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Efficacy of Subtype-Selective, Full vs Partial M4 Muscarinic Receptor Agonists in Modulating Amphetamine-Induced Brain Activity Assessed by Functional MRI (fMRI) in Rats

S. Bardehle,[1] G. L. Suidan,[1] H. Nguyen,[1]
S. Chakilam,[1] G. Gokulrangan,[1] R. Immonen,[2]
A. Shatillo,[2] P. A. Iredale[1]

[1]Cerevel Therapeutics, Cambridge, MA, USA;
[2]Charles River Discovery Services, Kuopio, Finland

Presenting Author: Sophia Bardehle, Sophia.Bardehle@cerevel.com

CONCLUSIONS

- Amphetamine (1 mg/kg; SC) induced highly significant increase in rCBV peaking at approximately 15 minutes post injection across multiple brain areas, with the strongest effects observed in the striatum (25%) and medial prefrontal cortex (35%)
- **CV-0000071 (partial agonist):** Test compound (TC) effect alone induced a transient, significant decrease of rCBV response in the high-dose (0.32 mg/kg) group. Amphetamine-induced increase in CBV response was significantly reduced in a dose-dependent manner in all major brain areas (mPFC and CPu shown) and sustained during the 45-min follow-up
- **CV-0000042 (full agonist):** TC effect alone resulted in rapid, sustained decrease in rCBV response in the high-dose group (0.32 mg/kg). The lower dose (0.032 mg/kg) showed rCBV decrease of lower magnitude. Amphetamine-induced increase in rCBV was significantly reduced in both CV-0000042 dose groups in all major brain areas
- The differential fMRI response profiles observed between compounds with different levels of M4 agonism suggest that there is an opportunity to dial in the most appropriate amount of intrinsic efficacy in a compound to match the desired downstream effect

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BACKGROUND

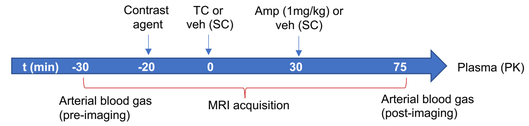
- The M4 muscarinic acetylcholine receptor (mAChR) is a transmembrane Gi-coupled protein expressed both pre- and postsynaptically in brain regions associated with psychotic and cognitive functions, including the striatum, cortex, and hippocampus
  - Amongst the mAChR subtypes (M1-M5), the unique brain expression profile of M4 mAChR, as well as its effects on neurotransmitter signaling, make it a compelling target for various psychiatric diseases
- Our hypothesis for activating M4 receptors is that the resultant reduction in acetylcholine (ACh) release can potentially correct the striatal hyperdopaminergic activity that has been linked to symptoms of psychosis
- The development of subtype-selective, potent M4 agonists provides therapeutic potential for treatment of psychosis symptoms observed in both schizophrenia and neurodegenerative conditions, such as Alzheimer's disease

Figure 1. MOA hypothesis.

OBJECTIVE & STUDY DESIGN

- To investigate the effects of systemic subcutaneous (SC) administration of 2 different test compounds (TC) – a full (CV-0000042) compared with a partial (CV-0000071) M4 receptor agonist – at 2 doses each (**Table 1**) on amphetamine-induced brain activity in anesthetized Sprague-Dawley rats (Crl:CD(SD)) using relative cerebral blood volume (rCBV) functional MRI (fMRI) readout

Figure 2. Schematic of design for rCBV fMRI study.



METHODS: Dosing and fMRI Protocol

- Adult male, cannulated, ventilated Sprague-Dawley (Crl:CD(SD)) rats (body weight, 345 ± 20 g) were imaged under medetomidine-isoflurane anesthesia. For a total duration of 1.75 hours, fMRI scans were acquired using a Bruker 7T MRI system (Bruker, Billerica, MA, USA). Physiological monitoring included pre- and post-imaging arterial blood gas and pH measurements, as well as continuous heart rate recording. Relative cerebral blood volume (rCBV) data were collected using high resolution T2*-weighted gradient-echo sequence (FLASH) with iron oxide contrast agent (ferumoxytol; intravenous). Test compounds were dosed 30 minutes prior to the amphetamine (Amp) challenge, and rCBV changes followed uninterrupted for 45 min post Amp injection. Terminal plasma samples were collected for PK analysis. All animal experiments were conducted in accordance with institutions' Institutional Animal Care and Use Committee or equivalent ethics committee(s)

Table 1. Experimental Groups for fMRI Study

Group	Treatment compound	Dose	Dosing route	Challenge compound	Group size
1	Vehicle (5% DMSO/5% Solutol/ 90% sterile water)	1 ml/kg	SC	Vehicle (Saline); SC	12
2	Vehicle (5% DMSO/5% Solutol/ 90% sterile water)	1 ml/kg	SC	Amphetamine; 1 mg/kg, SC	12
3	CV-0000042	0.032 mg/kg	SC	Amphetamine; 1 mg/kg, SC	12
4	CV-0000042	0.32 mg/kg	SC	Amphetamine; 1 mg/kg, SC	12
5	CV-0000071	0.032 mg/kg	SC	Amphetamine; 1 mg/kg, SC	12
6	CV-0000071	0.32 mg/kg	SC	Amphetamine; 1 mg/kg, SC	12

DMSO, dimethyl sulfoxide; SC, subcutaneous.

RESULTS

PLASMA PK DATA

Figure 3. Plasma concentrations in terminal plasma samples collected at the end of the rCBV MRI scan, 75-80 min after dosing.

