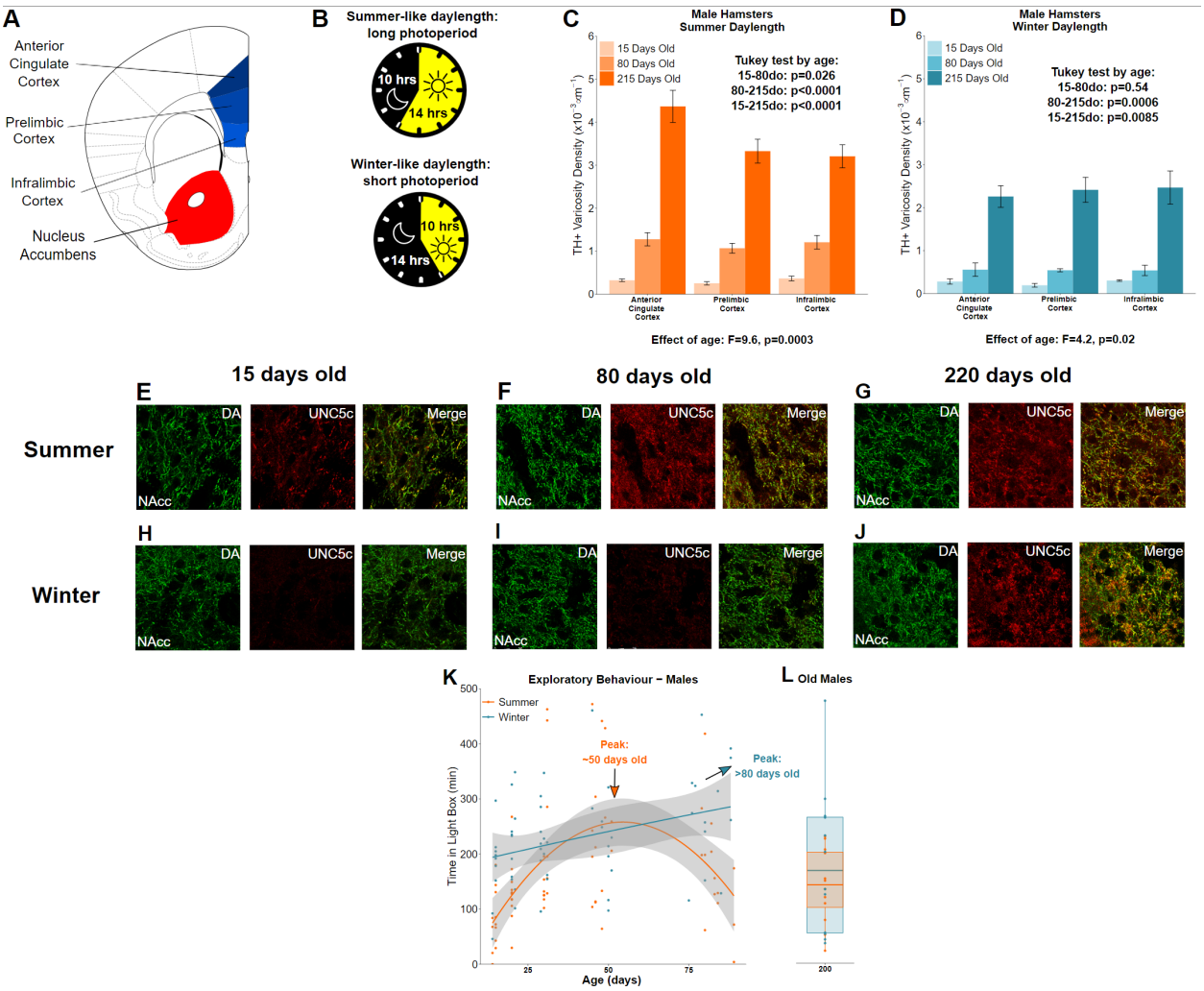


Figure 3 Plasticity of adolescent development in male Siberian hamsters according to seasonal phenotype. All results illustrated in this figure refer to results in male hamsters. **A,** Dopamine innervation was quantified in three subregions of the medial prefrontal cortex, highlighted in blue. UNC5C expression was examined in the nucleus accumbens, highlighted in red. Line drawing of a coronal section of the mouse brain was derived from Plate 19 of Paxinos and Franlkin (Paxinos and Franklin, 2013). **B,** Hamsters were housed under either summer-mimicking long days and short nights ("summer hamsters") or winter-mimicking short days and long nights ("winter hamsters"). **C,** In male hamsters housed under a summer-mimicking daylength there is an increase in dopamine varicosity density in the medial prefrontal cortex between 15 and 80 days old. Mixed-effects ANOVA, effect of age: F=9.6, p=0.000255. Tukey Test, 15-80 days old (do): p=0.026; 80-215do: p<0.0001; 15-215do: p<0.0001. **D,** In male hamsters housed under a winter-mimicking daylength there is no increase in dopamine varicosity density until

hamsters have reached 215 days of age. Mixed-effects ANOVA, effect of age: F=4.17, p=0.0205. Tukey Test, 15-80do: p=0.54; 80-215do: p=0.0006; 15-215do: p=0.0085. **E,** At 15 days old, dopamine axons in the nucleus accumbens of male summer-daylength hamsters largely do not express UNC5C. Scale bar = 20um (bottom right). **F-G,** At 80 (**F**) and 215 (**G**) days old, dopamine axons in the nucleus accumbens express UNC5C. **H-I,** At 15 (**H**) and 80 (**I**) days old, dopamine axons in the nucleus accumbens of male winter hamsters largely do not express UNC5C. **J,** By 215 days old there is UNC5C expression in dopamine axons in the nucleus accumbens of male winter hamsters. **E-J**, Representative images of the nucleus accumbens shell, 6 individuals were examined per group. **K,** Male hamsters house under a summer-mimicking daylength show an adolescent peak in exploratory behaviour in the light/dark box apparatus. Instead, those raised under a winter-mimicking photoperiod show a steady increase in exploratory behaviour over the same age range. Arrows indicate the ages at which exploratory behaviour peaks in summer (orange) and winter (blue) hamsters. Polynomial regression, effect of season: F=3.551, p=0.00056. **L,** In male hamsters, at 215 days of age, there is no difference in exploratory behaviour between hamsters raised under summer and winter photoperiods. T-test, effect of season: t=0.975, p=0.341. For all barplots, bars indicate group means and error bars show standard error means.

