

# 1 **Lasting effect of psilocybin on sociability** 2 **can be blocked by DNA methyltransferase inhibition**

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## 17 **Abstract**

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19 The recent renaissance in research on psychedelics such as psilocybin has highlighted their  
20 therapeutic potential including their lasting influences on brain function. Here we report that a  
21 single systemic administration of the serotonergic psychedelic psilocybin can durably promote  
22 social behaviour in the Cntnap2-knockout mouse model of autism. This effect can be blocked  
23 by pharmacological inhibition of DNA methyltransferase I, indicating an epigenetic mechanism  
24 underlying the long-lasting effect of psilocybin.  
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## 27 **Introduction**

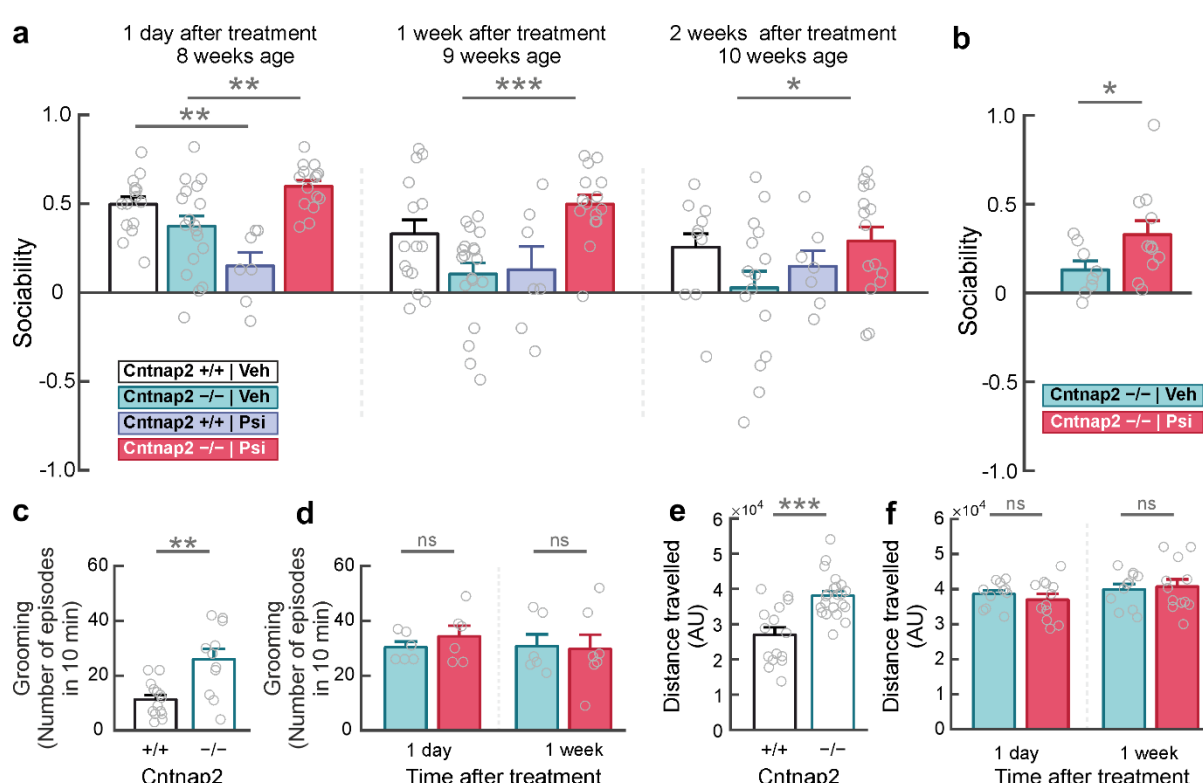
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29 The serotonergic psychedelic psilocybin has been shown to induce behavioural alterations  
30 outlasting the acute psychedelic effect. Clinical studies have revealed a therapeutic potential  
31 of psilocybin for the treatment of psychiatric disorders<sup>1</sup> and suggested beneficial effects on  
32 behavioural traits associated with and common to several neuropsychiatric conditions<sup>2</sup>.  
33 Intriguing but mechanistically unexplained are the therapeutic effects that last at least several  
34 weeks, for instance the alleviation of depression in treatment-resistant patients following a  
35 single dose of psilocybin-assisted treatment<sup>3–5</sup>. Reduced sociability is a common characteristic  
36 of all types of autism under the classification autism spectrum disorder (ASD) and this altered  
37 trait is replicated in several mouse models that mimic the genetics of ASD<sup>6–8</sup>. Here, we  
38 examined the effect of a single systemic injection of psilocybin in altering the social behaviour  
39 of the Cntnap2 knockout mouse model<sup>8</sup> of autism spectrum disorder and the durability of this  
40 effect.  
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## 43 **Results**

### 44 **Psilocybin durably restores sociability in Cntnap2<sup>-/-</sup> homozygous mutant mice.**

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47 We administered a single systemic injection of 1 mg/kg psilocybin or saline to adult Cntnap2  
48 homozygous mutant (Cntnap2<sup>-/-</sup> KO) and Cntnap2 homozygous wild-type isogenic control  
49 (Cntnap2<sup>+/+</sup> WT) mice at 8 weeks of age. We assessed the social behaviour of these mice  
50 using the three-chamber social behaviour test at 1 day, 1 week, and 2 weeks after the single

injection, corresponding to ages of postnatal week 8, 9 and 10 respectively. We quantified sociability as the amount of time a mouse approaches and explores a cup containing another mouse versus an empty cup. Similar to the reduced sociability shown in several previous studies<sup>8-10</sup>, saline treated homozygous mutant KO mice exhibited a reduced sociability compared to the isogenic wild-type control group ( $p = 0.042$ ,  $Cntnap2^{-/-}$  Vehicle vs  $Cntnap2^{+/+}$  Vehicle, Bonferroni's post-hoc). Importantly, however, psilocybin-treated homozygous mutant KO group showed a significant increase in sociability compared to the vehicle-treated  $Cntnap2^{-/-}$  Vehicle KO control group ( $p < 0.001$ ,  $Cntnap2^{-/-}$  Psilocybin vs  $Cntnap2^{-/-}$  Vehicle, Bonferroni's post-hoc, **Fig 1a**) and this effect was sustained for at least two weeks following the single psilocybin administration. Sociability of  $Cntnap2^{-/-}$  Psilocybin KO group was restored to a level similar to the isogenic wild-type control group ( $p = 0.796$ ,  $Cntnap2^{-/-}$  Psilocybin vs  $Cntnap2^{+/+}$  Vehicle, Bonferroni's post-hoc). The total time a mouse spent exploring any cup (either mouse-containing or empty) did not significantly differ between treatment of the same genotype groups ( $p > 0.999$ ,  $Cntnap2^{-/-}$  Psilocybin vs  $Cntnap2^{-/-}$  Vehicle;  $p = 0.390$ ,  $Cntnap2^{+/+}$  Psilocybin vs  $Cntnap2^{+/+}$  Vehicle, Bonferroni's post-hoc; **Extended Data Fig 1a**), indicating that the difference in sociability is due to a specific deficit in social behaviour rather than a variability in the drive to explore. We did not detect any significant effect of sex on our observations ( $p = 0.850$ , repeated measure ANOVA with Bonferroni's post-hoc).



