Drug effects on sleep architecture and qEEG: a cross-species comparison in mice, rats and dogs

Transpharmation
Science that translates into results

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INTRODUCTION

Electroencephalography (EEG) is often used for measuring brain penetrance and receptor-engagement in preclinical drug-discovery[1], and EEG recordings provide rich datasets which also include data on locomotor activity and sleep architecture. As an objective measure, the hope is that EEG results obtained in animal models will translate to the clinic. The NMDA receptor antagonist ketamine has been shown to have clear effects on gamma power in human EEG[2]. Here, to demonstrate the translational potential of EEG, we compared responses to ketamine in mice, rats and dogs.

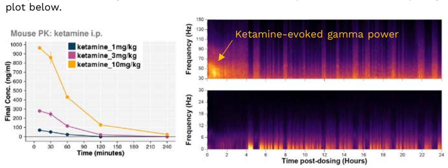
METHODS

Male C57bl/6 mice, male Sprague Dawley rats, and a mixed cohort of male and female beagle dogs were implanted for EEG, electromyography (EMG) and activity recording. Animal telemetry and ketamine dose details are described in **Table 1** below. EEG electrodes consisted of bipolar (DSI) or differential; (TaiNi[3]) pairs, with the positive pole over the prefrontal cortex and the negative pole over the occipital/parietal cortex. EMG electrodes were secured in the neck extensor muscles behind the head to allow quantification of muscle tone.

Species	N	Ketamine dose (mg/kg)	Route	Telemetry platform
Mouse	8	1, 3, 10, 30	i.p.	TaiNi: 20/16/12/SOA, head-mounted
Rat	8	0.3, 1, 3, 10	i.p.	DSI: HD-SO2, implantable
Dog	6	5, 7.5	i.m.	DSI: L03-F1, implantable

All animals were maintained on a 12-12 hour light-dark cycle, with dosing performed ~2 hours after lights-on. EEG and EMG data was bandpass filtered (0.1-100Hz) and digitised at 500Hz. Recording continued for ~22 hours after dosing to facilitate automated vigilance state scoring. All data was analysed using proprietary Transpharmation software and aligned to zero (the time of dosing), with 10-second epochs assigned to either the wakefulness, non-REM sleep or REM sleep vigilance state. EEG was analysed using short-time fast Fourier transform (FFT: amplitude/second), producing 1-100Hz spectra. Area under the curve was used to estimate quantitative EEG (qEEG) values for the following bands: delta (1-4 Hz) theta (4-10Hz) sigma/alpha (6-15 Hz) beta (12-30 Hz) gamma1 (30-50Hz) gamma2 (50-100Hz).

An analysis of ketamine plasma levels (below left) in a satellite cohort of mice indicated a relatively short half-life of approximately 45minutes, with almost complete clearance by 3 hours. This is evident in the representative time-frequency plot below.

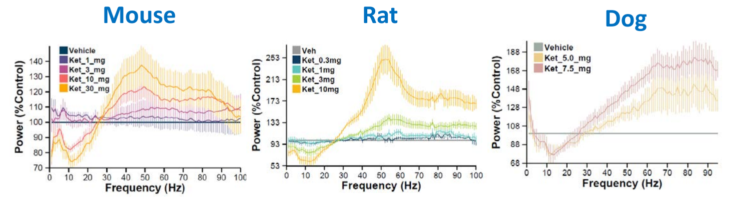


Statistical analysis: Based on the 3-hour clearance profile in mice, analysis was focussed on the first 3 hours of data, averaged in 30-minute non-overlapping bins. Effects of treatment and time were assessed with 2-way repeated-measures ANOVA (R version 3.6.3) following multiple-imputation (MICE package) if required to handle missing cases. The Greenhouse-Geisser correction for any violations of sphericity. For qEEG, separate analyses were conducted for each vigilance state. Post-hoc analysis of interaction contrasts was performed to compare each treatment with the control (Vehicle), with Dunnett's correction applied. Post-hoc results were only reported if either the main effect of treatment or the treatment x time interaction was significant.