We then examined UNC5C expression by dopamine axons in the nucleus accumbens in male summer and winter hamsters across age classes. UNC5C expression was apparent only after the onset of adolescence in summer hamsters (Figure 3E,F,G), as observed in male mice. However, UNC5C expression was delayed in male winter hamsters – this group did not show UNC5C expression in dopamine axons in the nucleus accumbens until after 80 days old (Figure 3H,I,J). This aligns with the delayed timing of mesocortical dopamine axon growth and pubertal onset in male winter hamsters and demonstrates that the emergence of UNC5C is a marker of adolescent onset in male mice.

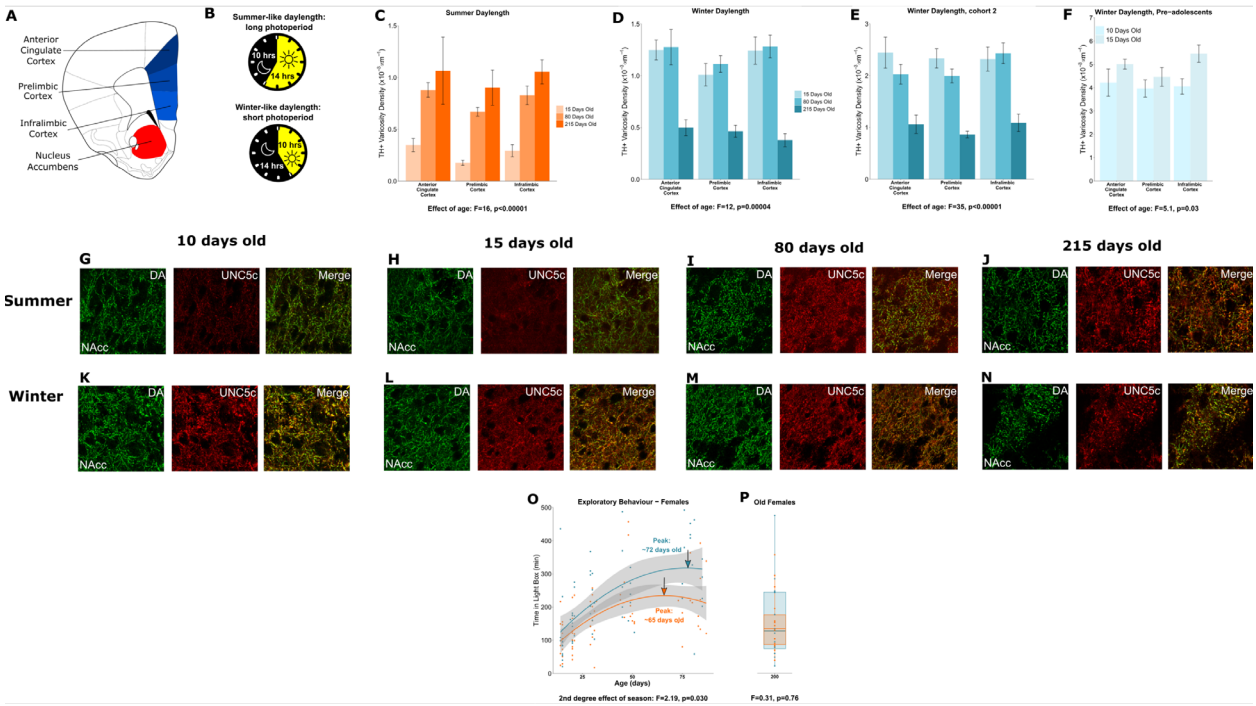
300 A behaviour characteristic of adolescence is increased willingness to enter a novel environment,
301 called exploratory behaviour (Arrant et al., 2013; Lynn and Brown, 2009); to measure this we
302 used the light/dark test (Bourin and Hascoët, 2003). Time spent in the light compartment is
303 dopamine-dependent (Bahi and Dreyer, 2019; Gao and Cutler, 1993) and peaks in adolescence
304 (Arrant et al., 2013). We assessed the developmental profile of exploratory behavior in the
305 light/dark box test in summer and winter hamsters across adolescence. In male summer
306 hamsters, the exploratory behavior increases across adolescence, peaks around 50 days, then
307 subsequently declines (Figure 3K). However, the adolescent increase in exploratory behavior is
308 protracted in winter hamsters: across the age range examined we observe a gradual, consistent
309 increase in exploratory behaviour rather than a peak and decline.

310 We next assessed a cohort of 215-day old hamsters, for which both summer and winter male
311 hamsters have undergone puberty and exhibit high levels of dopamine innervation of the
312 prefrontal cortex (Figure 3C,D,G,J, Supplementary Figure 2B). In these hamsters, we find no
313 difference in exploratory behaviour between the male summer and winter groups (Figure 3L),
314 demonstrating that, after 80 days, exploratory behaviour begins to decline in male winter
315 hamsters and that by 215 days it has declined to the same level as in summer hamsters. Male
316 hamsters raised under summer-mimicking long days and winter-mimicking short days both
317 ultimately make the transition to the adult behavioral phenotype.

318 **3.ii An extraordinary case of decoupling puberty and adolescence**

319 In parallel with males, we conducted equivalent experiments in female hamsters (Figure 4A,B).
320 Under a summer-mimicking daylength, dopamine innervation to the medial prefrontal cortex

321 increases between 15 and 80 days old, similar to male summer hamsters (Figure 4C). There is
322 no further increase in innervation density after 80 days old, consistent with earlier adolescent
323 development in females observed in other rodent species (Juraska and Willing, 2017; Kopec et
324 al., 2018; Reynolds and Flores, 2021; Spear, 2000; Westbrook et al., 2018). We confirmed that
325 puberty is delayed in female winter hamsters compared to summer hamsters by measuring
326 their uterine weights across ages (Supplementary Figure 2A).



327

328 **Figure 4** Plasticity of adolescent development in female Siberian hamsters according to seasonal phenotype. All
329 results illustrated in this figure refer to results in female hamsters. **A,** Dopamine innervation was quantified in
330 three subregions of the medial prefrontal cortex, highlighted here in blue. UNC5C expression was examined in the
331 nucleus accumbens, highlighted in red. Line drawing of a coronal section of the mouse brain was derived from
332 Paxinos and Franlkin (Paxinos and Franklin, 2013). **B,** Hamsters were housed under either a summer-mimicking or
333 winter-mimicking daylength. **C,** In female hamsters housed under a summer daylength dopamine varicosity density
334 in the medial prefrontal cortex increases between 15 and 80 days of age. Mixed-effects ANOVA, effect of age:

335 F=16.72, p<0.0001 **D,** In female hamsters housed under a winter daylength there is no increase in dopamine
336 varicosity density post-adolescence. Instead, there is a steep decline in density between 80 and 215 days of age.
337 Mixed-effects ANOVA, effect of age: F=12.33, p=0.000043. **E,** As our results in panel D were unexpected, we
338 replicated them with a second cohort of hamsters and found qualitatively identical results. Mixed-effects ANOVA,
339 effect of age: F=34.871, p<0.0001. **F,** To try and determine when dopamine varicosities innervate the medial
340 prefrontal cortex, we examined a cohort of 10- and 15-day-old hamsters. We found that varicosity density
341 increases in the medial prefrontal cortex during this time, indicating that dopamine innervation to the medial
342 prefrontal cortex is accelerated in female winter hamsters. Mixed-effects ANOVA, effect of age: F=5.05, p=0.03. **G-**
343 **H,** In 10- and 15-day-old female summer hamsters there is little UNC5C expression in nucleus accumbens
344 dopamine axons. Sample size: 4 (panel G) or 6 (panel H). **I-J,** By 80 days old (panel I), and continuing at 215 days old
345 (panel J), dopamine axons in the nucleus accumbens express UNC5C in female summer hamsters. Sample sizes: 6.
346 Scale bar = 20um (panel G bottom right). **K-N,** At all ages at which winter female hamsters were examined
347 dopamine axons in the nucleus accumbens express UNC5C in winter female hamsters. Sample sizes: 4 (panel K) or
348 6 (panels L-N). **O,** In female hamsters, those raised under summer and winter daylengths both show an increase in
349 exploratory behaviour over time. The winter hamsters peak later compared to the summer daylength hamsters.
350 Arrows indicate the ages at which exploratory behaviour peaks in summer (orange) and winter (blue) hamsters.
351 Polynomial regression, effect of season: F=3.305, p=0.00126. **P,** In female hamsters, at 215 days of age, there is no
352 difference in exploratory behaviour between hamsters raised under summer and winter photoperiods. T-test,
353 effect of season: t=0.309, p=0.76. For all barplots, bars indicate group means and error bars show standard error
354 means.

355

356 When housed under a winter-mimicking daylength, dopamine input density in the prefrontal
357 cortex of female hamsters is *not* delayed as in males, but rather reaches adult levels prior to 15
358 days old (Figure 4D). We replicated this unexpected finding in a separate, independent cohort

359 of female winter hamsters (Figure 4E). To our knowledge, this is the first time an intervention is
360 shown to accelerate adolescent cortical development.

361 We then measured dopamine axon density in female winter hamsters at two earlier ages: 10
362 and 15 days old. Dopamine innervation increases during this period (figure 4F), well before
363 normal adolescence and long before pubertal development. This is an extraordinary
364 phenomenon: a key marker of adolescent neurodevelopment is accelerated and dissociated
365 from puberty in female hamsters raised under winter-mimicking short days (Supplementary
366 Figure 2A).

367 The early increase in prefrontal cortex dopamine terminals in winter females is followed by a
368 dramatic reduction between 80 and 215 days old (Figure 4D,E). This overlaps with the delayed
369 timing of puberty in these females (Butler et al., 2007; Supplementary Figure 2A). Synaptic
370 pruning in the cortex is a well-known component of adolescent neural development across
371 species (Huttenlocher, 1984; Koss et al., 2013; Petanjek et al., 2011). Under normal conditions,
372 the effect of pruning on dopamine synapses is likely masked by the growth of new dopamine
373 axons to the prefrontal cortex (Manitt et al., 2013, 2011; Reynolds et al., 2018a). In the case of
374 female winter hamsters, we hypothesize that the growth of dopamine axons to the prefrontal
375 cortex occurs early while synaptic pruning, including of dopamine synapses, appears to occur
376 later. This leads to a remarkable dissociation between two cortical developmental processes
377 that are normally simultaneous, the behavioural implications of which are unclear.

378 If the developmental onset of UNC5C expression determines the timing of dopamine
379 innervation of the prefrontal cortex, then onset of UNC5C expression should also be advanced

380 in female winter hamsters. Hence, we examined UNC5C expression at the same ages as we
381 examined dopamine axon growth in female hamsters. At 10 and 15 days old, UNC5C
382 expression is present *only* in the winter hamsters (Figure 4G,H,K,L), but at 80 and 215 days old,
383 UNC5C expression is apparent in both summer and winter hamsters (Figure 4I,J,M,N).

384 We used the light/dark box test to examine potential exploratory behavioural implications of
385 the extraordinary developmental trajectory we observed in the prefrontal cortex of female
386 hamsters. In female summer and winter hamsters the adolescent increase and peak in
387 exploratory behaviour occurs between the ages of 15 and 80 days, as it does in summer
388 daylength males (Figure 4O). However, contrary to what we would expect, the peak in winter
389 females is delayed compared to summer females. When we assessed an independent cohort of
390 215-day-old female hamsters, we found no difference in exploratory behaviour between groups
391 (Figure 4R), indicating that, like males, female summer and winter hamsters both eventually
392 reach the same adult level of exploratory behaviour.

393 In both sexes, hamsters housed under a summer-mimicking daylength showed an adolescent
394 peak in exploratory behaviour at an age that we would predict based on results from other
395 rodents (Arrant et al., 2013; Pietropaolo et al., 2004; Tanaka, 2015). When raised under a
396 winter-mimicking daylength, hamsters of either sex show a protracted exploratory peak. In
397 males, it is delayed beyond 80 days old, but the delay is substantially less in females. For
398 dopamine and UNC5C neural markers, males delayed, whereas females advanced, adolescent
399 neural development. Based on the delayed developmental profile in exploratory behavior, we
400 predict that other adolescent neural changes are delayed in female winter hamsters. We

401 hypothesize that the advanced dopamine/UNC5C neural development lessens the delay in peak
402 exploratory behaviour of female winter hamsters compared to that of males.

403 **Conclusion**

404 Here we describe how the gradual growth of mesocortical dopamine axons marks adolescent
405 development, and how this process uses guidance cues and is sensitive to sex and environment.
406 Netrin-1 signalling provides the “stay-or-go” “decision making” conducted by dopamine axons
407 that innervate the nucleus accumbens at the onset of adolescence (Cuesta et al., 2020). UNC5C
408 expression by these dopamine axons marks the timing at which this decision is made. In mice,
409 UNC5C expression coincides with sex differences in both adolescent and pubertal development.
410 Females, which develop earlier, show earlier UNC5C expression in dopamine axons compared
411 to males.

412 In hamsters, behavioural and developmental shifts in response to environmental cues occur in
413 parallel with alterations in the timing of dopamine axon growth. As we show here, male
414 hamsters raised under a winter-mimicking daylength delay not only puberty, but also
415 adolescent dopamine and behavioural maturation. In contrast, female hamsters under identical
416 conditions delay puberty but accelerate dopamine axon growth, a key marker of adolescent
417 brain development. Behavioural shifts during adolescence appear to be delayed in these
418 females, but less substantially than in male hamsters. Notably, under all conditions, the
419 developmental timing of UNC5C expression corresponds to the timing of dopamine innervation
420 of the prefrontal cortex.

