A Pharmaco-EEG study for power spectral analysis dose response study of an acute treatment of AC101 compared to diazepam and vehicle in rats

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LIST OF ABBREVIATIONS

Abbreviation	Definition
%	Percent
°C	Degrees Celsius
EEG	Electroencephalogram
EMG	Electromyogram
g	Gram
hr	Hour
i.p.	Intraperitoneal
kg	Kilogram
mg	Milligram
min	Minutes
ml	Milliliters
mm	Millimeters
N	Number of animals per group
O2	Oxygen
PK	Pharmacokinetic
s.c.	Subcutaneous
IV	Intravenous
sec	Seconds
SD	Standard Deviation
SE	Standard error
SEM	Standard error of the mean
UP	Ultra pure water
w/v	Weight/Volume
Hz	Hertz

1. SUMMARY

The current set of experiments was designed to measure the power spectral changes using EEG following treatment with Sponsor test article, diazepam or vehicle. EEG activity was recorded via subcutaneously (s.c.) implanted transmitters, in a cross-over design with at least 5 days between each EEG session to allow for compound wash-out. Power spectral analysis was performed by CRL-SSF.

2. INTRODUCTION

2.1 General introduction

The objective of this study was to determine the dose response effect of AC101 in power spectral analysis compared to diazepam via radio telemetry EEG assessment. EEG activity was recorded via a subcutaneously (s.c.) implanted transmitter weekly during baseline day (4 hours) and treatment day (24 hours). AC101 and Diazepam were given IV and EEG was recorded for 4 hours post dosing. For each recording, 4 hours of data was analyzed for power-spectral changes.

2.2 Test facility and test site

The biotechnical experiments described in the present report were performed at Charles River Laboratories SSF.

2.3 Archiving

Raw EEG data is converted to EDF file, to be shared with the client. In addition to all data generated, Excel files and GraphPad Prism file. All these files will also be archived at Charles River Laboratories SSF by study number, Key 2192. Data will be stored for a period of 10 years after completion of the final report.

3. MATERIALS AND METHODS

3.1 Animals

Animals were provided by the CRL (n = 10, age 7-8 weeks). Upon arrival, rats were group-housed in polycarbonate cages (2-3 /cage) and acclimated for at least 4 days prior to commencing studies. Animals were housed in a 12 hr light/dark cycle with room temperature maintained at 22±2°C and approximately 50% humidity, and received food and water *ad libitum*, as well as a nylon bone (Bio-Serv®, K3580) and tunnel retreat (Bio-Serv®, K3245) for enrichment. Rats were tracked via unique identifying numbers. Age and identifier information can be found in Appendix I. At the start of the first EEG session, the rats were 10 weeks (+/- 3 days) of age. Experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of Charles River Laboratories SSF.

3.2 Transmitters

The EEG/EMG subcutaneous transmitters (model no.: HD-S02) were obtained from Data Sciences International™.

3.3 Surgery

Rats were anesthetized using isoflurane (2%, 800 mL/min O_2). Bupivacaine was used for local anesthesia and carprofen was used for peri-/post-operative analgesia.

A subcutaneous pocket was made close to the dorsal flank. The EEG transmitter, HD-S02, (Data Sciences International, MN, USA) was inserted into the pocket and leads were tunneled subcutaneously towards the head. The positive and negative EEG leads, Channel 1, were placed supradurally. The positive electrode was placed 2 mm anterior to the bregma and 2 mm from midline on the left hemisphere (frontal electrode) and the negative EEG lead, Channel 1, was placed 2 mm anterior to the lambda and 2 mm from midline on right hemisphere (parietal electrode).Channel 2 leads were used to measure EMG and were placed on either side of the musculus cervicoauricularis and sutured in place.

After surgery animals were housed individually in cages and provided food and water *ad libitum,* a nylon bone (Bio-Serv®, K3580) and paper towel as enrichment. Animals received a 5-day regime of antibiotics beginning 1 day prior to surgery. Pain management was maintained for 3 days beginning with the day of surgery. Initially, animals were monitored daily, then weekly for overall health and recovery purposes.

3.4 Test substance and formulation

The Sponsor's test article, AC-101 (BUS 382-001A, supplied by Dr. James. M. Cook, University of Wisconsin, Lot# MYM-III-92-2, purity: NA, storage: RT), and Diazepam (supplied by Sigma, Lot# 105F0451, purity >99%, storage: RT) were formulated fresh on day of use as described in Table 1. Route of administration for AC 101 and Diazapam was intraveneous, via the tail vein.
All substances are tracked via Smartsheet® inventory system, upon study completion all sponsor supplied substances were stored or disposed upon clients' request.