**Figure 1. Psilocybin durably restores sociability in  $Cntnap2^{-/-}$  homozygous KO mice.**

**a.** A single injection of psilocybin rescues the social deficits in  $Cntnap2^{-/-}$  KO mice for up to two weeks (**Extended Tables 1a, 2**). **b.** Psilocybin's sociability rescuing effect is observed in  $Cntnap2^{-/-}$  KO mice tested for the first time at two weeks after a single treatment. **c-d.** The significantly higher repetitive behaviour displayed by  $Cntnap2^{-/-}$  KO mice (**c**) is not altered by psilocybin (**d**). **e-f.** The significant hyperactivity displayed by  $Cntnap2^{-/-}$  KO mice (**e**) is not altered by psilocybin (**f**). Mean  $\pm$  SEM shown. Individual data point shown. For detailed statistics see **Extended Data Table 1-3**. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Further, we treated a subset of *Cntnap2*<sup>-/-</sup> KO mice with a single administration of psilocybin or saline vehicle, and tested for their sociability for the first time at two weeks after treatment. Here we also observed significantly higher sociability in psilocybin treated *Cntnap2*<sup>-/-</sup> KO mice ( $p = 0.025$ , *Cntnap2*<sup>-/-</sup>Psilocybin vs *Cntnap2*<sup>-/-</sup>Vehicle, **Fig 1b, Extended Data Table 2a**). This observation indicated that our observation of psilocybin effect was indeed a sustained rescue of social behaviour rather than an effect of a repeated testing design. Given this observation, we next sought to examine each timepoints of our repeated measure observation, and detected the greatest psilocybin rescuing effect at 1 week post-administration (at 1 day:  $p = 0.007$ , at 1 week:  $p < 0.001$ , at 2 weeks:  $p = 0.028$ , *Cntnap2*<sup>-/-</sup>Psilocybin vs *Cntnap2*<sup>-/-</sup>Vehicle, **Fig 1a, Extended Data Table 2b**).

### No detectable effects of psilocybin on two other behavioural measures.

Then, we explored whether psilocybin also affected other aspects of behaviour that are not directly related to sociability but are possibly affected in ASD model mice<sup>8,11</sup>. We examined repetitive behaviour by quantifying self-grooming activity during habituation. We observed that *Cntnap2*<sup>-/-</sup> homozygous mutant KO mice displayed significantly higher number of self-grooming episodes compared to *Cntnap2*<sup>+/+</sup> homozygous WT mice during the final habituation session prior to drug treatments ( $p = 0.002$ , *Cntnap2*<sup>+/+</sup> vs *Cntnap2*<sup>-/-</sup>, Mann Whitney, **Fig 1c**). No significant difference was detected in the number of self-grooming episodes displayed by *Cntnap2*<sup>-/-</sup> KO mice at 1 day after psilocybin treatment ( $p = 0.329$ , *Cntnap2*<sup>-/-</sup>Vehicle vs *Cntnap2*<sup>-/-</sup>Psilocybin, Mann Whitney, **Fig 1d**) or at 1 week after drug treatment ( $p = 0.461$ , *Cntnap2*<sup>-/-</sup>Vehicle vs *Cntnap2*<sup>-/-</sup>Psilocybin, Mann Whitney, **Fig 1d**). We therefore concluded that repetitive behaviour, although increased in *Cntnap2*<sup>-/-</sup> KO mice, is not affected by psilocybin.

Further, we tracked the positions of the test mice during the habituation phase without the introduction of a social partner. In line with previous reports of hyperactivity in *Cntnap2*<sup>-/-</sup> KO mice<sup>8</sup>, we observed that *Cntnap2*<sup>-/-</sup> KO mice displayed significantly higher distance travelled during the 10 minutes observation period compared to wildtype ( $p = 0.001$ , *Cntnap2*<sup>+/+</sup> vs *Cntnap2*<sup>-/-</sup>, Dunn's post-hoc, **Fig 1e**). Despite drug treatment, no significant difference was detected in the distance travelled by *Cntnap2*<sup>-/-</sup> KO mice at 1 day after drug treatment ( $p = 0.518$ , *Cntnap2*<sup>-/-</sup>Vehicle vs *Cntnap2*<sup>-/-</sup>Psilocybin, Mann Whitney) or at 1 week after drug treatment ( $p = 0.976$ , *Cntnap2*<sup>-/-</sup>Vehicle vs *Cntnap2*<sup>-/-</sup>Psilocybin, Mann Whitney). We therefore concluded that psilocybin has no lasting effect on spontaneous locomotory drive.

### Psilocybin does not enhance social behaviour in the *Cntnap2* genotypes without social deficit.

Interestingly, the single administration of psilocybin reduced sociability in *Cntnap2*<sup>+/+</sup> isogenic wild-type group, at 1 day post-treatment ( $p = 0.001$ , *Cntnap2*<sup>+/+</sup>Vehicle vs *Cntnap2*<sup>+/+</sup>Psilocybin). Psilocybin-treated isogenic wild-type mice remained lower in sociability scores at 1 week ( $p = 0.155$ , *Cntnap2*<sup>+/+</sup>Vehicle vs *Cntnap2*<sup>+/+</sup>Psilocybin) and at 2 weeks ( $p = 0.207$ , *Cntnap2*<sup>+/+</sup>Vehicle vs *Cntnap2*<sup>+/+</sup>Psilocybin) after treatment although no longer statistically significantly (**Fig 1a**). In line with the idea that *CNTNAP2* is a recessive gene<sup>12</sup>, heterozygous *Cntnap2*<sup>+/-</sup>Vehicle group did not show significantly altered sociability compared to wildtype *Cntnap2*<sup>+/+</sup>Vehicle group ( $p = 1.000$ , *Cntnap2*<sup>+/-</sup>Vehicle vs *Cntnap2*<sup>+/+</sup>Vehicle, Bonferroni's post-hoc, **Extended Data Table 1b**). *Cntnap2*<sup>+/-</sup> heterozygous mice also displayed reduced sociability after psilocybin treatment (**Extended Data Fig 1b**), even though not statistically significantly. These observations indicate that while the prosocial effect of psilocybin has potential to rescue abnormal behavioural traits, in the absence of behavioural deficits psilocybin does not enhance and may even reduce sociability.

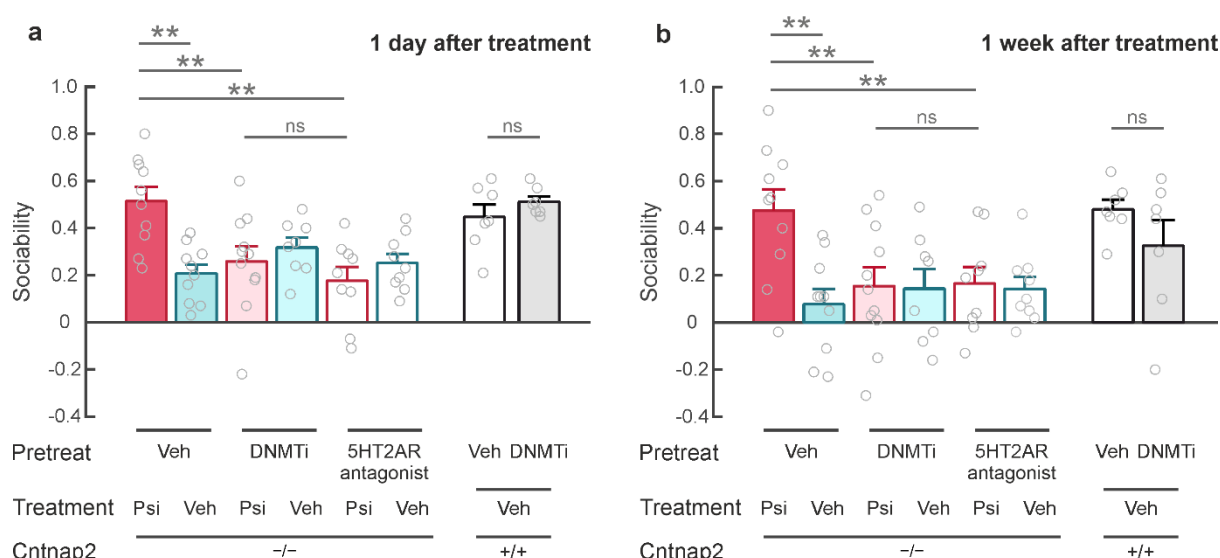
# **Lack of correlation between sociability and the acute effect of psilocybin.**

