1. Effects of cannabidiol (CBD) content in vaporized cannabis on tetrahydrocannabinol (THC)-induced impairment of driving and cognition

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BACKGROUND: As legal restrictions around recreational and medicinal cannabis are relaxed, the risks associated with driving under the influence of cannabis are of increasing community concern. Some evidence suggests that cannabidiol (CBD) may mitigate some of the adverse effects of delta-9-tetrahydrocannabinol (THC), implying that CBD enriched cannabis may differentially affect driving and cognition relative to pure THC (e.g. dronabinol), or prototypical THC-dominant chemovars.

OBJECTIVES: To test the effects of two cannabis cultivars (11% THC; <1% CBD; 11% THC; 11% CBD) and placebo (<1% THC/CBD) on simulated driving performance and cognition.

METHODS: Fourteen participants completed the study (11 males, 3 females). Participants were administered two active cannabis strains and placebo via vaporisation in a randomised and counter-balanced order over 3 sessions. Driving performance and cognition were measured at acute and post-acute intoxication phases (+0.5h, +3.5h). Plasma and saliva samples were taken before and after drug administration (-0.5h, +0.2h, +1h, +2h, +3h, +4h). Subjective drug effects and self-reported driving ability were also assessed.

RESULTS: Both active cannabis chemovars impaired driving during a car-following task and reduced performance on a Digit Symbol Substitution Task (DSST), Divided Attention Task (DAT) and Paced Auditory Serial Addition Task (PASAT). Impairment on the latter two tasks appeared to be worsened by CBD. Subjective drug effects (e.g. 'stoned') and confidence in driving ability did not vary with CBD content. There was also a significant increase in peak plasma THC concentrations following vaporization of THC/CBD equivalent cannabis relative to THC-dominant cannabis.

DISCUSSION: THC-induced driving impairment is pronounced under challenging conditions but may not be evident during monotonous and simple driving. Cannabis containing equivalent concentrations of CBD and THC is no less impairing than THC-dominant cannabis, and in some circumstances, CBD may in fact exacerbate THC-induced impairment.

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2. Raman spectroscopy: evaluation of a non-invasive technique for the detection of topically applied ketorolac tromethamine *in vitro* and *in vivo*

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Ocular pharmacokinetic studies investigate time- and dose dependent behavior of ophthalmic drugs. These studies are important to detect the maximum drug concentration (C_{max}), the time to reach C_{max} (T_{max}), half-life, and clearance of the drug. Based on those parameters, a dosage regimen can be created. Currently, the assessment of ocular pharmacokinetics is using tissues or fluids in a destructive test which comprises chemical pre-treatment followed by high-performance liquid chromatography (HPLC). A non-invasive pharmacokinetic assessment technique could resolve these issues. A technique that is potentially suitable for non-invasive detection of ocular pharmacokinetics is Raman spectroscopy. Raman spectroscopy identifies molecules, based on the specific inelastic scattering properties of their rotational and vibrational modes. This technique enables real-time detection of molecules without pre-processing and damaging tissue.

In this project, we evaluated the detection and quantification of ocular ketorolac tromethamine levels with confocal Raman spectroscopy after topical administration of Acular™. Confocal Raman spectroscopy and HPLC were compared in terms of sensitivity of detection. Enucleated pig eyes were treated with different concentrations of ketorolac. Hereafter, ketorolac concentrations in the aqueous humor of pig eyes were analyzed by confocal Raman spectroscopy and HPLC. Subsequently, twelve rabbits were treated with Acular™ for four weeks. At several time points, ketorolac concentrations in aqueous humor of the rabbits were measured by confocal Raman spectroscopy followed by drawing an aqueous humor sample for HPLC analysis.

In ketorolac treated pig eyes, both *ex vivo* Raman spectroscopy as well as HPLC were able to detect ketorolac in a broad concentration range. However, *in vivo* confocal Raman spectroscopy in rabbits was unable to detect ketorolac in contrast to HPLC.

To conclude, confocal Raman spectroscopy has the capacity to detect ketorolac tromethamine *in vitro*, but currently lacks sensitivity for *in vivo* detection.

3. Loss of Piccolo function in rats induces Pontocerebellar Hypoplasia type 3-like phenotypes.

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The presynaptic active zone protein Piccolo (Pclo) is best known for its role in the formation of active zones and regulation of neurotransmitter release. Through manipulation of the *PCLO* gene, we have observed a specific effect of Piccolo loss-of-function (LOF) on the unique cerebellar mossy fibre (MF) synapse. We observe a profound impact on the anatomical, functional and behavioural level.

Analysis of Piccolo knockout ($Pclo_{gt/gt}$) brains revealed a severe reduction in brain size in comparison to wildtype ($Pclo_{wt/wt}$) counterparts, with reduced size of cerebellar, pontine and cortical regions. Formation of MF afferents to the cerebellum appear to be disrupted, as $Pclo_{gt/gt}$ MF terminals are reduced to half of size of $Pclo_{wt/wt}$. Climbing fibre innervation of the molecular layer of $Pclo_{gt/gt}$ cerebella is increased, indicating peturbation of the cerebellar network. We also observe a reduction in the α6 subunit of the $GABA_A$ receptor, expressed at the MF, which could be a homeostatic downregulation to compensate for reduced glutamatergic input from MF boutons.

