



RESEARCH ARTICLE

REVISED Short- and long-term habituation of auditory event-related potentials in the rat [v2; ref status: indexed, <http://f1000r.es/3dv>]

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v2 First published: 11 Sep 2013, 2:182 (doi: [10.12688/f1000research.2-182.v1](https://doi.org/10.12688/f1000research.2-182.v1))
 Latest published: 01 May 2014, 2:182 (doi: [10.12688/f1000research.2-182.v2](https://doi.org/10.12688/f1000research.2-182.v2))

Abstract

An auditory oddball paradigm in humans generates a long-duration cortical negative potential, often referred to as mismatch negativity. Similar negativity has been documented in monkeys and cats, but it is controversial whether mismatch negativity also exists in awake rodents. To this end, we recorded cortical and hippocampal evoked responses in rats during alert immobility under a typical passive oddball paradigm that yields mismatch negativity in humans. The standard stimulus was a 9 kHz tone and the deviant either 7 or 11 kHz tone in the first condition. We found no evidence of a sustained potential shift when comparing evoked responses to standard and deviant stimuli. Instead, we found repetition-induced attenuation of the P60 component of the combined evoked response in the cortex, but not in the hippocampus. The attenuation extended over three days of recording and disappeared after 20 intervening days of rest. Reversal of the standard and deviant tones resulted in a robust enhancement of the N40 component not only in the cortex but also in the hippocampus. Responses to standard and deviant stimuli were affected similarly. Finally, we tested the effect of scopolamine in this paradigm. Scopolamine attenuated cortical N40 and P60 as well as hippocampal P60 components, but had no specific effect on the deviant response. We conclude that in an oddball paradigm the rat demonstrates repetition-induced attenuation of mid-latency responses, which resembles attenuation of the N1-component of human auditory evoked potential, but no mismatch negativity.

Article Status Summary

Referee Responses

Referees	1	2	3
v1 published 11 Sep 2013	 report	 report	 report
v2 published 01 May 2014 REVISED		 report	

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How to cite this article: Gurevicius K, Lipponen A, Minkeviciene R and Tanila H. **Short- and long-term habituation of auditory event-related potentials in the rat [v2; ref status: indexed, <http://f1000r.es/3dv>]** *F1000Research* 2014, 2:182 (doi: [10.12688/f1000research.2-182.v2](https://doi.org/10.12688/f1000research.2-182.v2))

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Grant information: The author(s) declared that no grants were involved in supporting this work.

Competing interests: The authors have no competing interests to declare.

First published: 11 Sep 2013, 2:182 (doi: [10.12688/f1000research.2-182.v1](https://doi.org/10.12688/f1000research.2-182.v1))

First indexed: 10 Mar 2014, 2:182 (doi: [10.12688/f1000research.2-182.v1](https://doi.org/10.12688/f1000research.2-182.v1))

REVISED Amendments from Version 1

We thank all the reviewers for taking time to review the manuscript and make suggestions for its improvement. Based on the reviewers' comments we made the following modifications: Firstly, we rewrote the introduction to clarify the objectives of the manuscript. In particular, we now stress the necessity to develop a non-anesthetized/unrestrained animal model of mismatch negativity (MMN) and clarify role of hippocampus in the generation of MMN. Secondly, we rewrote one paragraph in the discussion to make it more consistent with the general objectives of the manuscript outlined in the introduction. We also would like to thank the reviewers for pointing out several important references, which have been added through-out the manuscript. Thirdly, according to reviewers' comments, we slightly modified Table 1, and Figure 2–Figure 4. Finally, we clarified a number of small methodological details. In particular, the choice of reference and recording location, signal quality control, and the dataset used for some of the statistical comparisons.

See referee reports

Introduction

The auditory oddball paradigm, in which a series of repeated standard stimuli are interrupted by occasional deviant stimuli, has been used extensively in cognitive psychology to study early stages of auditory processing in humans¹. Typically a sufficiently rare deviant stimulus evokes a long-duration negative potential shift beginning 100–200 ms after the stimulus onset, referred to as mismatch negativity (MMN). MMN has been considered an electrophysiological correlate of a mismatch between the incoming stimulus and a sensory memory trace².

The underlying neuronal mechanisms of MMN have been extensively studied and electrical and magnetic recordings in human subjects have localized the MMN generator to the auditory cortex³, although a frontal component has also been observed⁴ (*see also review*⁵). In addition, a substantial amount of animal work has contributed to our mechanistic understanding of MMN. MMN-like responses have been reported in various animal species including cats⁶, guinea pigs⁷, rabbits⁸, monkeys⁹ and rats^{10,11}. Interestingly, animal studies have suggested that at least in some species subcortical brain regions, thalamus¹² and hippocampus^{11–13} might be involved in generating subcomponents of MMN. It is worth noting that hippocampus has been suggested to play a major role of detecting novelty^{14,15}. In support of this, posterior hippocampal lesion dramatically reduces the novelty related cortical P3a event-related potential and autonomic skin reaction¹⁶. However, it is still unclear whether MMN and P3a represent different functional outcome or whether they represent the short-term and medium-term trace of the same novelty/deviant detection mechanism¹⁵. In addition, severity of Alzheimer's disease, and presumable the progression of hippocampal damage, is related to the MMN amplitude decrease¹⁷. Nevertheless, the involvement of hippocampus in MMN is not supported by the intracranial EEG recording studies of neurological patients^{18,19} although the plausible confounding effect of the neurological disorder of subjects or medication to MMN cannot be ruled out.

The contribution of hippocampus to MMN has been studied in more detail in rats but only in anesthetized animals¹¹ (and Ruusuvirta

et al., submitted). Therefore, the role of anesthesia to MMN needs to be clarified. A chronic recording in non-anesthetized animals also opens possibilities for studying the effects of neuropharmacological manipulation without confounding effects of anesthesia. In this regard, an interesting target is the basal forebrain cholinergic projection system to the hippocampus and cortex, which degenerates early on in Alzheimer's disease²⁰. Only few studies so far have investigated the effects of cholinergic drugs on MMN. Scopolamine, a centrally acting cholinergic antagonist, reduced MMN amplitude to frequency change one hour after injection in young adults²¹, while no such change was observed in elderly subjects²². However, both studies reported some modulation effects of scopolamine on P50 and P100 components.

The main aim of this study was to develop a non-anesthetized rat model of cortical MMN. In addition, we wanted to clarify the putative role of the hippocampus in generation of MMN or its subcomponents in non-anesthetized rats. Second, this model allowed us to distinguish between MMN and long-term adaptation to standard auditory stimuli. Therefore, we repeated the oddball stimulus set on two daily sessions and on consecutive days to assess within-day and more long-term adaptation of the response. Finally, we wanted to elucidate the contribution of the cholinergic projection system in cortical and hippocampal MMN by the central muscarinic receptor antagonist scopolamine. We report evidence for repetition-induced attenuation of the mid-latency auditory ERPs in freely-moving rats but no correspondence to the sustained negativity around 100–200 ms in response to the deviant sound that is referred to as MMN in humans.