Psilocybin or its active metabolite psilocin, like other psychedelic 5HT2AR agonists, trigger an acute head twitch response (HTR)<sup>13</sup> that lasts for the duration of the agonist's bioavailability<sup>14</sup>. Immediately after psilocybin application, we assayed the HTR for a 20 minutes observation period to confirm the normal functioning of 5HT2ARs in psilocybin-treated mice (**Extended Data Fig 1c, Extended Data Table 4a**). No difference in HTR was observed between the different genotype groups without psilocybin ( $p = 0.825$ ,  $Cntnap2^{+/+}_{Vehicle}$  vs  $Cntnap2^{-/-}_{Vehicle}$ ;  $p > 0.999$ ,  $Cntnap2^{+/+}_{Vehicle}$  vs  $Cntnap2^{+/-}_{Vehicle}$ , Dunn's post-hoc), and psilocybin significantly increased HTR in all genotype groups ( $p < 0.001$ ,  $Cntnap2^{+/+}_{Vehicle}$  vs  $Cntnap2^{+/+}_{Psilocybin}$ ;  $p = 0.001$ ,  $Cntnap2^{-/-}_{Vehicle}$  vs  $Cntnap2^{-/-}_{Psilocybin}$ ;  $p = 0.006$ ,  $Cntnap2^{+/-}_{Vehicle}$  vs  $Cntnap2^{+/-}_{Psilocybin}$ ; Dunn's post-hoc) to a similar level ( $p > 0.9999$ ,  $Cntnap2^{+/+}_{Psilocybin}$  vs  $Cntnap2^{-/-}_{Psilocybin}$ ;  $p > 0.9999$ ,  $Cntnap2^{+/+}_{Psilocybin}$  vs  $Cntnap2^{+/-}_{Psilocybin}$ ; Dunn's post-hoc). Even though sex-related differences in HTR have been reported for other psychedelic compounds and analogues<sup>15,16</sup>, we did not detect any significant effect of sex on the HTR ( $p = 0.452$ ,  $Cntnap2^{-/-}_{Psilocybin::Male}$  vs  $Cntnap2^{-/-}_{Psilocybin::Female}$ , Mann Whitney; **Extended Data Table 4b**) which is similar to a lack of sex-specific effects on psilocybin-induced HTR as observed by others<sup>17</sup>. Moreover, we observed no significant correlation between acute HTR and sociability levels in the  $Cntnap2^{-/-}$  KO mice (**Extended Data Table 4c**). This indicates that the reduced sociability in  $Cntnap2^{-/-}$  KO mice cannot be explained by an altered acute function of 5HT2ARs.

# **DNA methyltransferase inhibition blocks psilocybin-induced sociability changes in $Cntnap2^{-/-}$ homozygous mutant KO mice to a similar extent as 5HT2AR antagonism.**

Given that the observed sociability-rescuing effect outlasts the bioavailability of psilocybin, we reasoned that the lasting alterations in sociability following psilocybin administration may originate from mechanisms other than sustained 5HT2AR activation<sup>18</sup>. We reasoned that epigenetic alterations may provide a potential mechanism for the observed lasting social deficit rescuing effects of psilocybin. We focused on changes in DNA methylation in view of reports showing differential DNA methylation patterns in autism spectrum disorder<sup>19,20</sup>.

We pretreated mice with systemic administration of RG108<sup>21-23</sup> - a small-molecule inhibitor of DNA methyltransferase I (DNMT1) - to test whether DNMT inhibition (DNMTi) prevents the effects from psilocybin administration. We then behaviourally tested the mice for sociability at 1 day and 1 week after treatment (**Fig. 2**). When pretreated with vehicle, psilocybin treatment rescues the reduced sociability in homozygous KO mice ( $p < 0.001$ ,  $Cntnap2^{-/-}_{Vehicle::Vehicle}$  vs  $Cntnap2^{-/-}_{Vehicle::Psilocybin}$ , Bonferroni's post-hoc), similar to our earlier observations. DNMTi pretreatment significantly reduced the psilocybin-induced increased sociability in  $Cntnap2^{-/-}$  KO mice ( $p = 0.003$ ,  $Cntnap2^{-/-}_{Vehicle::Psilocybin}$  vs  $Cntnap2^{-/-}_{DNMTi::Psilocybin}$ , Bonferroni's post-hoc) and psilocybin treatment is no longer effective ( $p = 1.000$ ,  $Cntnap2^{-/-}_{DNMTi::Psilocybin}$  vs  $Cntnap2^{-/-}_{DNMTi::Vehicle}$ , Bonferroni's post-hoc). DNMTi pretreatment is similarly effective at 1 day and 1 week after administration (**Extended Data Table 6**). This indicates that *de novo* DNA methylation is required for the sustained effect of psilocybin on sociability in  $Cntnap2^{-/-}$  KO mice. DNMTi pretreatment itself did not affect sociability in  $Cntnap2^{-/-}$  KO mice ( $p = 1.000$ ,  $Cntnap2^{-/-}_{Vehicle::Vehicle}$  vs  $Cntnap2^{-/-}_{DNMTi::Vehicle}$ , Bonferroni's post-hoc), or in  $Cntnap2^{+/+}$  isogenic WT mice ( $p = 0.526$ ,  $Cntnap2^{+/+}_{Vehicle::Vehicle}$  vs  $Cntnap2^{+/+}_{DNMTi::Vehicle}$ , Repeated measure ANOVA). Additionally, we confirmed that DNMTi pretreatment in isogenic WT mice (**Extended Data Table 7**) does not have an effect on the number of self-grooming episodes ( $p = 0.222$ ,  $Cntnap2^{+/+}_{Vehicle::Vehicle}$  vs  $Cntnap2^{+/+}_{DNMTi::Vehicle}$ , Mann Whitney), or total distance travelled ( $p = 0.128$ ,  $Cntnap2^{+/+}_{Vehicle::Vehicle}$  vs  $Cntnap2^{+/+}_{DNMTi::Vehicle}$ , Mann Whitney).



**Figure 2. Psilocybin's sociability rescuing effect is blocked by DNMTi.**

**a.** Psilocybin's sociability rescuing effect can be blocked by pretreatment of DNMTi to a similar extent as 5HT2AR antagonism. **b.** The pretreatment blockade effects are sustained at 1 week after treatment. Mean  $\pm$  SEM shown. Individual data point shown. For detailed statistics see **Extended Data Table 5-6**. \*\*  $p < 0.01$ .