421 In both mice and hamsters, the emergence of UNC5C expression coincides with the onset of
422 dopamine axon growth to the prefrontal cortex, a key characteristic of the adolescent transition
423 period. While previously we have shown that the Netrin-1 signalling in the nucleus accumbens
424 is responsible for coordinating *whether* dopamine axons grow in adolescence (Reynolds and
425 Flores, 2021), here we propose that Netrin-1 signalling is also key to determining *how* and *when*
426 this marker of adolescence occurs.

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High resolution figures and captions

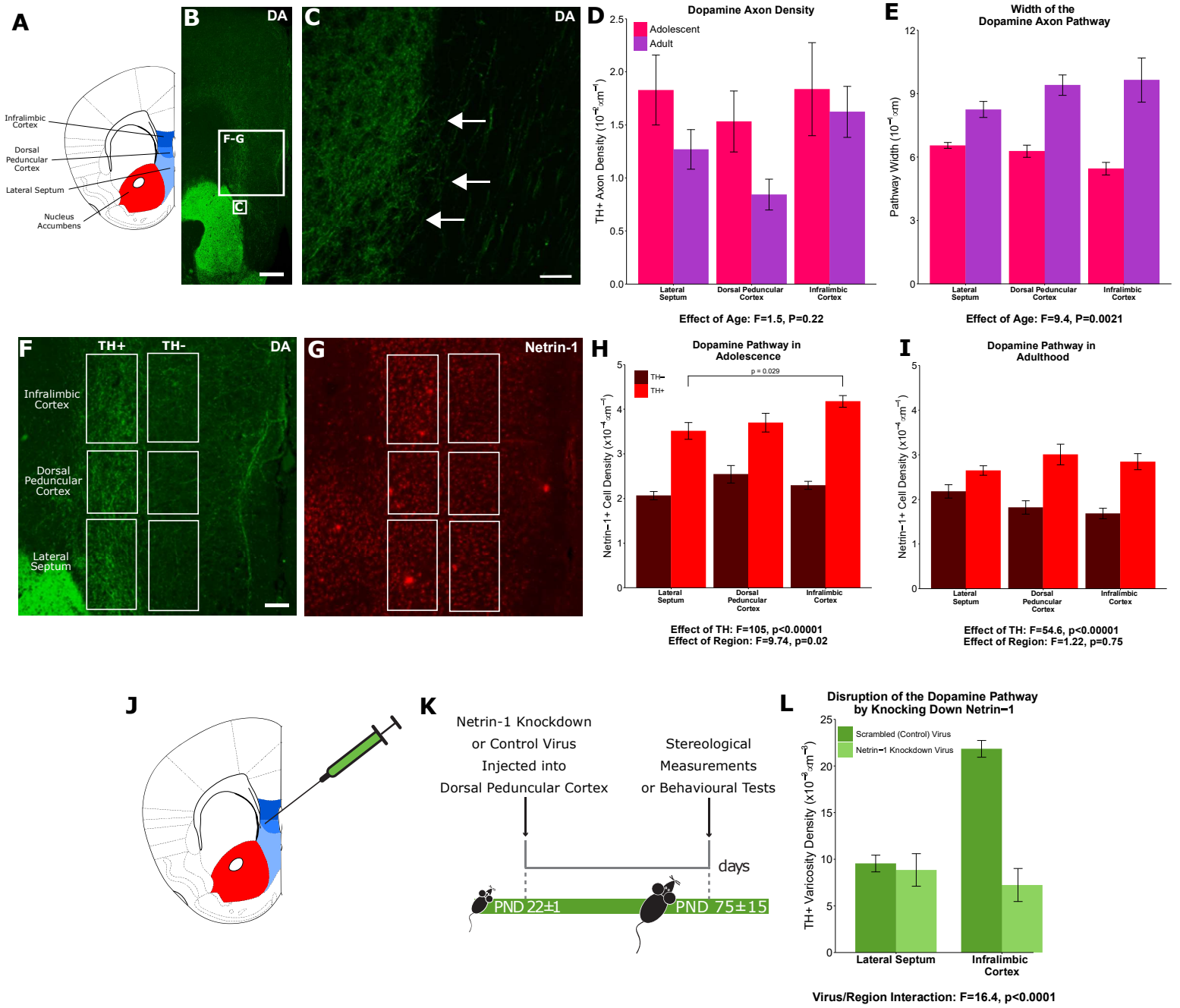
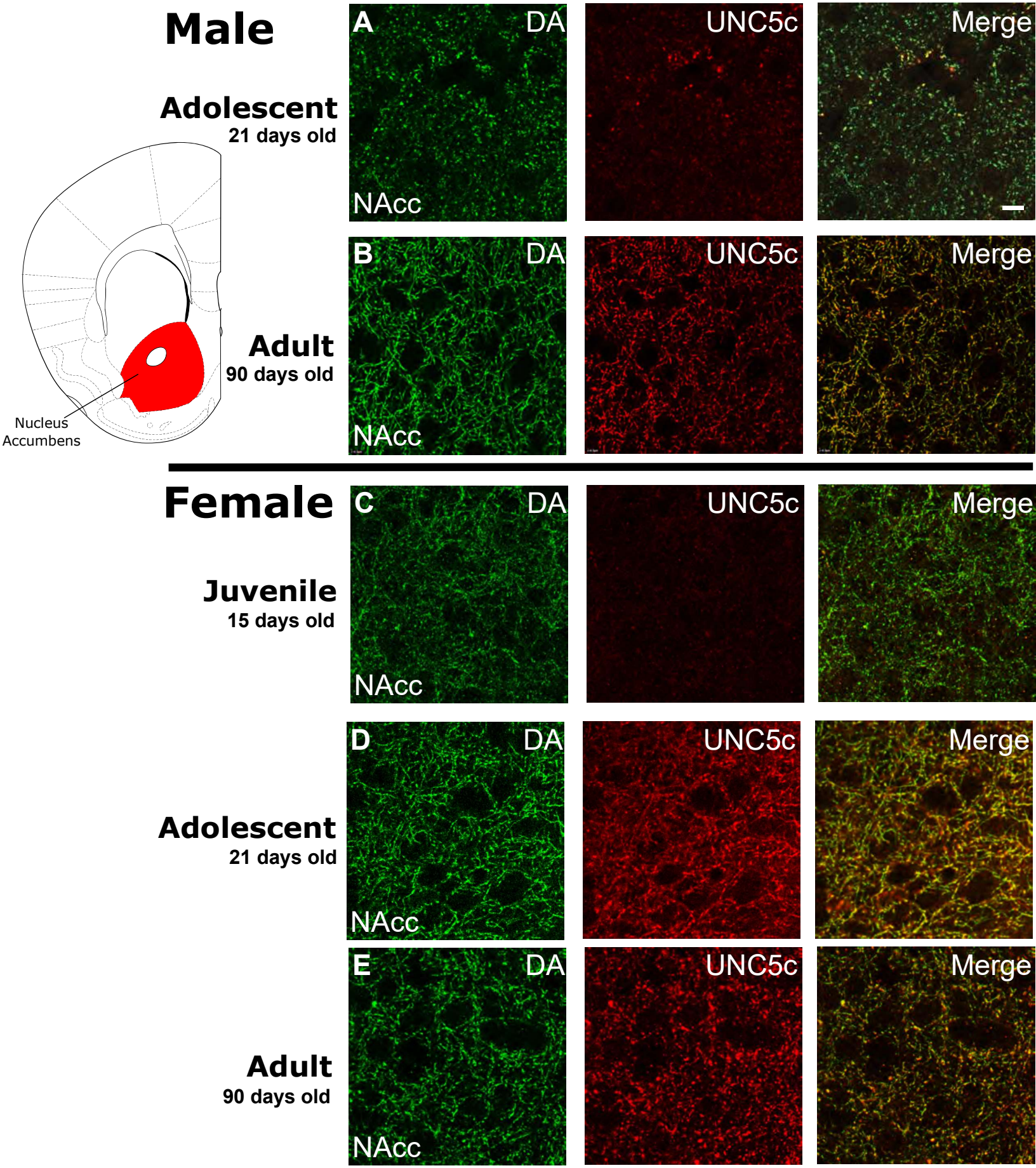