Table 1 : Compound Formulation

Substance	MW	Treatment Group	Concentration (mg/mL)	Route of Administration	Formulation
Vehicle	N/A	A	N/A	i.v.	85% DI water 14% Propylene Glycol 1% Tween-80
BUS 382-001 A **AC-101**	MW: 386.42 CF: N/A	B	1	i.v.	85% DI water 14% Propylene Glycol 1% Tween-80
		C	3	i.v.	
		D	10	i.v.	
B 065 **Diazepam**	MW: CF: N/A	E	0.5	i.v.	50% DI water 40% Propylene Glycol 10% alcohol
		F	1	i.v.	

3.5 EEG recordings

After at least 10 days of recovery after surgery, EEG recording began. Session 1, Day 1 was started on 08/12/20, and the last session ending on 09/25/20. Recordings were aquired using Ponemah Aquisition and Analysis software (Data Sciences International, MN, USA).

Animals were placed on the RPC-1 receivers (Data Sciences International, MN, USA) the night prior to the recording session to allow for acclimation to the recording locations. Rats were recorded weekly, with at least 5 days between each session with 8 rats recorded for each session. Details on the animals, EEG, dosing and clinical observations of each session was recorded (see Appendix I).

Day 1 (Baseline Recording) - At approximately 9 am, animals were recorded for 4 hours after acclimation. No treatment was administered.

Day 2 (Treatment) - At approximately 9 am, recording began. After 1 hour of recording, animals were injected with test article or vehicle via i.v. tail vein. Animals were then recorded for an additional 24 hours. Recording sessions were performed as described in Table 2.

Table 2: Recording Sessions and Treatment

Animal Identity	*Session 1*	*Session 2*	*Session 3*	*Session 4*	*Session 5*	*Session 6*
1	A	B	C	D	E	F
2	B	C	D	E	F	A
3	C	D	E	F	A	B
4	D	E	F	A	B	C
5	E	F	A	B	C	D
6	F	A	B	C	D	E
7	A	B	C	D	E	F
8	B	C	D	E	F	A

3.6 Post-mortem tissue collection

At the end of experiment, on 10/03/20, rats were euthanized via CO_2 asphyxiation and no terminal tissues were collected.

3.7 Analysis and Statistics

EEG for each treatment group was analyzed by CRL using NeuroScore (Data Sciences International, MN, USA). Frequency based analysis, i.e., looking at the frequency components of various band widths within the EEG waveform was performed for each animal. The data of the animals in each treatment group was combined of which the average relative power and average absolute power was calculated. The relative power is expressed as percentage (%) of each power band, e.g., Delta frequency. It is where the output values are normalized by representing the power band as a ratio (measure) between the desired power band being analyzed and the total power in the signal. The absolute power of the power band is expressed as μV^2.

Prism 9.0 software was used for graphs and run the appropriate statistical and post hoc tests. In this study Two Way ANOVA was used as the statistical test, followed by Tukeys multiple comparison test was performed.

4. RESULTS

5. CONCLUSION

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Title : **A Pharmaco-EEG study for power spectral analysis following acute treatment**

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Date : Dec 2020

Holden Janssens, Ph.D.

Director, Charles River Laboratories

Date : 12/20/2020

Place: South San Francisco

APPENDIXES

Appendix I – EEG Session and animal details

ID	JCMS ID	Pen	Strain	Sex	DOB	Coat Color	Date of Arrival	Date of surgery
2	CD_005781	18987	Sprague Dawley (CRL)	M	4-Jun-20	Albino	23-Jul-20	27-Jul-20
3	CD_005782	18988	Sprague Dawley (CRL)	M	4-Jun-20	Albino	23-Jul-20	27-Jul-20
4	CD_005783	18988	Sprague Dawley (CRL)	M	4-Jun-20	Albino	23-Jul-20	27-Jul-20
6	CD_005785	18989	Sprague Dawley (CRL)	M	4-Jun-20	Albino	23-Jul-20	28-Jul-20
7	CD_005786	18990	Sprague Dawley (CRL)	M	4-Jun-20	Albino	23-Jul-20	28-Jul-20
8	CD_005787	18990	Sprague Dawley (CRL)	M	4-Jun-20	Albino	23-Jul-20	28-Jul-20
9	CD_005788	18991	Sprague Dawley (CRL)	M	4-Jun-20	Albino	23-Jul-20	29-Jul-20
10	CD_005789	18991	Sprague Dawley (CRL)	M	4-Jun-20	Albino	23-Jul-20	29-Jul-20
11	CD_005802	19059	Sprague Dawley (CRL)	M	31-May-20	Albino	30-Jul-20	6-Aug-20
12	CD_005803	19059	Sprague Dawley (CRL)	M	31-May-20	Albino	30-Jul-20	6-Aug-20