Methods

Animals

Male Wistar rats (Laboratory Animal Center, University of Eastern Finland, Kuopio, Finland, n=12, weight 412 ± 9 g) were reared in groups of 2–4 until 5 months of age and individually thereafter in a controlled environment (temperature +21°C, lights on from 7:00 h to 19:00 h, water and food available ad libitum) Animals were housed in stainless steel metal cages, floor 31 cm × 45 cm, height 18 cm according to the guidelines of the Council of Europe ETS123. At the age of 5–6 months, the rats were chronically implanted with two recording electrodes made of 50 µm insulated stainless steel wire (California Fine Wire Company Co, Grover Beach, CA, USA) in the hippocampus at the following stereotaxic coordinates: AP (from Bregma) - 3.8, L (from Bregma) +3.1, V (from brain surface) - 3.1 with a vertical separation of the tips of 0.6 mm. In addition, two cortical screw electrodes (Wurth Electronics, Finland) were fixed on the (left and right) parietal bones (L ± 2.0 mm and A -7.5 mm from Bregma). A frontal screw was selected as the ground and a common reference electrode because in our previous unpublished studies the prelimbic or infralimbic cortices did not show AEP or MMN components in rats. The hippocampal electrode closest to the pyramidal cell layer (based on histological and electrophysiological markers) and the right parietal cortical electrode were selected for the final analysis of evoked potentials. The location of the parietal screw electrode was chosen based on our pilot studies such that it picks up the maximum AEP amplitude. This electrode location allowed us to indirectly record auditory cortical response while avoiding severe damage of large temporal muscles attached to the

skull above the auditory cortex in the rats. The rats were anesthetized with a mixture of pentobarbital and chloral hydrate (40 mg/kg i.p. each), and, for post-operative analgesia, they received 5 mg/kg of carprofen (Rimadyl®, Vericore, Dundee, UK) intraperitoneally. The rats were housed in individual cages after the surgery. Recordings started after at least 2 weeks of recovery period. Before the present series of experiments the rats participated in a pharmacological EEG study and thus had been extensively handled. Care was taken to have a washout period of at least three weeks before the current study on AEPs. All animal procedures were carried out in accordance with the guidelines of the European Community Council Directives 86/609/EEC and approved by the State Provincial Office of Eastern Finland.

Data acquisition

In total 10 rats were recorded for the study but due to poor signal (i.e. bad electrode contact) in some channels, the number of recorded animals in the analysis varied from 6 to 9. During the recordings the rat was able to freely move in a brown paste-board cylinder (70 cm diameter, 50 cm height) that was highly familiar to the rat due to previous EEG recordings. Two conventional speakers were placed on the opposite sides outside the cylinder. Auditory stimuli were created through a computer sound card (Sound Blaster 16, Creative Technology Ltd, Singapore, Singapore) and included pure sinusoidal tones of 7, 9 or 11 kHz pitch (tone duration 150 ms, 70 dB, rise/fall time 5 ms). The signal was analog filtered for the 1–1000 Hz band, amplified ($\times 1000$ – 5000), and digitized at 2 kHz per channel for further processing using a commercial software (Experimenter's Workbench, DataWave Technologies, Longmont, CO, USA).

At the end of the experiment, the rats were euthanized by an overdose of pentobarbital and chloral hydrate (each 80 mg/kg i.p.) and the sites of the electrode tips were marked by passing a 30 μ A anodal current for 5 s through each hippocampal electrode. Subsequently, the brains were immersion-fixed overnight with 4% formalin solution (Sigma-Aldrich) and sectioned at 50 μ m with a vibratome

(Leica VT1000s). The sites of the electrolytic lesions were verified in sections stained with cresyl violet (Sigma-Aldrich) by using a light Olympus CX microscope [Figure 1](#).

Study design

The basic study protocol was a conventional mismatch (or oddball) paradigm consisting of one standard tone and one or two deviant tones. Under most conditions, the standard was 9 kHz and the deviants were 7 and 11 kHz tones. Both a low and a high deviant were used to exclude the contribution of tonotopy to auditory evoked potential (AEP) amplitudes. Every run consisted of 400 repetitions with a 1-s inter-stimulus interval. The three tones (7, 9 and 11 kHz) were presented in a pseudo-random order, so that the proportions of the standard, deviant 1 and deviant 2 tones were 85%, 7.5% and 7.5%, respectively.

Experiment 1 consisted of three consecutive days with the 9 kHz tone as the standard, and 7 and 11 kHz tones as the deviants. Similar recordings were performed during Experiment 2 (three weeks after Experiment 1) that also consisted of three consecutive runs. Day 1 replicated Day 1 of the Experiment 1, and was followed by a similar run on Day 2. In addition, Day 2 included a second run with the mismatch contingency reversed, so that 7 kHz became the standard and 9 kHz the deviant. Experiment 3 (one week later) included pharmacological manipulations and consisted only of two runs, one on Day 1 and the second on Day 4. In the first run the standard tone was 9 kHz and the deviants 7 and 11 kHz. In the second run the standard tone was 7 kHz and the deviant 9 kHz. Four rats received scopolamine (0.2 mg/kg, s.c.; Sigma-Aldrich) 20 min before the first run, and five rats before the second run. Saline was used as control treatment.

Data analysis

First, all signals were corrected for amplification. Waveform averaging and AEP peak detection were conducted by custom made routines in Visual Basic under Microsoft Excel® (version 2002).