Psilocybin is known to act predominantly through the activation of serotonin 2A receptors (5HT2ARs)<sup>24-26</sup>. To test whether the observed effects of psilocybin were indeed initiated by 5HT2AR activation, we also performed similar experiments using pretreatment with a specific 5HT2AR antagonist volinanserin (MDL100907). We observed that 5HT2AR antagonist pretreatment prevented the psilocybin-induced increased sociability in *Cntnap2*<sup>-/-</sup> KO mice ( $p = 0.001$ , *Cntnap2*<sup>-/-</sup><sub>Vehicle::Psilocybin</sub> vs *Cntnap2*<sup>-/-</sup><sub>5HT2AR-ant::Psilocybin</sub> groups, Bonferroni's post-hoc; **Fig 2, Extended Data Table 5**). When comparing mice pretreated with DNMTi or 5HT2AR antagonist, we found no significant difference in psilocybin's effect on sociability ( $p = 1.000$ , *Cntnap2*<sup>-/-</sup><sub>DNMTi::Psilocybin</sub> vs *Cntnap2*<sup>-/-</sup><sub>5HT2AR-ant::Psilocybin</sub> groups, Bonferroni's post-hoc; **Fig 2, Extended Data Table 5**), which indicates that DNMTi is as effective as 5HT2AR antagonist in blocking psilocybin's effect on rescuing sociability. Further, we observed that the blocking effect of both pretreatment groups were sustained at 1 week after treatment (**Fig 2b; Extended Data Table 6**). This indicated that both *de novo* DNA methylation and 5HT2AR activation are required for the sustained effect of psilocybin on sociability in *Cntnap2*<sup>-/-</sup> KO mice.

While pretreatment with 5HT2AR antagonist effectively abolished the psilocybin-induced head-twitch response ( $p < 0.001$ , *Cntnap2*<sup>-/-</sup><sub>Vehicle::Psilocybin</sub> vs *Cntnap2*<sup>-/-</sup><sub>5HT2AR-ant::Psilocybin</sub> groups, Dunn's post-hoc), psilocybin-induced HTR was not affected by pretreatment with DNMTi ( $p > 0.999$ , *Cntnap2*<sup>-/-</sup><sub>Vehicle::Psilocybin</sub> vs *Cntnap2*<sup>-/-</sup><sub>DNMTi::Psilocybin</sub> groups, Dunn's post-hoc) or with vehicle ( $p > 0.999$ , *Cntnap2*<sup>-/-</sup><sub>Vehicle::Psilocybin</sub> vs *Cntnap2*<sup>-/-</sup><sub>DNMTi::Psilocybin</sub> groups, Dunn's post-hoc; **Extended Data Fig 2, Extended Data Table 8**). These observations indicate that the effect of pretreatment with DNMTi did not interfere with the normal acute effect of 5HT2AR activation by psilocybin, or spontaneous head-twitch events in *Cntnap2*<sup>-/-</sup> KO mice ( $p > 0.999$ , *Cntnap2*<sup>-/-</sup><sub>Vehicle::Vehicle</sub> vs *Cntnap2*<sup>-/-</sup><sub>DNMTi::Vehicle</sub> groups, Dunn's post-hoc; **Extended Data Fig 2, Extended Data Table 8**).



## Discussion

Taken together, our observations demonstrate that psilocybin rescues social deficits in the Cntnap2 knock-out mouse model of altered sociability in ASD, and this effect is sustained for at least up to two weeks. Our results further suggest that the long-lasting effect of psilocybin depends on *de novo* DNA methylation dynamics, since pretreatment of DNA methyltransferase inhibitor is as effective in blocking psilocybin's therapeutic effect as antagonism of psilocin's main binding target 5HT2A receptor. Our work here supports the rapidly expanding body of experimental evidence that psychedelics have the capability to exert sustained effects on brain function and behaviour, and an involvement of epigenetic mechanisms in this durable effect.

Despite this promising therapeutic effect, we also note that psilocybin does not improve sociability in mice that do not show behavioural deficits, such as that of the Cntnap2 isogenic WT or heterozygote groups here. Our observations even indicate that psilocybin exerts opposing effects, reducing sociability in the absence of abnormal sociability traits. Thus, it appears that psilocybin is not a general sociability enhancer, but rather is only beneficial when in deficit, a clinically relevant behavioural measure for brain disorders or mental health issues. When viewing psilocybin administration as a pharmacological perturbation, its counteracting effects suggest the existence of a setpoint that governs social behaviour. Our observation that DNA methylation plays a role in psilocybin's sustained social deficit rescuing effect further suggests that the setpoint for social behaviour may be controlled and/or reconfigured through epigenetic plasticity mechanisms. In line with this, others have observed that a single administration of other serotonergic psychedelic such as lysergic acid diethylamide (LSD) into control mice also did not enhance social behaviour<sup>27</sup>. However, social enhancement could be achieved through repeated LSD treatments<sup>27</sup>, and repeated LSD treatments into control mice has been separately reported to result in detectable changes in DNA methylation in the brain<sup>28</sup>. Considering this together with our observations, these experimental evidence collectively suggest that different behavioural setpoints may exist for normal vs deficit states, which may be adjusted with varying levels of ease.

Separately, the single administration of another serotonergic psychedelic 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) led to detectable changes in chromatin organisation and enhancer activity, associated with increased synaptogenesis<sup>29</sup>. Psilocybin may similarly lead to epigenomic changes including alteration of DNA methylation dynamics, which may underpin our observations here. In the case of the socially reduced Cntnap2<sup>-/-</sup> homozygous mutant KO mice, we observed that pretreatment with DNMTi is not the exclusive mechanism to block psilocybin's therapeutic effects, but it is as effective as pretreatment with 5HT2AR antagonism. This suggests that psilocybin-induced changes in DNA methylation dynamics is downstream of 5HT2AR activation. Future work will be necessary to identify the signalling pathways involved.

Further, we noted a general decline in sociability across postnatal weeks 8-10 for all experimental groups in line with previously reported age-dependent reduction in social-related behaviour in mice<sup>30</sup>. This decline is much more significant in Cntnap2 KO mice ( $p = 0.003$ , Cntnap2<sup>-/-</sup> vehicle:1 day vs Cntnap2<sup>-/-</sup> vehicle:1 week;  $p = 0.532$ , Cntnap2<sup>-/-</sup> vehicle: 1 week vs Cntnap2<sup>-/-</sup> vehicle: 2 weeks;  $p = 0.008$ , Cntnap2<sup>-/-</sup> vehicle:1 day vs Cntnap2<sup>-/-</sup> vehicle: 2 weeks; T test) and is delayed following psilocybin treatment ( $p = 0.120$ , Cntnap2<sup>-/-</sup> psilocybin:1-day vs Cntnap2<sup>-/-</sup> psilocybin: 1 week;  $p = 0.040$ , Cntnap2<sup>-/-</sup> psilocybin: 1 week vs Cntnap2<sup>-/-</sup> psilocybin: 2 weeks;  $p = 0.002$ , Cntnap2<sup>-/-</sup> psilocybin:1 day vs Cntnap2<sup>-/-</sup> psilocybin: 2 weeks; T test). It will be interesting to investigate whether psilocybin extends or even re-opens the critical developmental window where experience-dependent components of

sociability traits are the most plastic, and indeed, latest experimental observations by others indicate that psychedelics has the potential to re-open the critical period for social reward learning<sup>31</sup>. This appears to be in line with our observations here that while psilocybin was effective in rescuing social deficits, it did not alter the increased self-grooming repetitive behaviour or hyperactivity associated with this ASD model<sup>8</sup>, which may not involve critical periods.