On a functional level, $Pclo_{gt/gt}$ rats display impaired motor coordination, evidenced by failure in a rotarod task, despite adequate performance in tasks that reflect muscle strength and locomotion. We are currently undertaking electrophysiological recordings of MF boutons to further investigate the consequence of Piccolo LOF on on these unique synapse structures.

A mutation in the *PCLO* gene has been observed in patients with Pontocerebellar hypoplasia III (PCH III), a rare developmental disorder characterised by an abnormally small cerebellum and pons, severe developmental delay, motor deficits and seizures. As the human condition shares a number of anatomical and behavioural abnormalities with the $Pclo_{gt/gt}$ rats, we propose that the $Pclo_{gt/gt}$ mutation can be used as a model for PCH III, providing insights into how this AZ protein contributes to the formation and function of neural circuits during development.

4. "On cloud nine" or "in a funk"? conflicting effects of chronic adolescent exposure to HU-210 on tests of depressive-like behavior.

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Adolescent and adult rodents are known to have differential susceptibilities to the effects of cannabinoid receptor agonists. Indeed, chronic cannabinoid exposure has been shown to induce opposite long-lasting effects, as a function of rodent age, whereby it has prodepressant- and antidepressant-like effects in adolescent and adult animals, respectively. Interestingly, while chronic cannabinoid exposure also seems to lead to short-term antidepressant-like effects in adults, there is no equivalent data regarding adolescent animals.

Here, we report two separate experiments, designed to assess the short-term affective behavioral effects of chronic adolescent HU-210 exposure. For this, two separate series of adolescent female Sprague-Dawley rats were administered twice-daily intraperitoneal injections of HU-210, following an ascending dosing schedule (PND35-37: 25µg/kg; PND38-41: 50µg/kg; PND42-45: 100µg/kg), for 11 days. Starting 24-hours following the last injection animals were tested in Open Field Test (OFT) and mFST, or the Elevated Plus Maze (EPM) and the SPT. Furthermore, samples from the hippocampus and the PFC were collected for molecular analyses.

In line with previous adult studies, HU-210-treated animals showed a marked antidepressant-like profile in the mFST – with significantly decreased immobility, and increased climbing – without alterations of locomotor activity or anxiety-like behavior in the OFT. Contrastingly, in the SPT, HU-210-treated animals showed strong decreases in sucrose preference/intake, suggesting a marked prodepressant-like impact of treatment.

In addition to having important implications for the cannabinoid literature, our results also highlight the necessity of critically evaluating the results of behavioral tests in light of their known limitations, and of using multiple tests to perform more reliable assessments. Finally, our results also raise the need for a mechanistic explanation of how a single manipulation led to the markedly contrasting effects observed, regarding depressive-like behavior.

5. **Interoceptive associations in early onset consumption of smoked cocaine**

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Objectives: Neurocognitive plasticity is critical for maturation throughout adolescence. However, these adaptive processes also increase vulnerability for developing addictions. The connection between plasticity and vulnerability for addictions is not fully known. Smoked cocaine (SC) is the earliest intermediate product of cocaine hydrochloride (CC) production and represents a public health problem for teenagers in developing countries. SC is highly addictive mainly due to its fast administration route, which has been linked an increased ability to sense and process body signals (interoception). However, there is scant evidence about changes during adolescence and no report has assessed interoception in SC consumers. In this study, we implement a multimodal approach (behavioral, EEG, and neuroimaging) to study differences in interoceptive performance between adolescent consumers of SC, CC and controls (CTR).

Methods: We included 25 participants that smoked (SC), 22 that insufflated cocaine (CC), and 25 matched CTR. Cocaine consumption begun between ages 14-16. We applied a heartbeat-detection (HBD) task and measured modulations of the heart-evoked potential (HEP) during interoceptive conditions. We complemented these measures with structural (MRI) and functional connectivity (fMRI) analysis of the main interoceptive hubs (insular, ACC and somatosensory cortex).

Results: HBD and HEP results showed that only SC consumers presented ongoing psychophysiological measures of enhanced interoceptive accuracy. This pattern was associated with a structural and functional tuning of interoceptive networks.

Conclusions: Our findings provide the first evidence of an association between cardiac interoception and SC consumption in adolescents. They also support models that propose hyper-interoception as a key aspect of addiction while suggesting that this enhancement may depend on specific administration routes.

6. Self-Rated Effectiveness of Microdosing With Psychedelics for Mental and Physical Health Problems Among Microdosers

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There is a growing interest in the use of low (micro) doses of psychedelic substances for health related purposes, including symptom relief for disorders like anxiety, depression, and pain. Although the focus of recent clinical trials has been on high doses of psychedelics, empirical evidence regarding the efficacy of microdosing for symptomatic relief is lacking. The present study aimed to investigate, by means of an online questionnaire, the self-rated effectiveness (SRE) of microdosing with psychedelics (MDP) for mental and physiological disorders compared to the conventional prescribed treatment and to regular doses of psychedelics.