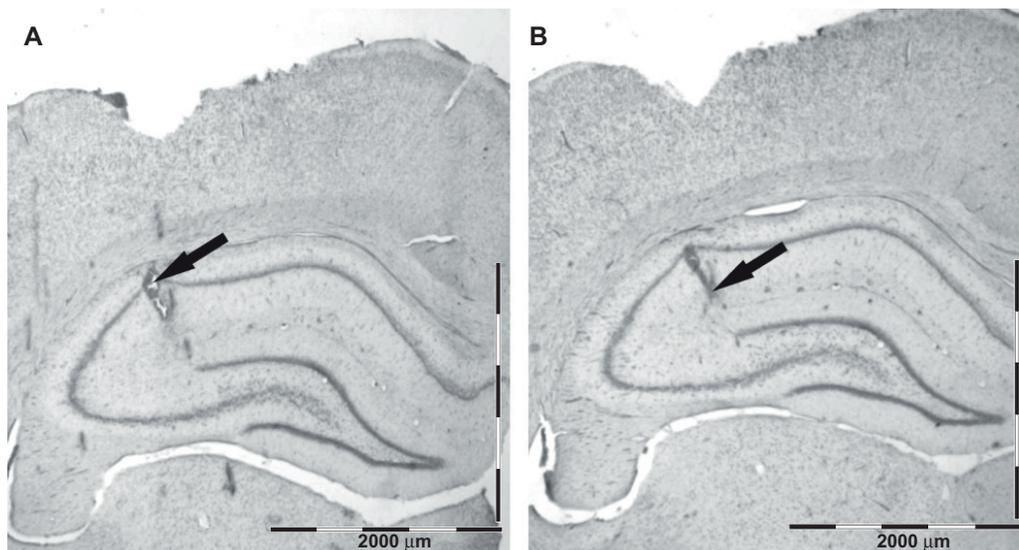


Figure 1. Histological verification of the electrode placement in the hippocampus. The arrows point to the lesion marks corresponding to the two electrode tips, the upper one in the alveus/oriens and the deeper one in the hippocampal fissure. Scale bar = 2000 μ m.

The AEP in a typical rat had three middle-latency components, N40, P60 and P110 (N40 means a negative deflection at 40 ms). In addition, these components were followed by a broad negativity from 150 ms to 250 ms after the stimulus onset (Figure 2A, B). The amplitude of these components was calculated as a maximum deviation from the baseline. The baseline was calculated for each rat from the averaged response between 0 and 100 ms before stimulus onset. When calculating mismatch effect between standard and deviant AEP, we focused on the middle-latency components only (N40, P60 and P110).

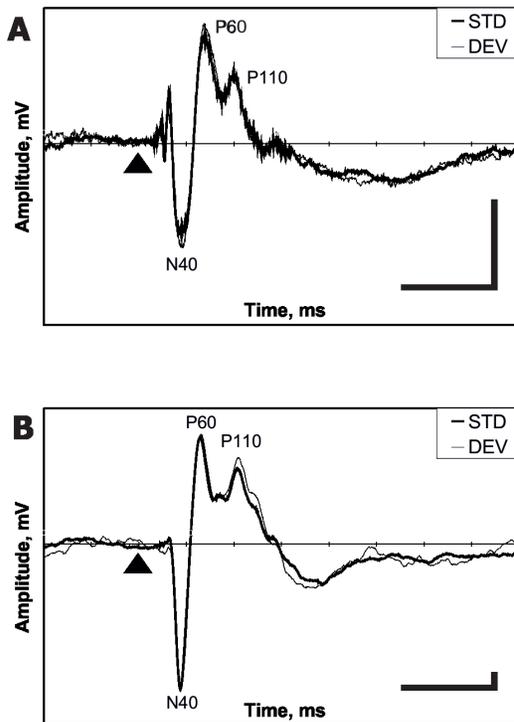


Figure 2. Representative examples of averaged AEPs obtained in the auditory oddball paradigm (grand average of the Day 1 in Experiment 1). Cortical (A) and hippocampal (B) AEPs. The thin line denotes the response to the deviant tone and the thick line the response to the standard tone. The triangle marks the tone onset. The horizontal bar corresponds to 100 ms, the vertical bar to 0.04 mV (scale for the cortex is five times smaller than that for the hippocampus). Negativity is downward.

The statistical analysis was conducted by using SPSS for Windows 11.5 software. The standard and deviant responses were compared within-subjects using ANOVA with repeated measures with the run (1–3) or drug (placebo or scopolamine) as additional within-subject factors. The threshold for significance was set to $p < 0.05$.

Results

Electrode location

Histology verified the location of the hippocampal electrodes in the intended layers: the top electrode in the stratum pyramidale – stratum radiatum and the deeper one in the hippocampal fissure – outer molecular layer of the dentate gyrus. The typical location of the hippocampal electrodes is illustrated in Figure 1.

AEP components

Representative examples of an averaged cortical and hippocampal AEPs obtained in the auditory mismatch paradigm are shown in Figure 2. The components N40, P60 and P110 were identified for each rat and pooled for standards and deviants for all drug-free days. The exact latencies of these components are summarized in Table 1 and their mutual correlations in Table 2. The mutual Pearson correlation coefficients were high and significant for all components of the hippocampal response (if the absolute value of one component grows there is a high probability that other components will also grow). This suggests that physiological sources of AEP components are not completely independent. On the other hand, only the mutual correlations of the P60–P110 components in the cortical response reached a comparable significance level. Furthermore, neither cortical P60 nor P110 correlated with any hippocampal component, which suggests that the cortical and hippocampal responses are largely independent, with the exception of the early N40 component.

Increased cortical response to the deviant tone

The overall analysis of all three days of Experiment 1 revealed larger cortical responses to the deviant tone compared to the standard tone (Figure 2A, and Figure 3). The difference was significant for N40 [$F(1,7) = 7.7, p = 0.03$] and P60 ($p = 0.04$) components and approached significance for P110 ($p = 0.06$). However, the shape of the average evoked response remained the same, and there was no evidence for the typical mismatch negativity as reported in human studies². In contrast, the hippocampal response did not differentiate between the standard and the deviant tones ($p \geq 0.10$ for all components). Together with the correlation table (Table 2) this finding

Table 1. Latencies for defined mid-latency components in [ms] (combined three days of Experiment 1).

	CORTEX			n = 26	HIPPOCAMPUS			n = 20
	N40	P60	P110		N40	P60	P110	
Mean Latency	44.42	70.82	98.26	DEV	44.11	66.72	103.37	
Sem	0.68	1.04	0.93		0.39	1.27	0.49	
Mean Latency	43.32	69.84	98.20	STD	43.50	65.96	103.35	
Sem	0.81	1.11	0.70		0.34	1.33	0.40	

Table 2. The correlation matrix for middle-latency components (pooled data from Experiment 1 & 2).

		N40 CTX	P60 CTX	P110 CTX	N40 HIPP	P60 HIPP	P110 HIPP
N40	Pearson Correlation	1	0.04	-0.15	0.26*	0.02	-0.35**
CTX	Sig. (2-tailed)	.	0.667	0.141	0.024	0.851	0.002
	N	94	94	94	76	76	76
P60	Pearson Correlation		1	0.41**	0.16	0.00	0.17
CTX	Sig. (2-tailed)		.	0.000	0.172	1.000	0.143
	N		94	94	76	76	76
P110	Pearson Correlation			1	0.32**	-0.17	-0.02
CTX	Sig. (2-tailed)			.	0.004	0.139	0.876
	N			94	76	76	76
N40	Pearson Correlation				1	-0.58**	-0.39**
HIPP	Sig. (2-tailed)				.	0.000	0.001
	N				76	76	76
P60	Pearson Correlation					1	0.54**
HIPP	Sig. (2-tailed)					.	0.000
	N					76	76
P110	Pearson Correlation						1
HIPP	Sig. (2-tailed)						.
	N						76

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

speaks against the notion that the cortical response is a simple volume conducted signal from the hippocampus.