Session 1

#	Animal ID	Receiver #	Weight (gr)	Tx Group	Date	Dose time	Experimental Notes
1	2	1	410	A	13-Aug-20	10:25 AM	
2	3	2	397	B	13-Aug-20	10:28 AM	
3	4	3	449	C	13-Aug-20	10:32 AM	
4	6	4	373	D	13-Aug-20	10:44 AM	
5	7	5	426	E	13-Aug-20	10:49 AM	
6	8	6	380	F	13-Aug-20	10:52 AM	During/post injection animal became catatonic and limp
7	9	7	387	A	13-Aug-20	10:57 AM	scrape on tail, not injection related
8	10	8	411	B	13-Aug-20	11:00 AM	

Session 2

#	Animal ID	Receiver #	Weight (gr)	Tx Group	Date	Dose time	Experimental notes
1	2	1	453	B	20-Aug-20	10:34 AM	Minor lesion near right ear (~3 mm diameter)
2	3	2	450	C	20-Aug-20	10:38 AM	
3	4	3	492	D	20-Aug-20	10:49 AM	
4	6	4	402	E	20-Aug-20	10:45 AM	
5	7	5	468	F	20-Aug-20	10:53 AM	
6	8	6	406	A	20-Aug-20	10:57 AM	
7	9	7	418	B	20-Aug-20	10:59 AM	
8	10	8	447	C	20-Aug-20	11:01 AM	

Session 3

#	Animal ID	Receiver #	Weight (gr)	Tx Group	Date	Dose time	Experimental notes
1	2	1	483	C	27-Aug-20	11:09 AM	
2	3	2	481	D	27-Aug-20	11:13 AM	
3	4	3	531	E	27-Aug-20	11:17 AM	
4	6	4	427	F	27-Aug-20	11:20 AM	
5	7	5	490	A	27-Aug-20	11:24 AM	switched out with #11 for next session due to tail injury
6	8	6	427	B	27-Aug-20	11:27 AM	
7	9	7	457	C	27-Aug-20	11:29 AM	
8	10	8	476	D	27-Aug-20	11:32 AM	

Session 4

#	Animal ID	Receiver #	Weight (gr)	Tx Group	Date	Dose time	Experimental notes
1	2	1	511	D	3-Sep-20	10:38 AM	
2	3	2	516	E	3-Sep-20	10:44 AM	lethargic after dosing
3	4	3	555	F	3-Sep-20	10:52 AM	lethargic after dosing
4	6	4	444	A	3-Sep-20	11:00 AM	
5	11	5	443	B	3-Sep-20	11:21 AM	
6	8	6	435	C	3-Sep-20	11:07 AM	
7	9	7	491	D	3-Sep-20	11:14 AM	
8	10	8	510	E	3-Sep-20	11:18 AM	lethargic after dosing

Session 5

#	Animal ID	Receiver #	Weight (gr)	Tx Group	Date	Dose time	Experimental notes
1	2	1	540	E	10-Sep-20	10:55 AM	
2	3	2	540	F	10-Sep-20	11:04 AM	
3	4	3	586	A	10-Sep-20	11:15 AM	
4	6	4	463	B	10-Sep-20	11:33 AM	
5	11	5	469	C	10-Sep-20	11:56 AM	
6	8	6	468	D	10-Sep-20	11:30 AM	
7	9	7	520	E	10-Sep-20	11:42 AM	
8	10	8	531	F	10-Sep-20	11:48 AM	

Session 6

#	Animal ID	Receiver #	Weight (gr)	Tx Group	Date	Dose time	Experimental notes
1	2	1	572	F	17-Sep-20	11:17 AM	
2	12	2	464	A	17-Sep-20	11:13 AM	
3	4	3	640	B	17-Sep-20	11:20 AM	
4	6	4	510	C	17-Sep-20	11:26 AM	
5	11	8	511	D	17-Sep-20	11:48 AM	
6	8	5	492	E	17-Sep-20	11:31 AM	
7	9	6	564	F	17-Sep-20	11:45 AM	
8	10	7	560	A	17-Sep-20	11:39 AM	

Session 7

#	Animal ID	Receiver #	Weight (gr)	Tx Group	Date	Dose time	Experimental notes
1	2	1	572	A	24-Sep-20	10:53 AM	
2	12	2	482	B	24-Sep-20	10:56 AM	
3	4	3	658	C	24-Sep-20	10:59 AM	
4	6	4	518	D	24-Sep-20	11:03 AM	
5	11	5	515	E	24-Sep-20	11:08 AM	
6	8	6	505	F	24-Sep-20	11:17 AM	
7	9	7	574	A	24-Sep-20	11:14 AM	
8	10	8	583	B	24-Sep-20	11:19 AM	