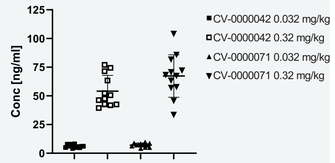
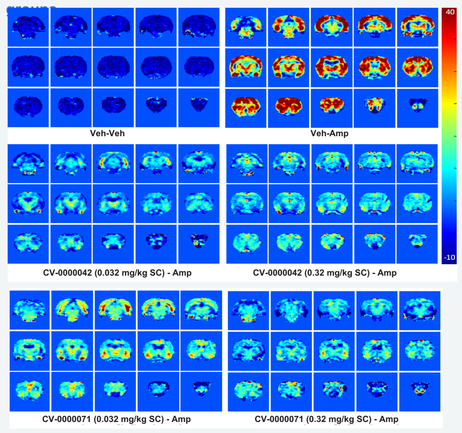


Table 2. PK Profile of CV-0000042 and CV-0000071 After Acute SC Dosing

Compound	Dose (mg/kg)	cu,p (nM)	cu,b (nM)	Change in cAMP, %
CV-0000042	0.032	4.07	4.71	**12**
CV-0000042	0.32	35.52	41.2	**58**
CV-0000071	0.032	3.65	4.85	**15**
CV-0000071	0.32	34.78	46.26	**63**

REPRESENTATIVE rCBV MRI MAPS

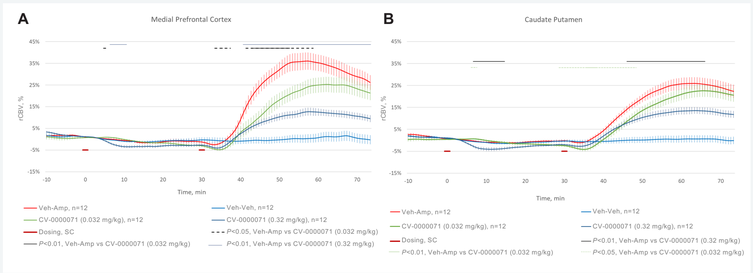
Figure 4. Representative rCBV maps showing brain activity of individual animals in Veh-Veh, Veh-Amp, CV-0000042 (0.032 or 0.32 mg/kg) - Amp, and CV-0000071 (0.032 or 0.32 mg/kg) - Amp groups



Color indicates high (red) or low (blue) median rCBV response over 45 minutes.

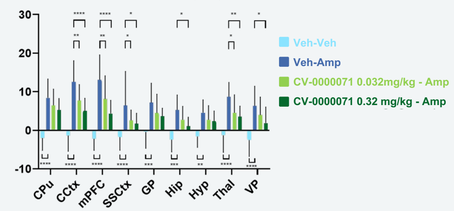
CV-0000071 REDUCED AMP-INDUCED rCBV RESPONSE IN A DOSE-DEPENDENT MANNER IN THE CORTEX AND STRIATUM

Figure 5. CV-0000071: Group rCBV signal time series from the low- and high-dose group in the medial prefrontal cortex (A) and caudate putamen (B).



Data are presented as mean ± SEM. Statistical analysis performed using point-wise one-way ANOVA; dashed lines, $P<0.05$; solid lines, $P<0.01$.

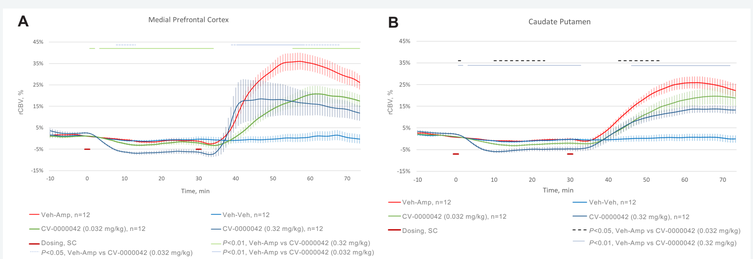
Figure 6. CV-0000071: Group rCBV area under the curve (AUC) values of the 45-min follow-up period after Amp injection from all brain regions for CV-0000071 treatment groups.



Data are presented as mean ± SEM. Statistical analysis performed using two-way ANOVA with Holm-Sidak's multiple comparisons test against the control (Veh-Amp) group.
*$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$

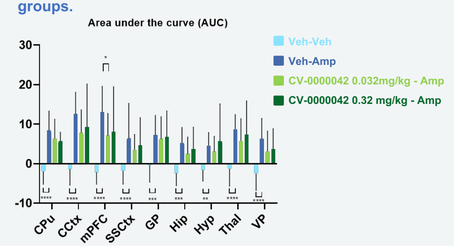
CV-0000042 REDUCED BASELINE rCBV RESPONSE AND AMP-INDUCED RCBV RESPONSE IN THE CORTEX AND STRIATUM

Figure 7. CV-0000042: Group rCBV signal time series from low-dose and high-dose groups in the medial prefrontal cortex (A) and caudate putamen (B).



Data are presented as mean ± SEM. Statistical analysis performed using point-wise, one-way ANOVA; dashed lines, $P<0.05$; solid lines, $P<0.01$.

Figure 8. CV-0000042: Group rCBV area under the curve (AUC) values of the 45-min follow-up period after Amp injection from all brain regions for CV-0000042 treatment groups.



Data are presented as mean ± SEM. Statistical analysis performed using two-way ANOVA with Holm-Sidak's multiple comparisons test against the control (Veh-Amp) group.
*$P<0.05$.