Repetition effect on the responses

The amplitude of cortical N40 response was relatively stable in Experiment 1, but the P60 component attenuated significantly between days [F(2,6) = 5.9, $p = 0.04$], and the P110 showed a similar, but non-significant trend [F(2,6) = 1.9, $p = 0.24$]. This trend could be observed for both standard and deviant tones (Figure 3). In contrast, none of the hippocampal components attenuated between days (all p values > 0.40).

The time dependency of AEP attenuation was further investigated in Experiment 2. First, we replicated the standard mismatch condition after 20 intervening days of rest. The cortical response to the standard tones reached the original (or higher) amplitude of Day 1 in Experiment 1 (Figure 3). The ANOVA for repeated measures revealed significant enhancement of cortical P60 [F(1,6) = 12.9, $p = 0.01$] and P110 ($p = 0.03$) components between Day 3 of Experiment 1 and Day 1 of Experiment 2. Interestingly, these were the same components that were also attenuated over three daily sessions in Experiment 1. Although a similar trend was observed in the N40 component in some animals, the difference did not reach significance at the group level ($p > 0.15$). The response enhancement after 20 intervening days could be observed to some extent for both standard and deviant stimulus (Figure 3). In contrast, hippocampal responses, which did not change significantly over the three days of Experiment 1, did not increase after the 20 intervening days of rest, either (all $p > 0.35$).

Next, we repeated the same mismatch condition on Day 2 of Experiment 2 to see whether this habituation of responses between days could be replicated. This time we saw an attenuation of cortical N40 [F(1,6) = 8.6, $p = 0.03$] and P60 [F(1,6) = 20.0, $p = 0.004$] components; and a similar, but not significant trend of P110 component [F(1,6) = 1.7, $p = 0.24$] (Figure 3). In addition, habituation of hippocampal N40 reached significance [F(1,5) = 12.9, $p = 0.02$]. Again habituation was similar for the standard and deviant responses. Furthermore, the difference between AEPs to the standard and deviant tones could be replicated. However, this time the most robust oddball effect was observed for cortical P110 [F(1,6) = 29.3, $p = 0.002$], while P60 showed only a trend ($p = 0.07$), and N40 no effect ($p > 0.30$). Unlike in Experiment 1, the hippocampal P60 component showed a clear oddball effect [F(1,5) = 15.2, $p = 0.01$].

Finally, we reversed the mismatch contingency on the second run of Day 2. The reversal resulted in a robust enhancement of both cortical [F(1,6) = 12.2, $p = 0.01$] and hippocampal N40 [F(1,5) = 28.7, $p = 0.003$] components, which increased even above the Day 1 (of Experiment 2) level (Figure 3). This change was observed for both the standard and deviant tones. No other cortical or hippocampal components were enhanced after the reversal (all $p > 0.14$), but the reversal removed the oddball effect for hippocampal P60 and cortical P110 components (Figure 3).

Scopolamine effect on the middle-latency components

Muscarinic receptors in the central nervous system (CNS) play an important role in the regulation of arousal, attention and synaptic

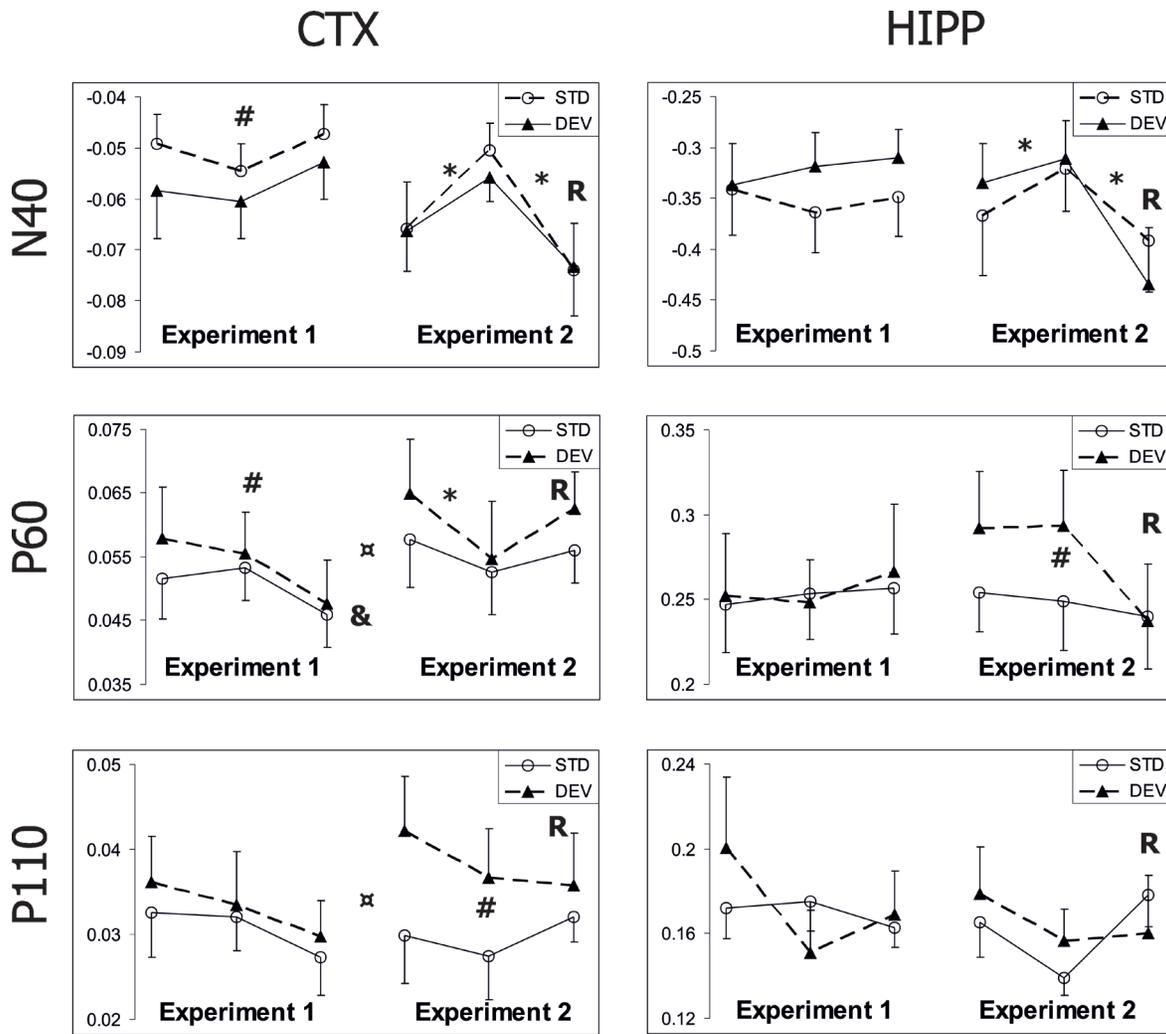


Figure 3. Effect of repetition on the AEP in response to the standard and the deviant tones. Mean amplitudes of AEP components (N40, P60, P110) ± SEMs are given. In each chart the x-axis represent different runs of the test. Note the break between Run 3 of Experiment 1 and Run 1 of Experiment 2 to indicate the intervening days.