A
Infralimbic Cortex
Dorsal Peduncular Cortex
Lateral Septum
Nucleus Accumbens
B
DA
F-G
C
C
DA
D
Dopamine Axon Density
Adolescent
Adult
TH+ Axon Density (10^-2 µm^-1)
Lateral Septum
Dorsal Peduncular Cortex
Infralimbic Cortex
Effect of Age: F=1.5, P=0.22
E
Width of the Dopamine Axon Pathway
Pathway Width (10^-1 µm)
Effect of Age: F=9.4, P=0.0021
F
TH+
TH-
DA
Infralimbic Cortex
Dorsal Peduncular Cortex
Lateral Septum
G
Netrin-1
H
Dopamine Pathway in Adolescence
TH-
TH+
p = 0.029
Netrin-1+ Cell Density (x10^-4 µm^-1)
Effect of TH: F=105, p<0.00001
Effect of Region: F=9.74, p=0.02
I
Dopamine Pathway in Adulthood
Effect of TH: F=54.6, p<0.00001
Effect of Region: F=1.22, p=0.75
J
K
Netrin-1 Knockdown or Control Virus Injected into Dorsal Peduncular Cortex
Stereological Measurements or Behavioural Tests
days
PND 22±1
PND 75±15
L
Disruption of the Dopamine Pathway by Knocking Down Netrin-1
Scrambled (Control) Virus
Netrin-1 Knockdown Virus
TH+ Varicosity Density (x10^-2 µm^-1)
Lateral Septum
Infralimbic Cortex
Virus/Region Interaction: F=16.4, p<0.0001
M
Go/No-Go Test
"Go" Trial
Nose-poke: Reward (Hits)
No nose-poke: Omission Error
Start Trial
"No-Go" Trial
No nose-poke: Reward
Nose-poke: Commission Error
N
GoNoGo Test: Commission Errors
Netrin-1 Knockdown Virus
Scrambled (Control) Virus
Percent Comission Errors
Upper Asymptote
ED50
Lower Asymptote
Go-No-Go Test Day
Effect of Day: F=68.32, p<0.0001
Virus/Day Interaction: F=9.00, p=0.0027
O
GoNoGo Test: Commission Errors
Upper Asymptote (Percent)
Netrin-1 Knockdown
Scrambled (Control)
Virus
t=5.18, p<0.0001
P
GoNoGo Test: Commission Errors
Inflection Point (Days)
Virus
t=0.97, p=0.35
Q
GoNoGo Test: Commission Errors
Lower Asymptote (Percent)
Virus
t=12.38, p<0.0001

Figure 1. A “pathway” of Netrin-1 expressing cells “paves the way” for dopamine (DA) axons growing from the nucleus accumbens to the medial prefrontal cortex during adolescence. **A**, The brain regions containing the bundle of dopamine fibres passing to the medial prefrontal cortex are highlighted in a line drawing of a coronal mouse brain section derived from Paxinos and Franklin (Paxinos and Franklin, 2013). **B**, An image of a coronal section through the forebrain of an adult mouse at low magnification (4x). Green fluorescence indicates immunostaining for tyrosine hydroxylase (TH), a marker for dopamine. The smaller and larger white squares indicate the regions enlarged in panel C and panels F & G, respectively. Scale bar = 500 µm. **C**, The nucleus accumbens (left) is densely packed with dopamine axons (in green). Some of these axons can be observed extending from the nucleus accumbens medially into a bundle of dopamine fibres (white arrows). Scale bar = 10 µm. **D**, Modified stereological quantification revealed no significant difference in dopamine axon density in the fibre bundle between adolescence (21 days old) and adulthood (75 days old). Mixed-effects ANOVA, effect of age: $F=1.53$, $p=0.22$; region by age interaction: $F=1.44$, $p=0.49$. **E**, The average width of the fibre bundle increased significantly from adolescence to adulthood, revealing that there is an increase in the total number of fibres passing to the medial prefrontal cortex during this period. Mixed-effects ANOVA, effect of age: $F=9.45$, $p=0.0021$; region by age interaction: $F=5.74$, $p=0.057$. **F**, In order to quantify the Netrin-1 positive cells along the dopamine fibre bundle, the bundle was contoured in each region, and a contour of equal area was placed medial to the fibre bundle as a negative control. Scale bar = 200 µm. **G**, Using quantitative stereology, Netrin-1 positive cell density was determined along and adjacent to the fibre bundle for each region. Red fluorescence indicates immunostaining for Netrin-1. **H**, In adolescent mice there are more Netrin-1 positive cells along the dopamine fibre bundle (“TH+”) than medial to it (“TH-“). This is what we refer to as the “Netrin-1 pathway”. Along the pathway, there is a significant increase in Netrin-1 positive cell density in regions closer to the medial prefrontal cortex, the innervation target. Mixed-effects ANOVA, effect of TH: $F=105$, $p<0.0001$. Effect of region: $F=9.74$, $p=0.021$. A post-hoc Tukey Test revealed a difference ($p = 0.029$) between the densities of the lateral septum and infralimbic cortex, but only within the dopamine fibre bundle. **I**, In adult mice the Netrin-1 pathway is maintained, however there is no longer an increasing density of Netrin-1 positive cells towards the medial prefrontal cortex. Mixed-effects ANOVA, effect of TH: $F=54.56$, $p<0.0001$. Effect of region: $F=1.22$, $p=0.75$. **J,** The virus injection location within the mouse brain. A Netrin-1 knockdown virus, or a control virus, was injected into the dopamine fibre