## **Methods**

### **Animals.**

Experimental procedures at Nanyang Technological University Singapore were performed in accordance with approved protocols following review by the Institutional Animals Care and Use Committee. Experimental procedures performed at Imperial College London UK in accordance with the United Kingdom Animal Scientific Procedures Act (1986), under Home Office Personal and Project Licences following appropriate ethical review. *Cntnap2*<sup>-/-</sup> homozygous mutant KO<sup>8</sup> transgenic mice (JAX017482, Jackson Labs USA) were maintained on a C57bl6/J background. Offsprings were weaned at P21 and housed in groups of up to five animals per cage after weaning. All animals were maintained in individually ventilated cages, on a 12/12 h light/dark cycle (light on between 07:00-19:00) at 21 ± 2°C and 55 ± 10% humidity. Water and food were provided ad libitum. 8-10 weeks old mice of both genders were used for behavioural experiments. Mice were group-housed where possible, with the same littermate mice both before and after treatments. Mice in the same cage typically included treatments of different conditions and/or included untreated companion mice. All experiments were conducted during the light phase.

### **Drug administration.**

Psilocybin (Usona Institute US) was dissolved in sterile saline solution (0.9% NaCl) to appropriate concentrations. 8-week old offspring were intraperitoneally injected either with saline or psilocybin (1 mg/kg) 24 hours prior to behavioural testing. We chose this dose of psilocybin based on our previous observations<sup>32</sup> as well as observations from several other groups<sup>17,33</sup>. For the duration of the acute drug effect, each mouse was placed in a separate chamber to assess the head-twitch response.

### **Head-twitch response.**

Head-twitch events were evaluated immediately after intraperitoneal administration of either saline or psilocybin for a period of 20 minutes. The mouse was placed into a customized behavioural box immediately after injection and was free to explore. The number of head-twitch events were counted by direct observation in 5-minute bins. The box is of size 40 cm X 20 cm X 20 cm [L x W x H] and the floor was covered with clean bedding. Experimenters were not blind to the treatment for head-twitch counts.

### **DNA methyltransferase I inhibition.**

We used the small-molecule DNMT1 inhibitor RG108 (Tocris, UK) to inhibit DNA methyltransferase I in vivo<sup>21-23</sup>. RG108 was dissolved in 10% DMSO then diluted using saline (final DMSO received by the mice was 0.75%). Two doses of 10 mg/kg RG108 or vehicle was systemically administered via intraperitoneal injections per test animal, first dose at 60 min

prior to psilocybin or saline treatment, second dose together with psilocybin or saline treatment. We selected RG108 due to its low cytotoxicity and its selective binding to DNA methyltransferase I.

## 5HT2A receptor antagonism.

We used the selective 5HT2A receptor antagonist MDL100907 (Tocris, UK) to inhibit 5HT2A receptor activity in vivo<sup>34</sup>. MDL100907 was dissolved in 10% DMSO then diluted using saline. One dose of 1 mg/kg MDL100907 or vehicle was systemically administered via intraperitoneal injections per test animal at 15 min prior to psilocybin or saline treatment.

## Behavioural testing.

Social behaviour was tested in a custom-built Plexiglass three-chamber box with matt white acrylic floor. The total arena (60 x 40 x 22 cm [L x W x H]) was divided in three smaller evenly sized chambers (20 x 40 x 22 cm [L x W x H]) interconnected by 4 x 4 cm doors. Transparent cylinders (10.5 cm internal diameter, 11 cm external diameter, 16 cm length) with 1-cm slits were used as cups. The box was placed in a darkened and quite room, illuminated from above with infrared LEDs located 1 meter over the arena.

Age-matched, sex-matched non-littermate mice with no social behavioural deficits (i.e. no homozygous mutant KO mice) from the same genetic background were used as social partners.

Animals were acclimatised to the testing room for at least one hour before the start of experiment on each day. Behavioural testing took place over 4 consecutive days in the first week. Each test mouse was placed into the centre chamber and allowed to freely explore and habituate to the three-chamber setup for 10 minutes over 3 consecutive days, doors to the side chambers were kept open. Social partner mice were individually pre-habituated to the cups also for 10 minutes over 3 consecutive days. On day 4, each test animal was allowed to freely explore all three empty chambers for 10 minutes, then driven to the central chamber with the doors closed while an age-, size-, and gender-matched unfamiliar non-littermate mouse (S) was placed into a cup in one side chamber, and an empty cup (C) was placed in the opposite side chamber. The side of the social partner chamber was randomised. The test mouse was then allowed to freely explore chambers for 10 minutes. Re-testing sessions at day 7 and day 14 after administration were performed using the day 4 protocol, with new stranger mice that the test mouse never encountered previously. Sex of the test mice were randomised where possible.

All behavioural sessions were video-recorded using a CMOS camera (Basler acA2000-165umNIR) and Basler software (Basler AG, Germany). Video recordings were used to track the position of the body of the animal in each chamber, as well as to manually time the duration of the test mouse's social exploratory behaviour by a blinded well-trained observer. We observed that the test mice frequently spent time in a side chamber while avoiding the interaction object, therefore we manually quantified the actual interaction duration of the test mouse with the cup quantified as either sniffing or crawling on a cup that is either empty or containing another mouse. We then derived a normalised sociability index using the equation:

$$\text{Sociability index} = \frac{\text{Interaction duration with S} - \text{Interaction duration with C}}{\text{Interaction duration with S} + \text{Interaction duration with C}}$$



## Position tracking and analysis.

We used DeepLabCut<sup>35,36</sup> to track the positions of the randomly selected test mouse during the habituation phase. Each mouse was labelled by a trained network to identify at least 4 body parts including the snout, two ears, tail base. Body positions were then calculated from the label coordinates and analysed using custom codes in MATLAB. Movement speed is calculated as the difference between the body positions between two consecutive image frames.

Chamber occupancy during the habituation phase of the test were calculated. No consistent side preference were detected either for an experimental group or for the same mouse on different test sessions.

## Grooming analysis.

Episodes of grooming behaviour were manually scored by an experimenter who was blind to the genotyping and treatment conditions.

## Statistical analysis.

Head twitch response data were analysed using non-parametric Kruskal-Wallis test with alpha-adjusted Dunn's multiple post-hoc comparisons, or Mann Whitney test as a priori specified. Data related to sociability index were analysed via three-factorial ANOVA with repeated measures on one factor and followed up on by Bonferroni-corrected multiple comparisons. Calculations were carried out using GraphPad Prism and SPSS, respectively. Correlations (Spearman's rho) were calculated using MATLAB.

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## Contributions

CS and TK designed the study. CS and TC performed the experiments. CS, TK and TB analysed the data. CS and TK supervised the project and wrote the manuscript with inputs from all authors.

## **Ethics declarations and competing interests**

The authors report no competing interests.

## **Data availability**

Data is available from the corresponding author upon reasonable request.