R under Run 3 of Experiment 2 indicates reversal of the mismatch contingency.

* significant difference between consecutive Runs;

α significant difference between Run 3 of Experiment 1 and Run 1 of Experiment 2;

significant difference between standard and deviant responses;

& significant repetition effect on component attenuation.

plasticity^{23,24}. To test the contribution of muscarinic receptors on the mismatch effect, we used the subtype nonspecific muscarinic antagonist, scopolamine²⁵, in Experiment 3.

Scopolamine resulted in general attenuation of the cortical response, with significant effects in the N40 and P60 components (Figure 4; $p = 0.03$ and $p = 0.04$, respectively). In the hippocampal response, only the P60 component decreased significantly ($p = 0.002$). In Experiment 3, differences were no longer detected between the responses to the standard and deviant sounds for any of the cortical or hippocampal components. Furthermore, the effect of scopolamine did not differ for the standard vs. deviant response (for all sound × drug interactions $p > 0.45$).

Summary of middle-latency Auditory Evoked Potential components in all Experiments

1 Data File

<http://dx.doi.org/10.6084/m9.figshare.785757>

Discussion

Mismatch negativity (MMN) is a well established phenomenon in humans and widely studied within the field of cognitive neuroscience and psychology. Numerous studies have verified that MMN or MMN-like phenomena also exists in different animal species⁶⁻¹¹ and some of these studies have implicated a role of subcortical structures

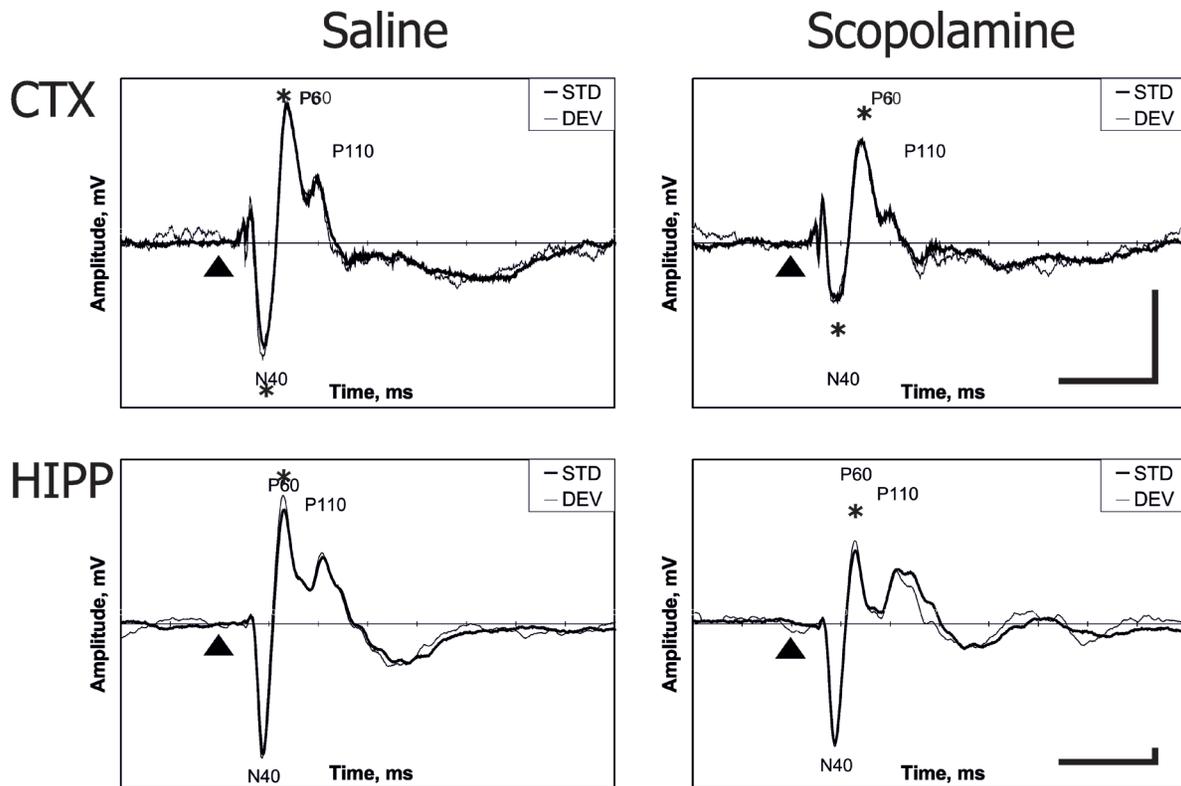


Figure 4. Scopolamine effect on cortical and hippocampus AEPs. Representative example obtained from one rat in the auditory oddball paradigm. The thin line indicates the response to the deviant tone and the thick line the response to the standard tone. The triangle marks the tone onset. Horizontal bar corresponds to 100 ms, vertical bar to 0.04 mV (cortical scale is 5.7 times smaller than that for the hippocampus). Negativity is downward.

* significant difference between scopolamine and saline runs.

in generating MMN or subcomponents of MMN. However, earlier studies in rats have been conducted in anesthesia, which may seriously confound the results. The present study in awake, freely moving rodents found evidence for repetition-induced attenuation of the mid-latency auditory ERPs (in cortex and hippocampus) but no correspondence to the sustained negativity around 100–200 ms in response to the deviant sound that is referred to as MMN in humans.

The sensitivity of the rat auditory system as a function of stimulus frequency is very different from that of humans. The human auditory system is sensitive to frequencies from about 20 Hz to a maximum of around 20,000 Hz, with a peak sensitivity between 2 and 5 kHz. In contrast, in rats the auditory evoked potential increases in amplitude from 2 to 8 kHz reaching a plateau until 20 kHz²⁶. Therefore, having the deviant sounds higher than the standards can yield a false impression of MMN. This possibility was excluded in the present study by using a balanced number of higher and lower deviants and averaging their responses when comparing them to the standard. Nevertheless, the cortical ERPs in Experiment 1 had higher amplitudes in response to the deviant than the standard tones. Notably, the overall shape of the ERP did not change, and we found no evidence for a sustained shift – whether negative of positive – that would resemble the human MMN. Interestingly, no

augmentation of the ERP to the deviant tone was observed in the hippocampus.