An online questionnaire was launched on several websites and fora between March and July 2018. Respondents who had consented, were 18 years of age or older, had experience with microdosing and were diagnosed with at least one disorder ($N = 410$; 7.2%) were included in the analyses. Odds ratio were calculated to compare the SRE of MDP with conventional treatment, and regular psychedelic doses for mental and physiological diagnoses for each of the three effectiveness questions ("Did it work," "Symptom disappear," "Quality of life improved").

Odds ratio showed that SRE of MDP was significantly higher compared to that of conventional treatments for both mental and physiological diagnoses; and that these effects were specific for ADHD/ADD and anxiety disorders. In contrast, SRE of MDP was lower compared to that of higher, regular psychedelic doses for mental disorders such as anxiety and depression, while for physiological disorders no difference was shown.

This study demonstrates that SRE of MDP to alleviate symptoms of a range of mental or physiological diagnoses is higher compared to conventionally offered treatment options, and lower than regular psychedelic doses. Future RCTs in patient populations should objectively assess the effectivity claims of psychedelics, and whether these are dose related, disorder specific, and superior to conventional treatments.

7. *In-vitro* validation of systems biology derived drug-discovery to regulate molecular signature after traumatic brain injury

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Traumatic brain injury (TBI) is a major cause of death and disability worldwide. Nearly 20 % of TBI patients die, while approximately 30% of survivors experience several health conditions such as amnesia and epileptogenesis. Existing treatments cannot prevent the progression of post-TBI sequela, hence, there is an unmet need of drugs that can halt secondary effects of TBI.

Our aim was to validate disease-modifying effects of *in-silico* discovered compounds with a predicted effect on gene networks regulated by TBI. We hypothesized that through systems-biology analysis, we would identify compounds which could address post-TBI sequela via their neuroprotective/anti-inflammatory/antioxidant properties.

Compounds were identified using our recently developed systems-biology pipeline which used TBI induced gene-expression signatures to perform LINCS-analysis. We selected compounds that have a potential in regulating post-TBI transcriptomics changes. *In-vitro* validation of chosen compounds was carried out in a neuron-BV2 microglia co-culture model, where acute neuroinflammation was induced. We assessed each compound's ability to promote neuronal viability, and to regulate tumor necrosis factor-alpha (TNF-α) and nitric oxide (NO) which are indicators of neuroinflammation and neurotoxicity.

From 20 potential candidate compounds we validated 5 *in-vitro.* At 50 μM compound A and B decreased percentage of NO ($p<0.001$) and TNF-α ($p<0.001$) and improved neuronal viability ($p<0.001$) while compound-C was efficient at 50nM ($p<0.001$ for all experiments). At 10nM, compound-D reduced nitric oxide ($p<0.01$) and improved the neuronal viability ($p<0.01$) while compound-E showed no therapeutic effect in the co-cultures.

Our *in-silico* approach identified compounds with a potential in promoting neuronal survival and reducing neuroinflammation *in-vitro*. The most promising compounds will be validated *in-vivo*, in a clinically relevant rat model of TBI, to evaluate their capacity in regulating the modifications occurring after TBI.

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8. Psychophysiogical interaction analysis: exploring ventral striatum functional connectivity during cognitive control following drug cue exposure

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Drug cues play a central role in maintaining addiction, by eliciting craving and triggering further drug seeking and taking. We investigated the effect of drug cue exposure on the neural mechanisms of cognitive control, using a drug word Stroop task. We hypothesised that drug cue exposure would enhance appetitive processing and weaken cognitive control. 20 newly abstinent detoxified opioid dependent individuals (ODI) carried out a heroin drug word Stroop on two separate occasions whilst undergoing fMRI scanning. On one occasion, participants viewed a drug cue video[1] immediately before the Stroop task and on another occasion, they viewed a neutral cue video before the task. Following the neutral cue video, the drug word Stroop recruited regions associated with appetitive processing, including the ventral striatum (VS). Against our prediction, this activation was not enhanced by the drug cue video, but instead the VS failed to be recruited by the drug word Stroop during the drug cue session, with the difference in VS activation between sessions reaching significance (SVC $p_{FWE}<0.05$).

An explanation for this is that the ODIs are maximally motivated to maintain abstinence due to recently completing rehabilitation. Rather than triggering drug seeking and craving, the drug cue video may therefore prime inhibition of appetitive processing in the VS. To confirm this hypothesis, we conducted psychophysiological interaction (PPI) analysis investigating functional connectivity between the VS and cognitive control regions involved in the inhibition of craving. A region of interest approach was taken using DLPFC coordinates from previous craving inhibition studies[2, 3]. A cluster within this region ($k_E = 6$, SVC $p_{FWE(cluster)} = 0.044$) had increased connectivity to the VS following the drug cue compared to the neutral cue video. Our results indicate top down control over appetitive processing by the DLPFC and evidence a prefronto-stratial pathway involved in the cognitive regulation of craving[4].