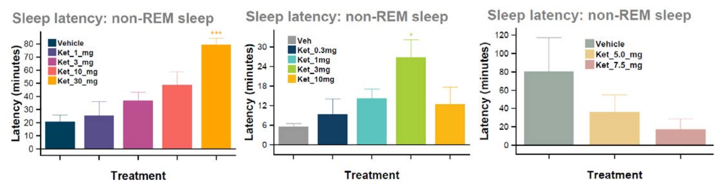
Data presentation: All data are presented as mean ± SEM (standard error of the mean). Statistical significance is represented as stars: *p<.05, **p<.01, and ***p<.001. Where data is presented as a time-series, x-axis labels refer to the beginning of each time-bin relative to dosing-time.

RESULTS: Power spectrum and non-REM sleep latency

Across species, spectral responses (all vigilance states) showed dose-dependent increases in the high frequency range (>30Hz). The response was particularly strong for rats.

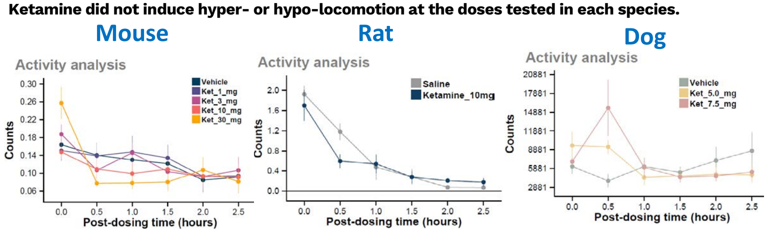


Ketamine produced a dose-dependent increase in latency to non-REM sleep in rats and mice only. Note that for rodents, dosing was during their non-active phase of the light-cycle.



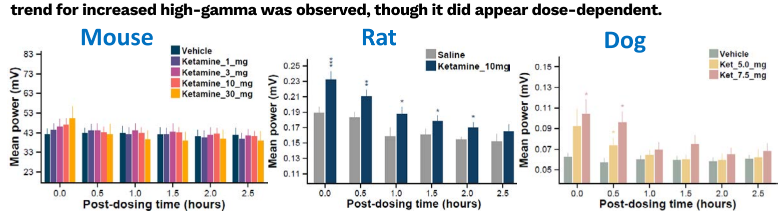
RESULTS: locomotor activity

Ketamine did not induce hyper- or hypo-locomotion at the doses tested in each species.



RESULTS: qEEG waking high-gamma (50-100Hz)

Rats and dogs saw a clear increase in high-gamma power in response to ketamine. In mice, only a trend for increased high-gamma was observed, though it did appear dose-dependent.



RESULTS: fingerprint analysis of effects across species (columns)

Analysis of effect size for a variety of measures and vigilance states (rows) highlights the consistency of effects like theta power reduction in REM sleep and enhanced gamma in wakefulness.

Cohen's D + Hedge's correction for small samples			
Small	0.20	-0.20
Medium	0.50	-0.50
Large	0.80	-0.80