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# **Lasting effect of psilocybin on sociability can be blocked by DNA methyltransferase inhibition**

## **Overview of Extended Data**

### **Extended Figures**

Extended Figure 1: Parameters related to psilocybin treatment of Cntnap2 mice.

Extended Figure 2: Head-twitch response related to the pretreatment experiment.

### **Extended Tables**

Extended Table 1: Statistics for durability of psilocybin-induced effects on sociability, relates to Fig 1.

Extended Table 2: Statistics for durability of psilocybin-induced effects on sociability by test session, relates to Fig 1.

Extended Table 3: Statistics for repetitive behaviour and locomotion, relates to Fig 1.

Extended Table 4: Statistics for head-twitch response, and HTR-SI correlations.

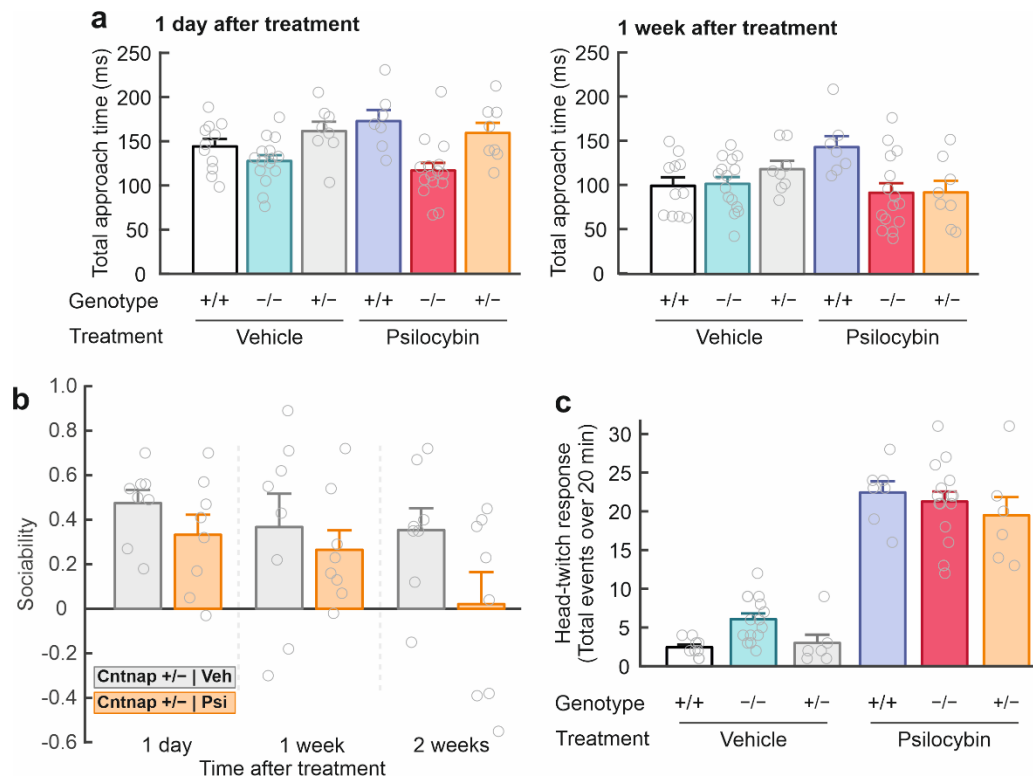
Extended Table 5: Statistics for either DNMTi or 5HT2AR antagonist pretreatment on psilocybin's sociability effect, relates to Fig 2.

Extended Table 6: Statistics for either DNMTi or 5HT2AR antagonist pretreatment on psilocybin's sociability effect by test session, relates to Fig 2.

Extended Table 7: Statistics for the effects of DNMTi on the behaviour of isogenic wild-type mice.

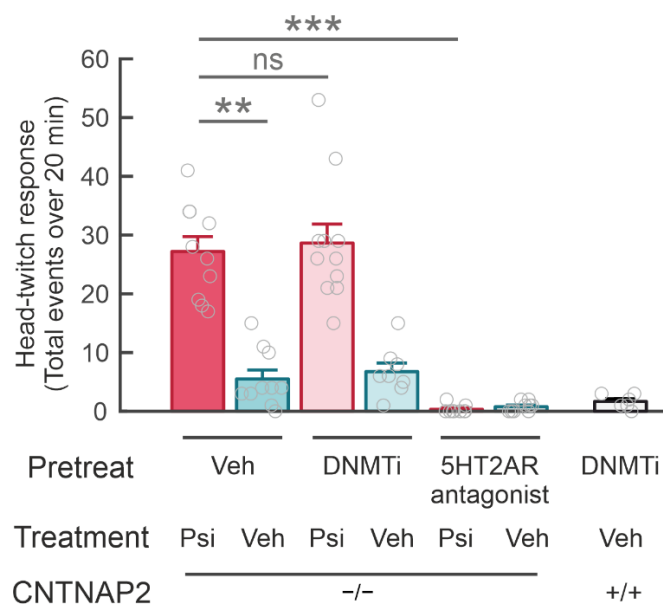
Extended Table 8: Statistics for either DNMTi or 5HT2AR antagonist pretreatment on psilocybin's effect on HTR.





### Extended Data Figure 1.

**a.** Total approach time for at 1 day (left) and 1 week (right) after treatment. **b.** Sociability at 1 day, 1 week and 2 weeks post-treatment (1 mg/kg psilocybin or vehicle control) for Cntnap2<sup>+/-</sup> heterozygote mice. **c.** Head-twitch response for all three Cntnap2 genotype groups. Mean  $\pm$  SEM.



### Extended Data Figure 2.

Head-twitch response for pretreatment experiments. Mean  $\pm$  SEM.

**Extended Data Table 1.**

**Statistics for durability of psilocybin-induced effects on sociability, relates to Fig 1.**

		Comparison group	Test used	Main effect	P value	Degrees of freedom
<b>Extended Data Table 1a. Effect of psilocybin treatment on sociability in Cntnap2(-/-) compared to Cntnap2(+/+) mice</b>						
Fig. 1a	Sociability index		Repeated measure ANOVA	Group	<0.001	3, 44
				Time	<0.001	2, 88
		Cntnap2(+/+) & Vehicle vs Cntnap2(-/-) & Vehicle	Bonferroni's post-hoc		0.042	
		Cntnap2(-/-) & Vehicle vs Cntnap2(-/-) & Psilocybin	Bonferroni's post-hoc		<0.001	
		Cntnap2(+/+) & Vehicle vs Cntnap2(-/-) & Psilocybin	Bonferroni's post-hoc		0.796	
		Cntnap2(+/+) & Vehicle vs Cntnap2(+/+) & Psilocybin	Bonferroni's post-hoc		0.106	

<b>Extended Data Table 1b. Effect of psilocybin treatment on sociability in Cntnap2(+/-) compared to Cntnap2(+/+) mice</b>						
Extended Data Fig. 1c	Sociability index		Repeated measure ANOVA	Group	0.049	3, 30
				Time	0.023	2, 60
		Cntnap2(+/+) & Vehicle vs Cntnap2(+/-) & Vehicle	Bonferroni's post-hoc		1.000	
		Cntnap2(+/-) & Vehicle vs Cntnap2(+/-) & Psilocybin	Bonferroni's post-hoc		0.368	
		Cntnap2(+/+) & Psilocybin vs Cntnap2(+/-) & Psilocybin	Bonferroni's post-hoc		1.000	

## Extended Data Table 2.

Statistics for durability of psilocybin-induced effects on sociability by test session, relates to Fig 1.