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9. The desipramine-evoked CaMKII phosphorylation depends on an intact alpha1A-adrenergic receptor subtype: study in mice

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Background: Various mental disorders, including depression, are associated with abnormalities in adrenergic signaling in the brain and many antidepressant drugs act on noradrenergic system. Among the adrenergic receptors the α1 family (α1-AR) consists of the α1A, α1B and α1D subtypes. All they are Gq/11 coupled receptors, their stimulation lead to the increase of intracellular Ca2+ level and may activate the calcium/calmodulin-dependent protein kinase II (CaMKII). However, the α1-ARs subtypes differ in transduction of intracellular signaling events. Many reports suggest also their different involvement in modulation of antidepressant-like behaviors and animals' depression.
Objectives: The aim of the current study was to evaluate the effects of selective knock-out of α1A- or α1B-AR and chronic antidepressant treatment on phosphorylation at Thr286 of CaMKIIα/ß in prefrontal cortex of female mice.
Methods: Female knock-out mice devoid of α1A-AR (α1A-KO) or α1B-AR (α1B-KO) and wild type controls (WT) were chronically treated (21 days) with desipramine (20mg/kg) or saline. The protein level and phosphorylation ratio of CaMKII isoforms were analyzed in the prefrontal cortex by Western blotting.
Results: We found that both the deletion of α1A-AR and the deletion of α1B-AR did not affects the level of CaMKIIα/ß phosphorylation. However after chronic treatment with desipramine, the deletion of α1A-AR prevents the increase in phosphorylation level of CaMKIIα/ß which was observed in WT mice. This effect was not visible in the case of the α1B-AR deletion. There was no statistically significant influence of α1A-KO or α1B-KO or chronic treatment with desipramine on the total level of CaMKII protein.
Conclusions: Our results indicate different involvement of the α1A-AR and α1B-AR subtypes in the mechanism of action of the classical antidepressant drug desipramine. The α1A-AR, in contrast to the α1B-AR, appears to be necessary to obtain the proper effects of chronic despiramine treatment in prefrontal cortex of female mice.

10. Effect of acute physical exercise on associative memory and motor sequence learning via endocannabinoid signaling

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Recent studies suggest that acute physical exercise improves memory functions by increasing plasticity in the hippocampus. In animals, a single session of physical exercise has been shown to boost endocannabinoids (such as anandamide (AEA)) which are involved in hippocampal synaptic plasticity.

Here, we combined circulating AEA levels, behavioral measures and functional MRI to assess the impact of acute physical exercise (of moderate and high intensity) on associative memory and motor sequence learning in humans. We tested eighteen young, fit males in a within-subjects design across three visits (a rest visit, a moderate intensity exercise and a high intensity exercise visit). We used an associative memory task where subjects had to learn sequences of images and a serial reaction time test (SRTT) where participants had to perform finger movements which followed a hidden sequence. Both tasks were composed of 2 parts (an encoding and a test part), separated by the physical exercise or rest session. We took blood samples at each visit right before and right after the physical exercise or rest session and report differences in AEA levels from the first blood sample to the second one.

We report an increase in AEA levels as a result of acute physical exercise, both for moderate and for high intensity exercise. This increase correlates with individual hippocampal activation measures during the associative memory task, meaning that the more participants increased their AEA levels, the more they activated their hippocampus during the memory task. Further this increase also correlated with the performance of the motor sequence learning task, where the larger the AEA increase, the better participants performed. These results highlight the overarching role of the endocannabinoid system in both memory systems, and may support the role of the endocannabinoid system in hippocampal plasticity mechanisms in humans.

11. The effect of adolescent Δ_9-tetrahydrocannabinol and cannabidiol exposure on adult neurogenesis

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INTRODUCTION: Adult neurogenesis is affected by many factors, including cannabinoids, according to recent studies. While adult cannabinoid exposure increases adult neurogenesis in animal studies, adolescent exposure impairs it. This is likely due to the fast developmental changes occurring during adolescence,that affect neuroplasticity, reward neurocircuitry, cognitive function, and emotional behavior- which are disrupted by cannabinoid exposure. As a result, adolescent cannabinoid exposure may lead to neuropsychiatric disorders that are directly linked with impaired adult neurogenesis.

PURPOSE: The purpose of this study is to evaluate the effect of low, escalating doses of Δ_9-tetrahydrocannabinol (THC) on adult neurogenesis. By using a protocol developed in our lab, which attempts to simulate adolescent cannabis use, we aim to study the effect of THC exposure on adult neurogenesis, as well as the potential protective or inhibitory effect of cannabidiol (CBD).

METHODS: In this protocol, we administer low, escalating doses of THC (PND 35-37, 0.3 mg/kg, PND 38-41, 1 mg/kg, PND 41-45, 3mg/kg; i.p, twice per day) +/- CBD (dose, i.p.) to male Sprague-Dawley rats during adolescence (PND 35-45). From PND 45-60, the rats were examined weekly and on PND 60-62, they received a daily i.p. injection of BrdU (dose), in order to label the hippocampal neural stem cells during their differentiation into neuronal cells. Finally, migration, maturation and incorporation of hippocampal neural stem cells will be evaluated in adulthood (PND 85).

RESULTS: The immunohistochemistry analysis is still in process.

12. A novel mouse model of Dravet syndrome: proteomic characterization

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Introduction: Dravet syndrome is known as a rare, severe pediatric form of epilepsy with intellectual and motor disabilities. Proteomic characterization of a novel mouse model of Dravet syndrome can show alterations in protein expression involved in epileptogenesis and indicate potential new targets for treatment of the syndrome.