bundle at the level of the dorsal peduncular cortex. **K,** Our experimental timeline: at the onset of adolescence a Netrin-1 knockdown virus, or a control virus, was injected, and then in adulthood the mice were sacrificed and stereological measurements taken. **L,** Dopamine varicosity density was quantified in the region below the injection site, in the lateral septum, and in the region above the injection site, in the infralimbic cortex. There was a significant decrease in dopamine varicosity density only in the infralimbic cortex. Mixed-effects ANOVA, virus by region interaction: $F=16.41$, $p<0.0001$. **M,** The experimental set-up of the final (test) stage of the Go/No-Go experimental protocol. A mouse that has previously learned to nose-poke for a reward in response to a visual cue (illuminated nose-poke hole), must now inhibit this behaviour when the visual cue is paired with an auditory cue (acoustic tone). **N,** Mice injected with the Netrin-1 knockdown virus show improved action impulsivity than controls because they incur significantly fewer commission errors across the Go/No-go task. Mixed-effects ANOVA, effect of day: $F=68.32$, $p<0.0001$. Day by virus interaction: $F=9.00$, $p=0.0027$. A sigmoidal curve is fit to each group of mice to determine how the two groups differ. Points indicate group means and error bars show standard error means. **O**, During the first days of Go/No-Go testing, both groups incur commission errors with high frequency, but, the Netrin-1 knockdown group has fewer errors than the control group (t-test, $t=5.18$, $p<0.0001$. **P,** The ED50 – the inflection point in each sigmoidal curve – are not different between groups, indicating that all mice improve their ability to inhibit their behavior at around the same time (T-test, $t=0.97$, $p=0.35$). **Q,** Mice microinfused with the Netrin-1 knockdown virus incur substantially fewer commission errors in the last days of the Go/No-Go task compared to mice injected with the control virus (T-test, $t=12.38$, $p<0.0001$). For all barplots, bars indicate group means and error bars show standard error means.


Male
Adolescent
21 days old
Adult
90 days old
Nucleus
Accumbens
A
B
DA
UNC5c
Merge
NAcc
Female
Juvenile
15 days old
Adolescent
21 days old
Adult
90 days old
C
D
E

49 **Figure 2** The age of onset of UNC5C expression by dopamine axons in the nucleus accumbens of mice is sexually
50 dimorphic. Images are representative of observed immunofluorescence patterns in the nucleus accumbens core
51 (approx. location highlighted in the coronal mouse brain section Plate 19, modified from Paxinos & Franklin, 2013).
52 For each row, six individuals were sampled. In males (**A-B**), UNC5C expression on dopamine fibres in the nucleus
53 accumbens appears during adolescence. **A,** At the onset of adolescence (21 days old) dopamine fibres do not
54 express UNC5C. Scale bar = 10 µm. **B,** By adulthood (90 days old), dopamine fibres express UNC5C. In females (**C-**
55 **E**), UNC5C expression on dopamine fibres in the nucleus accumbens appears prior to adolescence. **C,** In juvenile
56 (15 day old) mice, there is no UNC5C expression on dopamine fibres. **D,** By adolescence, dopamine fibres express
57 UNC5C. **E,** In adulthood, dopamine fibres continue to express UNC5C.