	Comparison group	N	Descriptive stats				Test used	P value
			Group	Mean	Median	SEM		
Extended Data Table 2a. Sociability first tested at 2 weeks after treatment								
Sociability index	Vehicle vs Psilocybin	15	Cntnap2(-/-) & Vehicle	0.129	0.101	0.050	Independent T test	0.025
		15	Cntnap2(-/-) & Psilocybin	0.328	0.260	0.079		

<b>Extended Data Table 2b. Sociability by test session</b>								
Sociability index	<b>1 day:</b> Cntnap2(-/-) & Vehicle vs Cntnap2(-/-) & Psilocybin	19	Cntnap2(-/-) & Vehicle	0.374	0.380	0.058	Independent T test	0.001
		15	Cntnap2(-/-) & Psilocybin	0.598	0.650	0.033		
	<b>7 days:</b> Cntnap2(-/-) & Vehicle vs Cntnap2(-/-) & Psilocybin	19	Cntnap2(-/-) & Vehicle	0.105	0.200	0.062	Independent T test	<0.001
		15	Cntnap2(-/-) & Psilocybin	0.498	0.500	0.053		
	<b>14 day:</b> Cntnap2(-/-) & Vehicle vs Cntnap2(-/-) & Psilocybin	15	Cntnap2(-/-) & Vehicle	0.028	0.100	0.104	Independent T test	0.028
		15	Cntnap2(-/-) & Psilocybin	0.291	0.380	0.080		
	<b>1 day:</b> Cntnap2(+/+) & Vehicle vs Cntnap2(+/+) & Psilocybin	15	Cntnap2(+/+) & Vehicle	0.497	0.510	0.041	Independent T test	0.001
		7	Cntnap2(+/+) & Psilocybin	0.151	0.130	0.076		
	<b>7 days:</b> Cntnap2(+/+) & Vehicle vs Cntnap2(+/+) & Psilocybin	15	Cntnap2(+/+) & Vehicle	0.331	0.260	0.080	Independent T test	0.107
		7	Cntnap2(+/+) & Psilocybin	0.129	0.020	0.131		
	<b>14 days:</b> Cntnap2(+/+) & Vehicle vs Cntnap2(+/+) & Psilocybin	10	Cntnap2(+/+) & Vehicle	0.256	0.340	0.093	Independent T test	0.207
		7	Cntnap2(+/+) & Psilocybin	0.149	0.140	0.088		

### Extended Data Table 3.

Statistics for repetitive behaviour and locomotion, relates to Fig 1.

		Test session	Comparison group		Test used	N	Descriptive stats			P value
			Genotype	Treatment			Mean	Median	SEM	
Extended Data Table 3a. Repetitive behaviour										
Fig. 1c	Self-grooming episodes	Before treatment	Cntnap2(+/+)	-	Mann Whitney	14	11.214	10.500	1.668	0.002
			Cntnap2(-/-)			11	25.909	28.000	3.843	
Fig. 1d	Self-grooming episodes	1 day after treatment	Cntnap2(-/-)	Vehicle	Mann Whitney	5	30.200	26.000	2.577	0.329
			Cntnap2(-/-)	Psilocybin		7	33.857	31.000	3.284	
		7 days after treatment	Cntnap2(-/-)	Vehicle	Mann Whitney	6	30.833	26.000	4.246	0.461
			Cntnap2(-/-)	Psilocybin		7	29.714	27.000	5.272	
Extended Data Table 3b. Locomotion										
Fig. 1d	Distance travelled	Before treatment	Cntnap2(+/+)	-	Kruskal-Wallis with Dunn's post-hoc	15	29200	28853	1737	0.001
			Cntnap2(-/-)			22	38099	38158	1294	
		Before treatment	Cntnap2(+/+)			15	29200	28853	1737	>0.999
			Cntnap2(+/-)			10	32375	33378	2265	
Fig. 1e	Distance travelled	1 day after treatment	Cntnap2(-/-)	Vehicle	Mann Whitney	11	38583	39653	1095	0.518
			Cntnap2(-/-)	Psilocybin		12	36990	37014	1620	
		7 days after treatment	Cntnap2(-/-)	Vehicle	Mann Whitney	11	39930	41439	1526	0.976
			Cntnap2(-/-)	Psilocybin		12	40714	38568	2069	

#### Extended Data Table 4.

#### Statistics for head-twitch response, and HTR-SI correlations.

		Comparison group		Test used	N	Descriptive stats			P value / Adjusted P value
		Genotype	Treatment			Mean	Median	SEM	
Extended Data Table 4a. Head twitch response									
Extended Data Fig. 1d	HTR			Kruskal-Wallis					<0.001
		Cntnap2(+/+)	Vehicle	Dunn's multiple comparison	9	2.444	2.000	0.341	0.825
		Cntnap2(-/-)	Vehicle		15	6.067	6.000	0.740	
		Cntnap2(+/+)	Vehicle	Dunn's multiple comparison	9	2.444	2.000	0.341	>0.999
		Cntnap2(+/-)	Vehicle		6	3.000	2.000	1.072	
		Cntnap2(+/+)	Vehicle	Dunn's multiple comparison	9	2.444	2.000	0.341	<0.001
		Cntnap2(+/+)	Psilocybin		7	22.429	23.000	1.462	
		Cntnap2(-/-)	Vehicle	Dunn's multiple comparison	15	6.067	6.000	0.740	0.001
		Cntnap2(-/-)	Psilocybin		15	21.267	22.000	1.300	
		Cntnap2(+/-)	Vehicle	Dunn's multiple comparison	6	3.000	2.000	1.072	0.006
		Cntnap2(+/-)	Psilocybin		6	19.500	18.500	2.332	
		Cntnap2(+/+)	Psilocybin	Dunn's multiple comparison	7	22.429	23.000	1.462	>0.999
		Cntnap2(-/-)	Psilocybin		15	21.267	22.000	1.300	
		Cntnap2(+/+)	Psilocybin	Dunn's multiple comparison	7	22.429	23.000	1.462	>0.999
		Cntnap2(+/-)	Psilocybin		6	19.500	18.500	2.332	

Extended Data Table 4b. HTR by sex									
	Male	Cntnap2(-/-)	Psilocybin	Mann Whitney	6	19.167	21.500	2.151	0.452
	Female	Cntnap2(-/-)	Psilocybin		9	22.667	22.000	1.546	

Extended Data Table 4c. HTR-SI correlations							
	1 day after treatment	Cntnap2(+/+)	Psilocybin	Spearman's rho		rho = -0.655	0.110
		Cntnap2(-/-)	Psilocybin			rho = -0.397	0.143
		Cntnap2(+/-)	Psilocybin			rho = 0.714	0.111
	1 week after treatment	Cntnap2(+/+)	Psilocybin	Spearman's rho		rho = -0.699	0.081
		Cntnap2(-/-)	Psilocybin			rho = 0.085	0.763
		Cntnap2(+/-)	Psilocybin			rho = 0.083	0.880
	2 weeks after treatment	Cntnap2(+/+)	Psilocybin	Spearman's rho		rho = -0.538	0.213
		Cntnap2(-/-)	Psilocybin			rho = 0.084	0.766
		Cntnap2(+/-)	Psilocybin			rho = 0.421	0.406