Methods: A novel, commercially available knock-in mouse model of Dravet syndrome, carrying the mutation in the *Scn1a*-A1783V gene, was used for seizure, behavioral and proteomic profiling. The left hippocampus was dissected from two (prior to spontaneous seizures onset) and four (following the spontaneous seizures onset) week-old male mice and analysed using LC-MS/MS with label-free quantification. Immunohistochemical staining was performed in the right brain hemisphere. ConsensusPathDB pathway tool was used for pathway enrichment analysis.

Results: Dravet mice showed an increased susceptibility for hyperthermia-induced seizures, development of spontaneous seizures, high incidence of SUDEP and hyperactivity, therefore showing an excellent face validity of this model for Dravet syndrome. Proteomic analysis of the hippocampus distinguished around 4000 proteins: 208 significantly changed in two-week-old, 881 significantly changed in four-week-old Dravet mice. Pathway analysis identified 16 and 127 significantly regulated pathways at the early and late time point, respectively. Interestingly, several regulated pathways in four-week-old mice were involved in glutamatergic, calcium and phosphatidylinositol signalling. When compared to a post-SE electrical rat model, only a small group of overlapping proteins was identified in this genetic mouse model. Differential expression of selected proteins was confirmed by immunohistochemical staining.

Conclusion: This study demonstrated marked proteome differences between Dravet and wildtype mice as well as between two- and four-week-old Dravet mice. These differences point out specific alterations in neurotransmission, which may contribute to the course of the disease and may guide future target identification.

13. Exploring modulatoin of neuroinflammation by ketamine in an animal model of depression

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Introduction: About 350 millions of people suffer from major depressive disorder (MDD) worldwide, with more than ⅔ of patients being resistant to treatment. Ketamine, a glutamatergic receptor antagonist, confers a rapid (within hours) antidepressant effect, even in treatment-resistant patients. However, mechanisms by which ketamine exerts its antidepressant effects are not fully understood. Therefore, we investigated whether ketamine's antidepressant effect is associated with modulation of neuroinflammation (microglia and macrophages activation) in the repeated social defeat (RSD) model of depression in rats.

Materials and Methods: All animal experiments were approved by the Central Authority for Scientific Procedures on Animals (CCD) of the Netherlands. Three experimental groups of 12 rats were used: control+vehicle, RSD+vehicle and RSD+ketamine. Experimental rats were submitted to a 5-day RSD protocol followed by one acute injection of ketamine (20 mg/kg). Behavior was assessed using the sucrose preference test (SPT) and open field test (OFT) for depressive-like and anxiety-like behavior. Positron emission tomography (PET)-scans were performed to measure changes in neuroinflammation, using the radiotracer 11C-PK11195 to mark TSPO protein overexpression in activated glial cells. One-way ANOVA, two-way ANOVA and Spearman were used as statistical tests.

Results: After RSD, rats showed a significant decrease in sucrose consumption and weight gain ($p<0.01$). This trend was not affected by ketamine injection.. In addition, PET imaging showed an increase in neuroinflammation in the insula and enthorhinal cortex for RSD+vehivle and RSD+ketamine, and in basal ganglia only for RSD+ketamine ($p<0.05$). There were no significant differences in PET tracer uptake between the RSD+ketamine and the RSD+vehicle group.

Discussion: Our results showed increased anhedonia due to RSD, which was not affected by ketamine injection. Additionally, RSD alone or in combination with ketamine caused neuroinflammation in the insula and basal ganglia, whereas RSD+ketamine, but not RSD alone, also induced neuroinflammation in the basal ganglia.

14. Soluble guanylate cyclase stimulator vericiguat enhances memory processes through GluA1-AMPA receptor trafficking

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Cognitive impairment is one of the main symptoms of Alzheimer's disease, which negatively impacts the quality of life of patients. Therefore, a pharmacological intervention that has memory enhancing effects would be beneficial to patient outcomes. Previous studies have implicated the importance of the intracellular cGMP-PKG signaling pathway in memory processes. This pathway is initiated through the activation of soluble guanylate cyclase (sGC) by nitric oxide (NO). sGC stimulators enhance sGC activity by directly stimulating its production while also increasing sGC sensitivity to endogenous NO. In this experiment we hypothesized that sGC stimulator vericiguat could have beneficial effects on memory functioning through enhanced cGMP-PKG signaling and subsequent increased GluA1-AMPA receptor (AMPAR) trafficking.

To evaluate the effects on long-term memory functioning in rats, different oral dosages of vericiguat were administered 30 minutes before T1 of a 24h inter-trial interval object location task (OLT) to investigate memory acquisition processes. To evaluate the effects on GluA1-AMPAR trafficking, an acute mouse hippocampal slice model was used to chemically induce long-term potentiation (chemLTP). The slices were incubated with vericiguat immediately before chemLTP induction to investigate acquisition-like processes, or 10 minutes after chemLTP induction to investigate early consolidation-like processes. GluA1 subunit dynamics were measured using western blotting.

It was found that 0.3 and 1 mg/kg vericiguat were able to significantly improve long term memory performance in the OLT. Additionally, treatment with 10 nM vericiguat increased chemLTP-induced trafficking to the membrane of a pre-existing pool of GluA1-AMPARs in acquisition-like processes only, which was found to be independent of phosphorylation of the receptor on S845.