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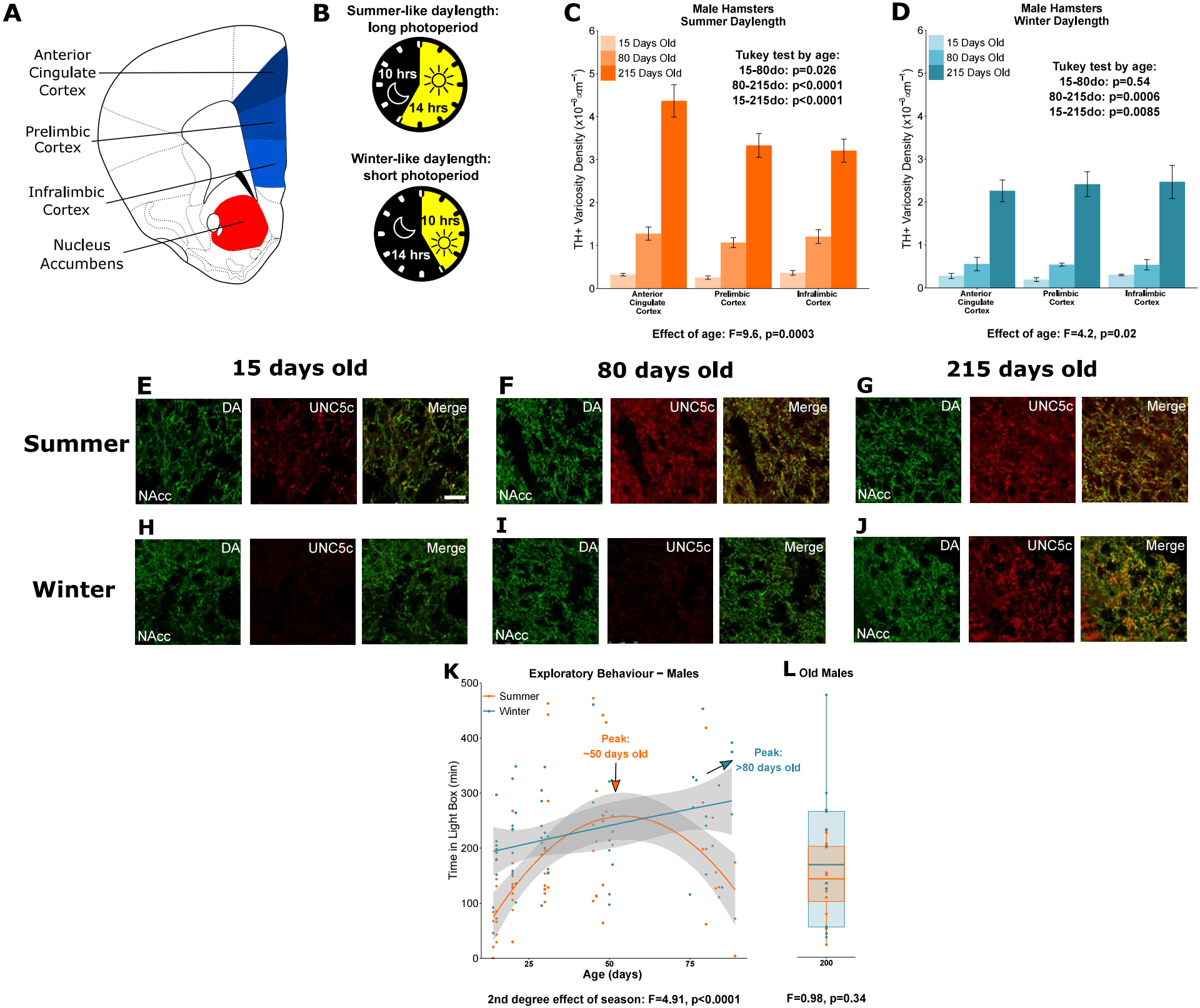
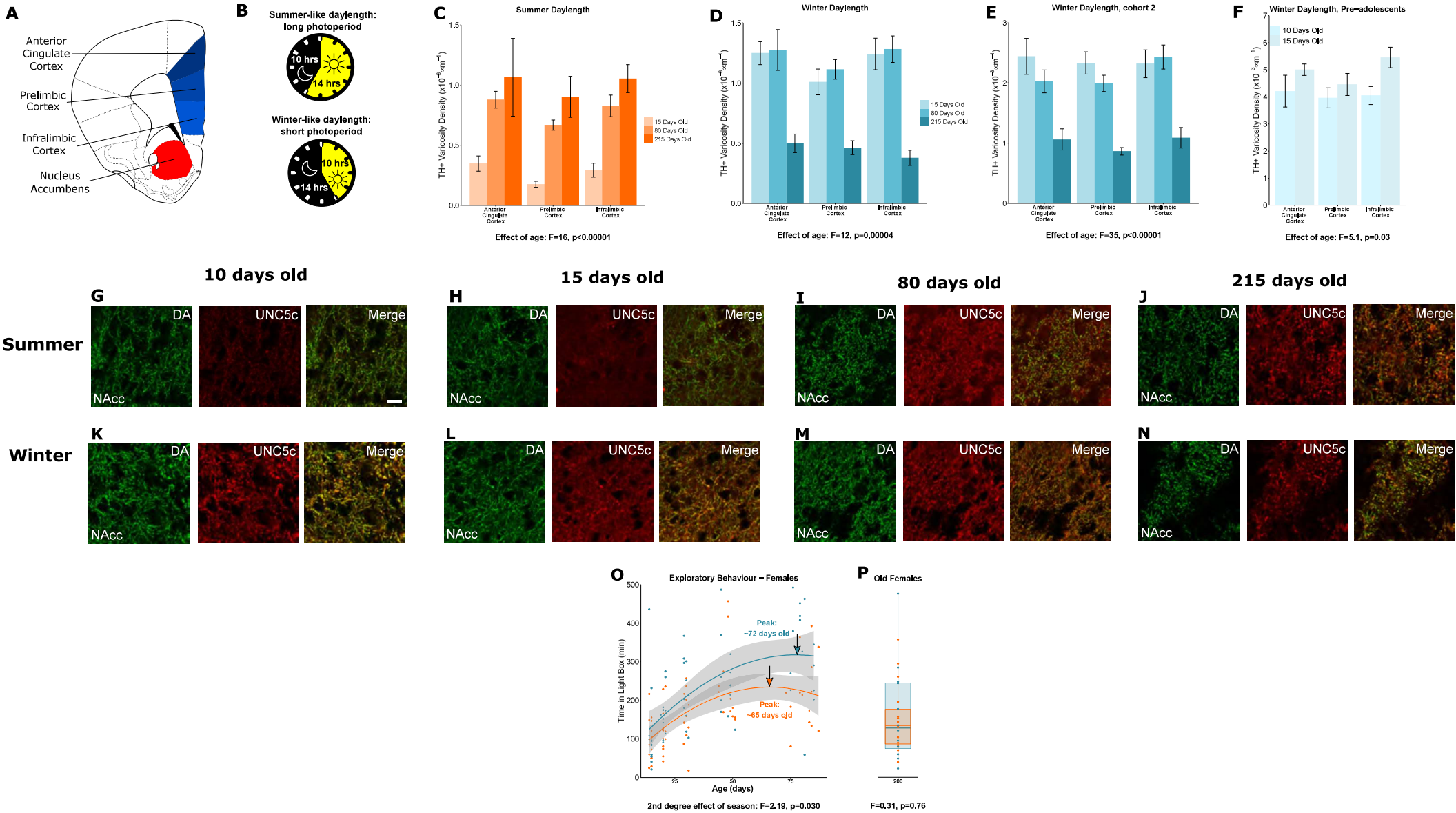

A
Anterior Cingulate Cortex
Prelimbic Cortex
Infralimbic Cortex
Nucleus Accumbens
B
Summer-like daylength: long photoperiod
10 hrs
14 hrs
Winter-like daylength: short photoperiod
10 hrs
14 hrs
C
Male Hamsters Summer Daylength
15 Days Old
80 Days Old
215 Days Old
Tukey test by age:
15-80do: p=0.026
80-215do: p<0.0001
15-215do: p<0.0001
TH+ Varicosity Density (x10^-3 µm^-1)
Anterior Cingulate Cortex
Prelimbic Cortex
Infralimbic Cortex
Effect of age: F=9.6, p=0.0003
D
Male Hamsters Winter Daylength
15 Days Old
80 Days Old
215 Days Old
Tukey test by age:
15-80do: p=0.54
80-215do: p=0.0006
15-215do: p=0.0085
TH+ Varicosity Density (x10^-3 µm^-1)
Anterior Cingulate Cortex
Prelimbic Cortex
Infralimbic Cortex
Effect of age: F=4.2, p=0.02
15 days old
80 days old
215 days old
E
F
G
H
I
J
Summer
Winter
DA
UNC5c
Merge
NAcc
K
Exploratory Behaviour – Males
Summer
Winter
Peak: ~50 days old
Peak: >80 days old
Time in Light Box (min)
Age (days)
2nd degree effect of season: F=4.91, p<0.0001
L
Old Males
F=0.98, p=0.34