**Extended Data Table 5.**

**Statistics for pretreatment on psilocybin's effect on sociability, relates to Fig 2.**

		Comparison group		Comparison group	Test used	Main effect	P value	Degrees of freedom
		Genotype	Pretreatment					
Extended Data Table 5. Effect of pretreatment on psilocybin's effect on sociability in Cntnap2(-/-) mice								
Fig. 2	Sociability index				Repeated measure ANOVA	Group	<0.001	6, 57
						Time	0.001	1, 57
		Cntnap2(-/-)	Vehicle	Vehicle	Bonferroni's post-hoc		<0.001	
		Cntnap2(-/-)	Vehicle	Psilocybin				
		Cntnap2(-/-)	Vehicle	Psilocybin	Bonferroni's post-hoc		0.003	
		Cntnap2(-/-)	DNMTi	Psilocybin				
		Cntnap2(-/-)	Vehicle	Psilocybin	Bonferroni's post-hoc		0.001	
		Cntnap2(-/-)	5HT2AR-ant	Psilocybin				
		Cntnap2(-/-)	DNMTi	Psilocybin	Bonferroni's post-hoc		1.000	
		Cntnap2(-/-)	DNMTi	Vehicle				
		Cntnap2(-/-)	Vehicle	Vehicle	Bonferroni's post-hoc		1.000	
		Cntnap2(-/-)	DNMTi	Vehicle				
		Cntnap2(-/-)	Vehicle	Vehicle	Bonferroni's post-hoc		1.000	
		Cntnap2(-/-)	5HT2AR-ant	Vehicle				
		Cntnap2(-/-)	DNMTi	Psilocybin	Bonferroni's post-hoc		1.000	
		Cntnap2(-/-)	5HT2AR-ant	Psilocybin				

# Extended Data Table 6.

Statistics for pretreatment on psilocybin's sociability effect by test session, relates to Fig 2.

			Comparison group			N	Descriptive stats			Test used	P value
			Genotype	Pretreatment	Treatment		Mean	Median	SEM		
Extended Data Table 6. Sociability by test session											
Fig. 2	Sociability index	1 day post-treatment	Cntnap2(-/-)	Vehicle	Vehicle	10	0.207	0.220	0.038	Independent T test	<0.001
				Vehicle	Psilocybin	10	0.514	0.530	0.060		
				Vehicle	Psilocybin	10	0.514	0.530	0.060	Independent T test	0.005
				DNMTi	Psilocybin	11	0.258	0.290	0.065		
				Vehicle	Psilocybin	10	0.514	0.530	0.060	Independent T test	<0.001
				5HT2AR-ant	Psilocybin	9	0.177	0.210	0.059		
				DNMTi	Psilocybin	11	0.258	0.290	0.065	Independent T test	0.450
				DNMTi	Vehicle	8	0.318	0.330	0.041		
				Vehicle	Vehicle	10	0.207	0.220	0.038	Independent T test	0.067
				DNMTi	Vehicle	8	0.318	0.330	0.041		
				Vehicle	Vehicle	10	0.207	0.220	0.038	Independent T test	0.432
				5HT2AR-ant	Vehicle	9	0.251	0.230	0.039		
				DNMTi	Psilocybin	11	0.258	0.290	0.065	Independent T test	0.362
				5HT2AR-ant	Psilocybin	9	0.177	0.210	0.059		
		7 days post-treatment	Cntnap2(-/-)	Vehicle	Vehicle	10	0.077	0.110	0.066	Independent T test	0.001
				Vehicle	Psilocybin	10	0.475	0.525	0.090		
				Vehicle	Psilocybin	10	0.475	0.525	0.090	Independent T test	0.008
				DNMTi	Psilocybin	11	0.155	0.140	0.080		
				Vehicle	Psilocybin	10	0.475	0.525	0.090	Independent T test	0.007
				5HT2AR-ant	Psilocybin	9	0.166	0.190	0.069		
				DNMTi	Psilocybin	11	0.155	0.140	0.080	Independent T test	0.926
				DNMTi	Vehicle	8	0.144	0.155	0.082		
				Vehicle	Vehicle	10	0.077	0.110	0.066	Independent T test	0.537
				DNMTi	Vehicle	8	0.144	0.155	0.082		
				Vehicle	Vehicle	10	0.077	0.110	0.066	Independent T test	0.435
				5HT2AR-ant	Vehicle	9	0.143	0.100	0.050		
				DNMTi	Psilocybin	11	0.155	0.140	0.080	Independent T test	0.918
				5HT2AR-ant	Psilocybin	9	0.166	0.190	0.069		

## Extended Data Table 7.

### Statistics for the effects of DNMTi on behaviour of Cntnap2(+/+) mice.

	Comparison group			Test used	Main effect	P value	Degrees of freedom
	Genotype	Pretreatment	Treatment				
Extended Data Table 7a. Effect of DNMTi pretreatment on sociability of Cntnap2(+/+) mice							
Sociability index	Cntnap2(+/+)	Vehicle	Vehicle	Repeated measure ANOVA	Group	0.526	1, 12
		DNMTi	Vehicle		Time	0.230	1, 12

	Test session	Comparison group			Test used	N	Descriptive stats			P value
		Genotype	Pretreatment	Treatment			Mean	Median	SEM	
Extended Data Table 7b. Effect of DNMTi pretreatment on other behaviour of Cntnap2(+/+) mice										
Self-grooming episodes	1 day after treatment	Cntnap2(+/+)	Vehicle	Vehicle	Mann Whitney	7	9.571	7.000	2.608	0.222
			DNMTi	Vehicle		7	11.857	11.000	1.844	
Locomotion			Vehicle	Vehicle	Mann Whitney	7	29791	27924	1521	0.128
						DNMTi	Vehicle	7	25134	

# Extended Data Table 8.

Statistics for either DNMTi or 5HT2AR antagonist pretreatment on psilocybin's effect on HTR.

		Comparison group			Test used	N	Descriptive stats			Adjusted P value
		Genotype	Pretreatment	Treatment			Mean	Median	SEM	
Extended Data Table 8. Head twitch response										
Extended Data Fig. 2	HTR				Kruskal-Wallis					<0.001
		Cntnap2(-/-)	Vehicle	Vehicle	Dunn's multiple comparison	10	5.500	4.000	1.529	0.0208
		Cntnap2(-/-)	Vehicle	Psilocybin		10	27.200	27.000	2.542	
		Cntnap2(-/-)	Vehicle	Psilocybin	Dunn's multiple comparison	10	27.200	27.000	2.542	<0.001
		Cntnap2(-/-)	5HT2AR-ant	Psilocybin		9	0.333	0.000	0.236	
		Cntnap2(-/-)	Vehicle	Psilocybin	Dunn's multiple comparison	10	27.200	27.000	2.542	>0.999
		Cntnap2(-/-)	DNMTi	Psilocybin		11	28.636	26.000	3.231	
		Cntnap2(-/-)	Vehicle	Vehicle	Dunn's multiple comparison	10	5.500	4.000	1.529	0.335
		Cntnap2(-/-)	5HT2AR-ant	Vehicle		9	0.778	1.000	0.278	
		Cntnap2(-/-)	Vehicle	Vehicle	Dunn's multiple comparison	10	5.500	4.000	1.529	>0.999
		Cntnap2(-/-)	DNMTi	Vehicle		8	6.750	6.000	1.461	