These data suggest that vericiguat enhances memory function in rats and that the in vivo memory improvement is acquisition driven.

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15. TrkB receptor agonist as a new therapy for treatment of depression – screening platform

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Brain derived neurotrophic factor also known as BDNF, is a member of the neurotrophin family and acts as a key regulator of many neuronal processes. BDNF is a ligand for TrkB receptor which has tyrosine kinase activity. BDNF has been reported to be involved in pathogenesis of many neuropsychiatric diseases. Targeting the BDNF-TrkB pathway by the small molecular compounds may have antidepressant and procognitive effects. Here we would like to present the screening platform designed for selection of active and selective TrkB receptor agonists.

The primary screening of compound library is performed by using Microscale Thermophoresis (MST) method for identification compounds interacting with the extracellular domain of TrkB receptor. Then, selected molecules are screened *in vitro* using SN56 cell line overexpressing TrkB and the ELFI method (Enzyme-linked fixed cell immunoassay) to determine orthosteric activity and allosteric modulation of the tested compounds. Compounds are further tested in differentiated SH-SY5Y cell line model for analyzing downstream protein activation by immunoblotting. Preliminary specificity of selected compounds is assessed using SN56 TrkA cell line or by use of Trk's inhibitors (K252a). Subsequently, the ability of compounds to provoke TrkB dimerization is monitored by native electrophoresis. The presented approach has allowed to identify so far five orthosteric agonists and one positive allosteric modulator compounds.

16. Quantification of methamphethamine self-administration in *Drosophila's per* and *tim* circadian mutants

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Addiction is a complex neuropsychiatric disorder caused by repeated illicit use of addictive drugs, such as methamphetamine (METH). Psychostimulants cause changes in the brain functioning through a mechanism of drug-induced neuronal plasticity. Self-administration is one of the behavioral endophenotypes connected to addiction and serves as a measure for the rewarding effect of the drugs. Several studies have shown the role of circadian genes in the direct regulation of dopaminergic reward circuitry. That indicates the potential involvement of circadian genes in the neuronal plasticity connected to of regulation of voluntary drug consumption which contributes to addiction in general.

To improve over existing CAFE assay used to measure liquid food consumption, we developed FlyCafe, a high-throughput method using the concept of CAFE assay in combination with Drosophila Activity Monitoring system (DAMs). In this new assay for each fly we objectivly quantify: amount of psychostimulant that flies self-administer, changes in the locomotor activity during the consumption and percentage of time spent close to the food capillaries. Using FlyCafe assay we tested the voluntary METH consumption in *wild-type Drosophila* males and compared that to *period* (*per*) and *timeless* (*tim*) circadian mutants.

Our results showed distinct preference for METH over sugar-based food in *wt* flies, suggesting that METH activates motivational and reward circuits in *Drosophila's* brain. Circadian mutants showed different pattern, developing the preference for METH only on the fourth day of the self-administration, suggesting a role for circadian genes in the regulation of rewarding effects of psychostimulants.

Our findings will provide the basis for further investigation of genetic mechanisms that influence long-term neuronal plasticity induced by addictive drugs in order to uncover new therapeutic targets aimed at the treatment of substance abuse disorders.

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17. Locus impairment of DISC1 results in cognitive deficiency in rodents

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Introduction: Microglia are responsible for excessive synaptic loss in schizophrenia and Alzheimer's disease. *Disrupted-in-schizophrenia-1* (DISC1) is a gene implicated in both neuropathologies, yet its function has only been studied in neurons where it interacts with phosphodiesterase 4 (PDE4). Interestingly, both DISC1 and PDE4 are expressed in microglia. PDE4 inactivates cAMP, a second messenger needed for phagocytosis. We hypothesized that the interaction of DISC1 with PDE4 regulates microglia-mediated synaptic elimination in the hippocampus, resulting in cognitive deficits in DISC1 locus impairment (LI) mice.
Materials & methods: 10-week old DISC1 LI mice were compared to WT mice in a battery of behavioral tests (n = 15/group), including the object location task (OLT), marble burying, nestlet shredding and tube dominance test. The effect of DISC1 LI on PDE4B isoform expression in primary microglia was investigated using qPCR. Statistical significance of gene expression levels and representative outcome measures for cognition, social and instinctive behavior was evaluated using the Student's t-test.
Results: DISC1 locus impairment significantly hampered cognitive capacity and instinctive mouse behavior. In the OLT, DISC1 LI mice spent equal amounts of time on both objects, indicating premature lapse in memory and impaired hippocampal functioning. Failure to exhibit instinctive behavior, as evidenced in the nestlet shredding test, confirmed this lack of hippocampal functioning. Furthermore, the social phenotype was altered, indicating neuronal dysfunction in the prefrontal cortex. *In vitro* analysis using qPCR revealed differential expression of various isoforms of PDE4B, which constitutes the highest expressed PDE4 gene product, in microglia.
Discussion & conclusions: The differential expression of PDE4B points towards cAMP as a potential downstream effector mediating synaptic elimination. *Post mortem* analyses investigating synapses in implicated brain regions will allow to elucidate microglial contribution in the impairment. This data would implicate PDE4B as a therapeutic downstream target to negate the effects of mutations in DISC1.