Whereas the number of high vs. low deviants was balanced in the present study, the standard and deviant responses differed in an important parameter, the repetition rate. The standard was presented at the proportion of 85%, while each deviant was presented only at 7.5%. One of the studies in anesthetized rats²⁷ reported augmented responses to deviant sounds, which the authors interpreted in terms of repetition rate. In the present study, the cortical ERPs gradually decreased over three daily sessions (Experiment 1) and returned to the original levels after a three-week break between Experiments 1 and 2. The decrement of ERP from session to session was again replicated in Experiment 2. Notably, this decrement in ERP amplitude was roughly the same for the standard- and deviant-evoked responses. The most parsimonious interpretation to these findings is that both the response enhancement to deviant stimuli and general ERP decrement over time reflect gradual attenuation of auditory ERPs to stimulus repetition. This interpretation is also consistent with the disappearance of all differences between standard- vs. deviant-evoked responses after the standard and deviant stimuli were reversed. Namely, after the reversal the cumulative number of the former deviant stimuli soon approached that of the standard for that

session. Thus our findings largely support the conclusion of Lazar and Metherate²⁷ that the enhanced response to the deviant sound in an oddball paradigm can be attributed to differences in repetition rate.

Some of the present findings, however, cannot be explained by differences in repetition rate. First, after reversal of the task contingency, the N40 responses (for both the standard and the deviant tone) increased markedly in amplitude. A change in repetition rate could explain why the responses increased to the 9 kHz stimulus, the former standard that now became the deviant (proportion change from 85% to 15%, as only one deviant was used in this part of the experiment). However, this enhancement was also found for the 7 kHz stimulus that became much more frequent (7.5% vs. 85%). Moreover, the enhancement could be observed not only in the cortical channel that was sensitive to the repetition rate, but also in the hippocampus. A similar response to the reversal in the cortex and hippocampus may reflect general arousal or response enhancement in the thalamus or brainstem. A second finding that is at odds with the repetition rate hypothesis was the enhanced deviant-evoked hippocampal P60 and cortical P110 responses. It is possible that these changes after a three-week break in the experiment reflect a 'declarative' kind of memory recall as opposed to gradual response attenuation as a function of stimulus presentation. This finding warrants further studies.

Overall, our conclusion is that no auditory MMN exists in awake rats in contrasts with other studies conducted in anesthetized rats^{10,11,28} or with non-anesthetized mice²⁹. The discrepancy between the results on non-anesthetized and anesthetized rats can be ascribed to the confounding effect of anesthesia on neuronal functions, as evidenced in a human anesthesia study³⁰. Although Umbricht and coworkers²⁹ were able to show MMN-like activity to duration deviants in mice, they could not rule out the possibility that the MMN-like response emerged due to both duration and intensity changes. However, in the same study frequency deviants yielded similar ERPs as the standard stimuli, which is in line with findings our current study.

Due to lack of MMN-like response in our study we can provide only limited remarks on the role of cholinergic system in cortical and hippocampal MMN. However, we can conclude that blocking the central cholinergic muscarinic receptors with scopolamine has a clear attenuating effect on cortical N40 and P60, and on hippocampal P60 components. Previous studies with human subjects have shown that scopolamine modulates cortical P50 and N100 components. In a MEG study of healthy subjects scopolamine increased P50 amplitude and delayed N100²¹ whereas in combined MEG and EEG study of elderly subjects scopolamine delayed P50 and N100 responses²². However, P_a and N_b (peaking approximately 30 ms and 45 ms from stimulus onset, respectively) are augmented after administration of scopolamine³¹. Because of the diversity of these findings we can conclude that we were able to replicate the modulation of auditory ERP by scopolamine, but determination of direction of the effects needs further studies. Thus the rat provides a model to study neuropharmacological regulation of the human N1-component, but other animal models need to be employed for the modeling of human MMN.

Author contributions

K.G. and H.T. designed the experiments. K.G. analyzed the data. K.G., H.T. and A.L. wrote the paper. A.L. carried out experiments. R.M. manufactured the electrodes, implanted them and conducted the brain histology.

Competing interests

The authors have no competing interests to declare.

Grant information

The author(s) declared that no grants were involved in supporting this work.

Acknowledgements

We thank Dr. M. Penttonen for constructive comments on the manuscript.

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Current Referee Status:

Referee Responses for Version 2



Timm Rosburg

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Approved with reservations: 30 June 2014

Referee Report: 30 June 2014

doi: [10.5256/f1000research.4387.r4615](https://doi.org/10.5256/f1000research.4387.r4615)

Gurevicius and colleagues provided a revised version of their study, but I consider the improvements as marginal and, unfortunately, many issues I raised in my previous review have not been addressed.

Furthermore, an inspection of the submitted data file revealed some inconsistencies with the reported statistics.

Gurevicius and colleagues make two claims: (1), there is no evidence for an MMN in the hippocampus of the rat. (2), there is a 'long-term habituation' effect of the cortical P60 component. However, there is a considerable lack of transparency in the data presentation; on the basis of the provided information, the reader cannot fully evaluate the reported results.

1. Figure 2 has now been re-labelled, as showing grand average data from Day 1. At the same time, the graphs are still labelled as 'representative examples'. This is not as clear as it should be. First, it is not clear what is meant by 'representative'. Second, grand average data should not be called 'examples'. Moreover, in Figure 2 the absolute amplitudes of the cortical P60 are larger than the absolute amplitudes of the cortical N40. This is somewhat in contrast to Figure 3 that shows similar absolute amplitudes for the two components. Finally, given the significant difference in the hippocampal P60 between standards and deviants, grand average data of Experiment 2 need to be depicted, as previously requested.
2. The authors do not describe how artefacts were identified and on what basis data were excluded. It is insufficient to state that data were excluded due to a "poor signal (*i.e. bad electrode contact*)". Such a description hinders any replication of the study.
3. It is still not fully clear what reference electrode was used. The authors state a "common reference electrode" was used but they do not describe which electrodes were used for calculating it.
4. As also previously criticized by other reviewers, the description of the conducted statistics is incomplete with some F values missing, but there are some inconsistencies as well.

(a), Table 1 implies that one data point each was excluded from the analysis of Experiment 1. However, the submitted data file does not contain missing values. Consequently, the data file is hardly suited to reproduce the study results.

(b), it is also worth noting that the algebraic signs are reversed in this data file (with positive values for the N40 and negative for the P60 and P110 amplitude values).