Figure 3 Plasticity of adolescent development in male Siberian hamsters according to seasonal phenotype. All results illustrated in this figure refer to results in male hamsters. **A,** Dopamine innervation was quantified in three subregions of the medial prefrontal cortex, highlighted in blue. UNC5C expression was examined in the nucleus accumbens, highlighted in red. Line drawing of a coronal section of the mouse brain was derived from Plate 19 of Paxinos and Franlkin (Paxinos and Franklin, 2013). **B,** Hamsters were housed under either summer-mimicking long days and short nights ("summer hamsters") or winter-mimicking short days and long nights ("winter hamsters"). **C,** In male hamsters housed under a summer-mimicking daylength there is an increase in dopamine varicosity density in the medial prefrontal cortex between 15 and 80 days old. Mixed-effects ANOVA, effect of age: $F=9.6$, $p=0.000255$. Tukey Test, 15-80 days old (do): $p=0.026$; 80-215do: $p<0.0001$; 15-215do: $p<0.0001$. **D,** In male hamsters housed under a winter-mimicking daylength there is no increase in dopamine varicosity density until hamsters have reached 215 days of age. Mixed-effects ANOVA, effect of age: $F=4.17$, $p=0.0205$. Tukey Test, 15-80do: $p=0.54$; 80-215do: $p=0.0006$; 15-215do: $p=0.0085$. **E,** At 15 days old, dopamine axons in the nucleus accumbens of male summer-daylength hamsters largely do not express UNC5C. Scale bar = 20um (bottom right). **F-G,** At 80 (**F**) and 215 (**G**) days old, dopamine axons in the nucleus accumbens express UNC5C. **H-I,** At 15 (**H**) and 80 (**I**) days old, dopamine axons in the nucleus accumbens of male winter hamsters largely do not express UNC5C. **J,** By 215 days old there is UNC5C expression in dopamine axons in the nucleus accumbens of male winter hamsters. **E-J**, Representative images of the nucleus accumbens shell, 6 individuals were examined per group. **K,** Male hamsters house under a summer-mimicking daylength show an adolescent peak in exploratory behaviour in the light/dark box apparatus. Instead, those raised under a winter-mimicking photoperiod show a steady increase in exploratory behaviour over the same age range. Arrows indicate the ages at which exploratory behaviour peaks in summer (orange) and winter (blue) hamsters. Polynomial regression, effect of season: $F=3.551$, $p=0.00056$. **L,** In male hamsters, at 215 days of age, there is no difference in exploratory behaviour between hamsters raised under summer and winter photoperiods. T-test, effect of season: $t=0.975$, $p=0.341$. For all barplots, bars indicate group means and error bars show standard error means.


A
Anterior Cingulate Cortex
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Infralimbic Cortex
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B
Summer-like daylength: long photoperiod
10 hrs
14 hrs
Winter-like daylength: short photoperiod
10 hrs
14 hrs
C
Summer Daylength
TH+ Varicosity Density (x10^-3 um^-1)
15 Days Old
80 Days Old
215 Days Old
Anterior Cingulate Cortex
Prelimbic Cortex
Infralimbic Cortex
Effect of age: F=16, p<0.00001
D
Winter Daylength
Effect of age: F=12, p=0.00004
E
Winter Daylength, cohort 2
Effect of age: F=35, p<0.00001
F
Winter Daylength, Pre-adolescents
10 Days Old
15 Days Old
Effect of age: F=5.1, p=0.03
10 days old
15 days old
80 days old
215 days old
Summer
Winter
G
H
I
J
K
L
M
N
DA
UNC5c
Merge
NAcc
O
Exploratory Behaviour – Females
Time in Light Box (min)
Peak: ~72 days old
Peak: ~65 days old
Age (days)
2nd degree effect of season: F=2.19, p=0.030
P
Old Females
F=0.31, p=0.76

84 **Figure 4** Plasticity of adolescent development in female Siberian hamsters according to seasonal phenotype. All
85 results illustrated in this figure refer to results in female hamsters. **A,** Dopamine innervation was quantified in
86 three subregions of the medial prefrontal cortex, highlighted here in blue. UNC5C expression was examined in the
87 nucleus accumbens, highlighted in red. Line drawing of a coronal section of the mouse brain was derived from
88 Paxinos and Franlkin (Paxinos and Franklin, 2013). **B,** Hamsters were housed under either a summer-mimicking or
89 winter-mimicking daylength. **C,** In female hamsters housed under a summer daylength dopamine varicosity density
90 in the medial prefrontal cortex increases between 15 and 80 days of age. Mixed-effects ANOVA, effect of age:
91 $F=16.72$, $p<0.0001$ **D,** In female hamsters housed under a winter daylength there is no increase in dopamine
92 varicosity density post-adolescence. Instead, there is a steep decline in density between 80 and 215 days of age.
93 Mixed-effects ANOVA, effect of age: $F=12.33$, $p=0.000043$. **E,** As our results in panel D were unexpected, we
94 replicated them with a second cohort of hamsters and found qualitatively identical results. Mixed-effects ANOVA,
95 effect of age: $F=34.871$, $p<0.0001$. **F,** To try and determine when dopamine varicosities innervate the medial
96 prefrontal cortex, we examined a cohort of 10- and 15-day-old hamsters. We found that varicosity density
97 increases in the medial prefrontal cortex during this time, indicating that dopamine innervation to the medial
98 prefrontal cortex is accelerated in female winter hamsters. Mixed-effects ANOVA, effect of age: $F=5.05$, $p=0.03$. **G-**
99 **H,** In 10- and 15-day-old female summer hamsters there is little UNC5C expression in nucleus accumbens
100 dopamine axons. Sample size: 4 (panel G) or 6 (panel H). **I-J,** By 80 days old (panel I), and continuing at 215 days old
101 (panel J), dopamine axons in the nucleus accumbens express UNC5C in female summer hamsters. Sample sizes: 6.
102 Scale bar = 20um (panel G bottom right). **K-N,** At all ages at which winter female hamsters were examined
103 dopamine axons in the nucleus accumbens express UNC5C in winter female hamsters. Sample sizes: 4 (panel K) or
104 6 (panels L-N). **O,** In female hamsters, those raised under summer and winter daylengths both show an increase in
105 exploratory behaviour over time. The winter hamsters peak later compared to the summer daylength hamsters.
106 Arrows indicate the ages at which exploratory behaviour peaks in summer (orange) and winter (blue) hamsters.
107 Polynomial regression, effect of season: $F=3.305$, $p=0.00126$. **P,** In female hamsters, at 215 days of age, there is no
108 difference in exploratory behaviour between hamsters raised under summer and winter photoperiods. T-test,
109 effect of season: $t=0.309$, $p=0.76$. For all barplots, bars indicate group means and error bars show standard error
110 means.