18. Curcumin: Novel treatment in neonatal hypoxic-ischaemic brain injury

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Hypoxic-ischaemic encephalopathy (HIE) is a major cause of mortality and morbidity in neonates, with an estimated global incidence of 3/1000 live births. HIE brain damage is associated with an inflammatory response and oxidative stress, resulting in the activation of cell death pathways. At present, therapeutic hypothermia is the only clinically approved treatment available for HIE. This approach, however, is only partially effective. There is therefore an unmet clinical need for the development of novel therapeutic interventions for the treatment of HIE.

Curcumin is an antioxidant reactive oxygen species scavenger, with reported anti-tumour and anti-inflammatory activity. Curcumin has been shown to attenuate mitochondrial dysfunction, stabilise the cell membrane, stimulate proliferation, and reduce injury severity in adult models of spinal cord injury, cancer, and cardiovascular disease. The role of curcumin in neonatal HIE has not been widely studied due to its low bioavailability and limited aqueous solubility. The aim of this study was to investigate the effect of curcumin treatment in neonatal HIE, including time of administration and dose-dependent effects.

Our results indicate that curcumin administration prior to HIE in neonatal mice elevated cell and tissue loss, as well as glial activation compared to HI alone. However, immediate post-treatment with curcumin was significantly neuroprotective, reducing grey and white matter tissue loss, TUNEL+ cell death, microglia activation, reactive astrogliosis and iNOS oxidative stress when compared to vehicle-treated littermates. This effect was dose-dependent, with 200μg/g body weight as the optimal dose-regimen, and was maintained when curcumin treatment was delayed by 60min or 120min post-HI. Cell proliferation measurements showed no changes between curcumin and HI alone, suggesting that the protective effects of curcumin on the neonatal brain following HI are most likely due to curcumin's anti-inflammatory and antioxidant properties, as seen in the reduced glial and iNOS activity

19. The selective muscarinic type 1 receptor antagonist, biperiden, does not impair episodic novelty memory: An EEG study

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The muscarinic antagonist, scopolamine has long been used as a model of episodic memory decline, as seen in Alzheimer's disease (AD). However, due its non-selective profile it is known to impair attention and cause severe side effects. Since, memory has been largely found to rely on the muscarinic M1 subtype receptors, a more selective M1 antagonist, such as biperiden might be a better alternative. Furthermore, previous research has shown that novelty processing is impaired in AD. Therefore, we investigated the effects of 4 mg orally administered biperiden on episodic novelty memory according to a double-blind, placebo-controlled, 2-way cross-over design in a population of healthy young volunteers. Memory was tested using a three-phase novelty paradigm with abstract figures and pseudo words. The behavioral and electrophysiological effects were investigated using signal detection theory and EEG. Four evoked response potential components of interest were the N200, P300, N400 and P600. Moreover, early and late old/new effects were examined. Additionally, we measured possible side effects. According to our results biperiden did not impair novelty memory, and did not cause any severe side effects. However, compared to placebo it slowed reaction times with respect to recognition of the abstract figures but not to the pseudo words. This indicates possible impairing effects of this higher dose on attention. As such, our behavioral results do not support the application of biperiden for modeling novelty memory problems as seen in AD. The underlying electrophysiological results are being currently analyzed.

20. Circulating microRNA as potential biomarkers for psychiatric and neurodegenerative disorders

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Circulating microRNAs (cimiRNAs) are a class of non-encoding RNAs found in body fluids such as blood, cerebrospinal fluid (CSF) and tears. CimiRNAs have been implicated as promising biomarkers for central nervous system (CNS) disorders because they are actively secreted as messengers and are profoundly involved in fine-tuning of developmental and differentiation processes. Furthermore, these are attractive biomarkers because they are extremely stable, tissue enriched and can be determined in a quantitative manner. This review aims to provide a comprehensive assessment on the current progress regarding the potential value of cimiRNAs as CNS biomarkers. Within this framework five CNS disorders were explored which share a common pathological hallmark namely cognitive impairment. The CNS disorders include Major depression disorder (MDD), Bipolar disorder (BD), Schizophrenia (SZ), Alzheimer's disease (AD) and Parkinson disease (PD). The similarities and differences between altered cimiRNAs in the different disorders are presented. The miR-29 family, miR-34a-5p and miR-132-3p are further explored as common dysregulated cimiRNAs found in the CNS disorders. Furthermore, it is shown that the type of body fluid used for measuring cimiRNAs is important as inconsistencies in cimiRNAs expression directions are found when comparing CSF, blood cell-free and blood cell-bound samples.