Time: 0-1 Hours

	state	Mouse 1mg/kg	Mouse 3mg/kg	Mouse 10mg/kg	Mouse 30mg/kg	Rat 0.3mg/kg	Rat 1mg/kg	Rat 3mg/kg	Rat 10mg/kg	Dog 5mg/kg	Dog 7.5mg/kg
Activity	All	-0.1695	-0.1182	-0.6191	0.509	0.7056	0.6956	1.3218	1.4913	**2.6352**	**2.2313**
Temperature	All									0.5079	1.175
Vigilance state %	Wake	-0.5083	0.2305	**2.7491**	**4.662**	0.5273	0.2384	**1.887**	0.6203	-0.6393	-1.0952
	NREM	0.5252	-0.6426	**-2.6591**	**-7.4602**	-0.5376	-0.9638	**-2.5509**	**-1.8116**	0.6444	1.1903
	REM	-0.364	0.3548	-1.2904	-1.331	0.1618	0.4511	0.0603	1.1707	-0.4015	0.7953
Sleep Latency	NREM	0.2939	1.406	**1.7836**	**6.2934**	0.6153	**2.2991**	**3.0228**	0.997	-0.8087	-1.4076
	REM	-0.3168	-1.127	0.3162	**2.0766**	0.6748	0.3571	-0.9783	**-1.8868**	1.7491	**-3.5436**
Sleep bout-length	Total	0.0867	-1.116	-1.2303	**-2.2292**	1.335	0.9004	-0.7607	-1.1339	-0.1687	1.2328
	NREM	-0.3226	-0.8517	**-1.5816**	**-1.8738**	0.6109	0.6432	0.4159	**-2.9266**	0.9524	**2.2068**
	REM	**-1.8262**	-1.4479	-0.4948	**-1.6833**	0.6413	0.5569	-0.036	-0.6406	0.0604	1.1785
qEEG Delta	Wake	0.0778	0.32	0.4006	0.9807	0.018	0.1324	0.4029	0.1411	1.2388	2.0187
	NREM	-0.2052	0.431	-1.2917	**-2.0728**	0.1111	-0.0166	-0.0859	-1.509	0.2423	0.1142
	REM	-0.2406	-0.3567	**-2.1582**	-1.0462	-0.9449	-0.9455	-0.6071	-0.743	0.7292	**3.3964**
qEEG Theta	Wake	0.1867	0.2919	-0.0494	-0.2061	-0.0371	-0.0775	0.0545	-0.0812	0.7128	1.1988
	NREM	-0.1919	0.4236	-0.7978	**-2.6756**	0.0029	-0.0393	-0.1911	-1.7593	-0.915	-1.5777
	REM	-0.0814	-0.0805	0.3653	-0.6354	-0.4821	-0.6729	**-1.7872**	-1.3549	0.3791	**-2.3366**
qEEG Sig.Alpha	Wake	0.2645	0.2852	-0.5167	-0.7612	-0.1521	-0.1949	-0.0383	-0.4089	-0.3236	-0.4372
	NREM	-0.2646	0.3602	-0.9601	**-2.9797**	-0.0113	-0.1044	-0.2837	**-2.0627**	-1.0425	-2.0679
	REM	0.1831	-0.3554	-0.2212	-0.3841	-0.5946	-0.8858	**-2.0836**	**-1.9929**	-0.9619	**-2.6415**
qEEG Beta	Wake	0.1771	0.1258	-0.406	-0.615	-0.2163	-0.2449	-0.2335	-0.4499	-1.038	-1.4272
	NREM	-0.022	0.2566	-0.8648	-1.3941	-0.0186	-0.0675	-0.2211	-1.1242	-0.9777	**-2.1671**
	REM	0.6443	0.2637	-1.1945	-1.5658	-0.7221	-0.8346	-1.5558	-1.6275	-1.3312	**-2.5682**
qEEG Gamma1	Wake	0.3199	0.2836	0.6775	0.7634	0.0869	0.1999	0.2949	1.4158	0.6512	**2.1545**
	NREM	0.2402	0.1882	0.6379	0.3858	0.0053	0.1458	0.2862	**1.807**	0.0388	1.6523
	REM	0.5691	-0.339	1.598	0.2168	-0.4946	-0.5562	-0.4572	0.8104	0.6391	1.7927
qEEG Gamma2	Wake	0.263	0.3975	0.5144	0.4058	0.2161	0.4215	0.7798	**1.9936**	2.0045	**3.0499**
	NREM	0.3009	0.1614	0.6142	1.0829	0.0461	0.2655	0.4613	**2.263**	**2.6983**	**4.6102**
	REM	-0.1408	-0.0451	0.9225	-0.0398	-0.654	-0.4265	0.115	1.5254	1.5468	**3.5254**

CONCLUSIONS

- Ketamine administered at sub-anaesthetic doses in mice, rats and dogs elicited a significant increase in qEEG gamma power (50-100 Hz).
- Rats appear to be particularly sensitive to these effects, and mice were the least sensitive, despite large effect-sizes seen at some doses.
- Additional analyses suggest that reductions in power in the theta, alpha and beta bands may also be cross-species indicators of NMD receptor activation.
- The wake-promoting effects of ketamine at the tested doses was only evident in mice and rats, with the drug competing against the natural sleep-drive of the rodents early in the light-cycle.
- EEG is a powerful physiological biomarker for target-engagement. Gamma power emerges as a reliable end-point across mammalian species used in preclinical research, in agreement with published human data.

REFERENCES

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