(c), furthermore, attempts to reproduce the study results are hampered by the fact that sometimes the degrees of freedom are incorrectly reported (in particular for the repetition effects). Moreover, for the repetition effects, it is unclear whether the reported results of the ANOVA were Greenhouse-Geisser corrected when necessary. This needs to be indicated. - When I re-calculated the repetition effect of the cortical P60 in an ANOVA with Day (Day 1 vs. Day 2 vs. Day 3) and ODDBALL (standards vs. deviants) as within-subjects factors with the provided data, I revealed an insignificant main effect of DAY [$F(2, 16) = 3.451$, $p = 0.094$ after Greenhouse-Geisser correction, $\epsilon = 0.556$].

5. As previously mentioned, the concepts of 'long-term habituation', 'short-term habituation' and 'long-term adaptation' are poorly described and no explicit operational definitions are provided. On the basis of previous 'long-term habituation' studies in humans, I would not expect to see response decrements from day to day but within each experimental day (i.e. within an experimental session); on the basis of the provided data, I am little convinced that there were actually any substantial response decrements from day to day in Experiment 1 (see comment point 4 c).
6. The authors argue that the contrast between the present and previous studies "*can be ascribed to the confounding effect of anesthesia on neuronal functions, as evidenced in a human anesthesia study*". This is not particularly convincing since the cited study of Heinke *et al.* (2004) showed that the human MMN is diminished by anesthesia rather than the other way around. Moreover, the authors claim in the Introduction that the "*severity of Alzheimer's disease, and presumable the progression of hippocampal damage, is related to the MMN amplitude decrement*", referring to the study of Pekkonen *et al.* (1994). However, the severity of Alzheimer's disease and the progression of hippocampal damage were not related to the MMN amplitude decrement in this study.
7. The authors need to clarify to what data the correlation matrix in Table 2 refers to, as previously noted.
8. The title of the study needs to be re-considered, as previously outlined.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Referee Responses for Version 1



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Approved with reservations: 10 March 2014

Referee Report: 10 March 2014

doi:10.5256/f1000research.2055.r2955

I have read the previous two referee reports, which were made several months ago, so I will try to confine myself to new points.

Overview:

This paper is acceptable but needs improvements, including a better narrative. In its current state, it will not be easy for a reader to know exactly how the paper adds to the current state of knowledge. The presentation is a little confusing, sometimes focused on the negative result that there is no hippocampal MMN, sometimes on the claim that there is no awake-rat MMN at all, sometimes on habituation of auditory ERP components that can be observed. The strands of all these stories should run throughout the report, including the Introduction, Results, and Discussion.

The authors seem to claim that they show that there is no MMN in the awake rat ("Our conclusion that no auditory MMN exists in non-anesthetized Rats..."). This claim needs further justification and clarification.

Introduction:

The introduction should better set out what *has* been established about the electrophysiological correlates of the hippocampus in novelty, and why it might be reasonable to test if there is a hippocampal MMN. For instance in the rat, the species examined here, various investigators find increased theta power and/or reduced theta frequency and/or theta reset in novelty. (Theta reset is important to their arguments on the bottom of page 7.) In humans, Knight (1996) produced evidence that a P3 component of the novelty reaction was hippocampus dependent. These and other such evidences would link the hippocampus to novelty, and where available to 'mismatch novelty', and perhaps suggest some larger novelty-related function of the hippocampus. This kind of intellectual context would improve the paper. The introduction should then go on to set out a better rationale for why specifically the authors thought the MMN should be studied in a rat, and why they should look in the hippocampus for this. To my knowledge, it is not a standard current view to suggest that the hippocampus might be a generator of the MMN, but presumably some kind of case can be made? Much more reference to the literature is required. If the aim was simply to detect a rodent MMN, why not look at the auditory and frontal cortex?

- *Electrical and magnetic recordings in human subjects have localized the MMN generator to the auditory cortex, although a frontal component has also been observed. In addition, there are some speculations on subcortical generators, especially the hippocampus, but those cannot be indisputably verified in noninvasive recordings^{5,6}.*

This is misleading. The last two references are not attempts to detect hippocampal origins of the MMN.

- *Therefore, the aim of the present study was to address a number of unresolved issues related to MMN in the rat. First, anesthesia was reported to attenuate MMN in the cat⁷. Therefore, we wanted to test whether MMN can be evoked in freely moving rats^{14,15} rather than in the anesthetized preparation^{11,12}. Second, we compared the event-related potentials (ERPs) recorded using cortical and hippocampal electrodes to reveal a possible hippocampal generator. Third, to distinguish between MMN and long-term adaptation to standard auditory stimuli as suggested by Lazar and Metharate¹³, we repeated the oddball stimulus set on two daily*

sessions and on consecutive days. Fourth, to shed light on the neuropharmacology of MMN, we manipulated the cholinergic input to the cortex and hippocampus by systemic administration of scopolamine."

Add test/rewrite to incorporate the idea that the paper does not have ANY results about the MMN, and to study the auditory-evoked components that are seen.

Methods:

- "The hippocampal electrode closest to the pyramidal cell layer and the right parietal cortical electrode were selected for the final analysis of evoked potentials"

How was it determined which electrode was closest to the layer - purely by histology? Did this turn out to matter much?

- "The rats were involved in an EEG study for three weeks before the current study on evoked potentials."

The procedure of the previous study should be briefly described in a supplementary note together with a comment that they think the other study made no difference to this (assuming the authors think that).

- "In total 10 rats were recorded for the study but due to poor signaling some channels, the number of records in the analysis varies from 6 to 9."

What were the minimum threshold criteria used to determine acceptability of signal?

Results:

- I would suggest splitting up the results by cortical and hippocampal regions even more to avoid ambiguity and perhaps using results headings which summarise the results. That might improve the readability of this paper.
- The figures and indeed main text should state more clearly what values are being entered into averages and so on. E.g. Figure 2, it says 'averaged AEPs'. How many trials per rat, how many rats, an equivalent number of trials per rat?
- Figure 3 and Results text 2nd paragraph in Repetition effect on the responses: "*The cortical response to the standard tones reached the original (or higher) amplitude of Day 1, in Experiment 1 (Figure 3). The ANOVA for repeated measures revealed significant enhancement of cortical P60 [$F(1,6) = 12.9, p = 0.01$] and P110 ($p = 0.03$) components between Day 3 of Experiment 1 and Day 1 of Experiment 2.*"

I suggest they use a different statistical symbol than a star to make this point in Figure 3, emphasising the between-experiment changes. The star is already used.

- Table 2 legend. What is the measure being correlated (one amplitude value, mean amplitude over a set period?), and from which datasets? How does the $n = 94$ and 76 break down?

Discussion:

- "Our conclusion that no auditory MMN exists in non-anesthetized rats contrasts with another studies conducted in anesthetized rats^{11,12,20}."