21. Neurodevelopment and pharmaceuticals: *in vitro*, *in vivo* or organoids?

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According to recent epidemiological studies, brain-related illnesses encompassing for example depression, epilepsy and brain neoplasms will take the leading position in the global burden of disease by the 2020s. Many neurological diseases begin during embryonic and fetal life. Some of these can be caused or precipitated by drug use during pregnancy and pharmaceuticals taken by mothers may affect fetus neurodevelopment. For this reason, we need platforms for testing pharmaceuticals in the developing brain – the only way to do that is by employing adequate *in vitro* and *in vivo* models. In our work, we are aiming to approach to neurodevelopment and pharmaceutical interactions through the use of several experimental models, such as immortalized neuron-like cells and differentiated neurons derived from human iPSCs as *in vitro*, but also developing chicken embryo as *in vivo* model and human organoids of different brain regions. Based on the human iPSC, we already succeeded and recapitulated this new approach for so-called brain organoids. We managed to culture organoids by the days-in-vitro 18 and we showed that early brain development markers (such as Brna3a and FoxP1) are expressed even at this stage of growth. It's also been shown in our lab that several potential candidate genes for changing in expression might be employed for neuropharmacotoxicological analysis, e.g. pax6, mmp9, pcna. Moreover, developing chicken brain and *in ovo* as an alternative *in vivo* model was employed. The chicken egg is trustful and easy to maintain animal model, where all the events of neurodevelopment can be recapitulated under drug treatment. Based on the facts, that many different neuropathological deviations take place during intrauterine development and the last studies which show that many drugs might cause pathological changes in the developing brain – testing of the neuropharmaceuticals is still a big issue. The question is which model system to use – 2D *in vitro* or 3D organoids or *in vivo* or all of them at once?

22. In-vivo treatment with the mGluR5 negative allosteric modulator CTEP ameliorates the disease progression in the SOD1G93A mouse model of amyotrophic lateral sclerosis.

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease leading to motor neurons (MNs) death and to glial cell damage. The aetiology of the disease is still poorly defined, although glutamate (Glu)-mediated excitotoxicity is assumed to represent one major cause of MN damage. Group I metabotropic glutamate receptors (mGluR1 and mGluR5) are implicated in Glu-mediated excitotoxicity in ALS, since they are involved in prominent cellular processes and largely over-expressed during disease progression. In this context, we recently demonstrated that activation of presynaptic mGluR1 and mGluR5 produced abnormal Glu release in the SOD1G93A mouse model of ALS and that halving or abolishing their expression significantly delays the disease onset and prolongs survival, ameliorates the disease progression by postponing the decline of motor abilities, preserves the spinal cord MNs from death and reduces the astrocytes and microglia activation in SOD1G93A mice.

Due to these encouraging results, we investigated here the effects of the in vivo pharmacological treatment of SOD1G93A mice with 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy) phenyl)-1H-imidazol-4-yl) ethynyl) pyridine (CTEP), a negative allosteric modulator of mGluR5. We treated 90 days old symptomatic SOD1G93A mice with CTEP (2mg/kg/48h or 4mg/kg/24h) or vehicle, by gavage, until death. CTEP dose dependently ameliorated the clinical features in SOD1G93A mice. The lower dosage barely produced positive effects. The higher dosage significantly delayed the disease onset, increased survival and improved motor abilities in treated mice. The in vivo treatment also preserved of MNs from death, decreased the activation of astrocytes and microglia and reduced the abnormal Glu release in spinal cord. All these effects were more marked in female than in male SOD1G93A mice.

In conclusion, our previous and present results suggest that mGluR5 represents a promising target for the treatment of ALS and the CTEP in vivo effects in SOD1G93A mice support this translational perspective.

23. GABAergic neurotransmission in the human brain characterized by single- and paired-pulse TMS with EEG co-registration and pharmacological GABAA activation

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All information transmission in the brain depends on the current level of neuronal excitability. Amongst numerous neurotransmitters that maintain this dynamic state, γ-aminobutiric acid (GABA) is of particular importance, as it represents the main inhibitory neurotransmitter of the central nervous system. The present study aims to characterize the state of GABAergic neurotransmission in the human brain using a non-invasive way that will subsequently allow comparing various patient groups to healthy controls.
We combined transcranial magnetic stimulation (TMS) over the left primary motor cortex (M1) with electroencephalography (EEG) and recorded TMS evoked potentials (TEPs) before and after pharmacological activation of GABAA receptors with alprazolam. In addition, we applied paired-pulse TMS in order to explore the phenomenon of short-latency intracortical inhibition (SICI), which represents the manifestation of local inhibition mediated by GABAA receptors. 20 healthy young volunteers participated in two sessions, each consisting of a baseline recording followed by administration of alprazolam or active placebo (cetirizine) and a post-medication recording. The TMS coil was kept on the target using MRI–based neuronavigation. Three types of TMS stimuli were applied: suprathreshold single-pulse (120 % resting Motor Threshold - rMT), subthreshold single-pulse (80% rMT), and paired-pulse (80% + 120% rMT, 2.5 ms inter-stimulus interval).

Our results show that alprazolam modulates amplitudes of early TEP waves following a single suprathreshold TMS stimulus. At baseline, the paired-pulse TMS evokes substantially reduced MEPs and amplitudes of early TEP waves when compared to a suprathreshold single-pulse. The effect of SICI in TEPs is reduced following the administration of alprazolam. In MEPs, alprazolam causes an amplitude decrease after a suprathreshold stimulus, while the response to paired-pulse TMS remains unchanged. Altogether, selective effects of alprazolam vs. cetirizine are clearly observable in TEPs and MEPs, which could reflect the state of GABAergic neurotransmission.