Overclaiming? On what basis can the authors say that "no auditory MMN exists in non-anesthetized rats", when they have not recorded from the auditory cortex? Or are they claiming they did sample the auditory cortex in some way? See the queries in Reviewer Rosburg Point 3b.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

**Timm Rosburg**

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Approved with reservations: 20 December 2013

Referee Report: 20 December 2013

doi:[10.5256/f1000research.2055.r2641](https://doi.org/10.5256/f1000research.2055.r2641)

The mismatch negativity (MMN) represents the cortical response to sound deviance in an otherwise uniform stimulation. This component of auditory evoked potentials (AEPs) has gained large interest in clinical neurophysiology. In order to understand underlying cortical mechanisms of the MMN, it is of great importance to further establish animal models that allow, for example, investigation into the effects of pharmacological interventions on MMN generation.

Given this, the study of Gurevicius and co-workers addresses an important issue of clinical neurophysiology. In their study, AEPs in response to standard tones and deviants were recorded from the hippocampus and parietal cortex of awake, freely moving rats. However, as a main finding, a MMN-like signal could not be observed. Overall, I think the study would benefit from a clearer description of the relation between MMN findings in humans and animals, a clearer outline of the study purpose, the clarification of some methodological details, re-calculation of the amplitude values, and some stronger focus on significant results (rather than on null-findings). I have the following additional comments:

1. The relation between human and animal MMN data is not sufficiently described: Invasive AEP recordings in humans did not reveal any evidence that the MMN is generated in the hippocampus (Halgren *et al.*, 1995; Kropotov *et al.*, 1995; 2000; McCarthy *et al.*, 1989; Rosburg *et al.*, 2007). In contrast, MMN-like signals in humans have been recorded from the temporal cortex (Kropotov *et al.*, 1995; 2000) and in some few instances also from the frontal cortex (Rosburg *et al.*, 2005). Consequently, I find it a little surprising that the authors did not observe a MMN-like hippocampal signal in rats. Moreover, it is not fully clear to me whether the authors doubt the value of other, already established animal models on MMN generation, like the mice model of Umbricht *et al.* (2005) or the cat model of Csepe *et al.* (1987).

2. The study is entitled as “*Short- and long-term habituation...*”, and indeed much space is dedicated to the description on how the recorded AEPs varied from one recording day to the next or from one experiment to the next. However, it is not evident what the authors actually mean by ‘short-term’ and ‘long-term’. In human recordings, ‘short-term decrement’ is usually conceptualized as response decrease from one stimulus to the next. For this kind of decrement, it has been argued that it reflects a consequence of refractoriness (rather than a process of habituation) (Barry *et al.*, 1992; Budd *et al.*, 1998; Rosburg *et al.*, 2004; 2006; 2010; 2013). However, Gurevicius and co-workers did not assess this kind of response decrements, concentrating exclusively on different forms of long-term decrement. Such long-term decrements might reflect a process of habituation, albeit further studies are warranted to support this notion (Rosburg *et al.*, 2002). In consequence, the title of the study should be modified. Furthermore, I propose that the term ‘long-term decrement’ should be used, rather than ‘long-term habituation’.
3. The authors present only exemplary AEP data, but no grand average data. Since the statistics are based on group data, the grand average AEP data need to be depicted (at least across experiment 1). Full evaluation of the study is not possible on the basis of exemplary data. Moreover, there are a couple of technical details that require clarification:
 - (a) The morphology of the depicted AEPs in the hippocampal and cortical recordings look rather similar. Based on the study of Ruusuvirta *et al.* (2013), I would have expected to find clear phase differences between the two recording sites.
 - (b) Related to this issue, the choice of the active and reference electrode sites needs to be justified. Is the parietal electrode assumed to record activity from the auditory cortex? Is the frontal reference electrode assumed to be electrically silent for auditory stimulation?
 - (c) Only analog filtering of the recordings (1-1000 Hz) is mentioned. Were data additionally offline-filtered?
 - (d) The authors mention that some recordings were excluded due to “poor signal” (p.2). What were the exact criteria for excluding data?
 - (e) The authors do not describe how the data were screened for artefacts and how artefacts were handled.
 - (f) According to the text, Table 1 refers to correlations between peak latency values. Is this really the case? Moreover, it is not clear on what data Tables 1 and 2 are based. Finally, the peak labels in Table 1 do not correspond to the labels used in the rest of the study.
 - (g) The authors should describe how the stimulus intensity was measured.
4. The N40, P60, and P110 peaks were defined as maximum deviations from the baseline. This kind of quantification introduces a bias for obtaining higher (absolute) amplitude values for AEPs to deviants than to standards because the noise levels affect peak amplitude measures (the maximum peak amplitude is increased by the overlying noise signal, and the noise level decreases with the number of trials, used for calculating the AEP). Thus, the peaks should be quantified as mean amplitudes instead. Moreover, the authors should not imply the existence of differences

when the statistical analysis revealed non-significant results (e.g. “the P110 showed a similar, but non-significant trend [$F(2,6) = 1.9, p = 0.24$ ”, p. 4).

5. Scopolamine had no differential effect on the AEPs to standards and deviants, but scopolamine resulted in general reductions of AEP components (N40, P60). However, the current study cannot differentiate whether this effect is due to peripheral or central effects of the drug. In human recordings, scopolamine administration often leads to delayed AEP responses, in particular of the N100 (e.g. [Pekkonen et al., 2005](#)), but not to an amplitude reduction. One study even described an enhancement of middle-latency auditory evoked neuromagnetic fields ([Jääskeläinen et al., 1999](#)). This divergence between human and animal data should be discussed.
6. There were some AEP differences between standards and deviants in the 2nd experiment. For evaluation, these differences should be depicted as waveform.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.



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Approved: 23 September 2013

Referee Report: 23 September 2013

doi: [10.5256/f1000research.2055.r1785](https://doi.org/10.5256/f1000research.2055.r1785)

This paper provides data on auditory evoked potentials in rats using a mismatch (oddball) paradigm and also tests the role of scopolamine on the evoked potentials. The work appears to have been conducted well and analyzed appropriately.

I have a few minor suggestions for improvement.

1. Fig 2A. 'P6' should be 'P60'
2. How much of the AEP was due to the reference screw v.s. the recording electrode? This is an issue that the authors may wish to address, as the reference screw presumably contributed to the evoked potentials measured in the differential recordings.
3. Table 1. How were the n's determined in this table? Is this the total number of recording sites? Are any recording sites counted multiple times?
4. Need to provide F values and d.f. for all comparisons, not just some, in the paragraph under the heading "Increased cortical response to the deviant tone" as well as in other places in the manuscript. The authors should make sure that the appropriate test statistics and degrees of freedom are provided for all of the measurements not just the resulting p values.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.
