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ORIGINAL INVESTIGATION

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Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD

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Abstract *Rationale:* Exaggerated acoustic startle is a prominent symptom of post-traumatic stress disorder (PTSD); however, its physiological basis is not well understood, and there are few available treatments. Neurobiological research has suggested that anti-kindling agents and/or glutamate antagonists can attenuate the acoustic startle response (ASR) in animal models. The anticonvulsant topiramate is an AMPA antagonist that also demonstrates potent anti-kindling effects and may, therefore, have promise in treating trauma-enhanced ASR. *Objective:* To evaluate the ability of topiramate to attenuate stress-induced increases in ASR in a previously validated animal model of PTSD. *Methods:* Male Sprague-Dawley rats (n=36) served as controls or received single prolonged stress (SPS). SPS consisted of 2 h restraint, forced swim and ether anesthesia, then a 7-day "undisturbed" period. Animals then received vehicle, 10 mg/kg or 30 mg/kg of topiramate orally, twice daily for 7 days. ASR was assessed for all animals before and after the study, in light and dark environments. *Results:* SPS produced a sustained increase in the ASR in both environments, an effect that was significantly reduced by topiramate. Meanwhile the ASR of control animals remained unaffected by topiramate. *Conclusions:* The current results provide one of the few demonstrations of a single stress episode producing sustained enhancement of ASR. In addition, topiramate demonstrates promise in treating exaggerated acoustic startle symptoms in PTSD or other stress-related disorders.

Keywords PTSD · Startle · Topiramate · Single prolonged stress

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Introduction

Exaggerated acoustic startle is a highly prevalent behavioral symptom of post-traumatic stress disorder (PTSD) (Shalev et al. 1992; Orr et al. 1995). However, the physiological relationship between traumatic stress and the acoustic startle response (ASR) remains poorly understood and there are few available treatments. It has been suggested that the amygdala, a limbic region with an established role in mediating the fear response, becomes kindled or sensitized following exposure to traumatic events (Liberzon et al. 1999b; Rauch et al. 2000), and long-term amygdala kindling does produce a significant increase in ASR in rodent models (Ménard et al. 2002). In concert, in animal models of ASR, blockade of the excitatory amino acid glutamate, using AMPA antagonists, attenuates fear-potentiated acoustic startle when injected into the central nucleus of the amygdala and light-potentiated acoustic startle (LPAS) when administered into the bed nucleus of the stria terminalis (Walker and Davis 1997). When administered into the basolateral amygdala, it attenuates both phenomena (Walker and Davis 1997). Thus, compounds demonstrating glutamate antagonism and/or anti-kindling effects may offer promise in attenuating exaggerated startle symptoms.

In this regard, the anticonvulsant topiramate demonstrates potent anti-kindling properties in rodent models (Wauquier and Zhou 1996) and produces a variety of inhibitory pharmacological and physiological effects relevant to ASR, including AMPA antagonism (Gibbs et al. 2000), $GABA_A$ stimulation (White et al. 2000) and blockade of voltage-gated Na^+ channels (Taverna et al. 1999). Although topiramate has shown initial promise in attenuating some PTSD symptoms, such as flashbacks and negative thought intrusions (Berlant and van Kammen 2002), its effectiveness in attenuating trauma-enhanced ASR has yet to be tested.

Recently, a rat model of single prolonged stress (SPS) was developed which successfully reproduced neuroendocrine characteristics of PTSD, including enhanced HPA negative feedback (Liberzon et al. 1997). Preliminary

data in our laboratory also suggested it produced an exaggerated ASR under certain conditions such as in the LPAS paradigm. LPAS assesses the ASR in an unconditioned aversive environment (bright lights) which is relevant to humans in that hyperarousal symptoms appear particularly sensitive in stressful environmental contexts (Grillon and Amelie 1998). Similarly in animal studies, ASR is reliably enhanced in brightly lit environments as compared to dark environments (Walker and Davis 2002). The purpose of the current study was thus to validate the ability of SPS to influence ASR in the LPAS paradigm, and to assess the attenuating effects of the anticonvulsant topiramate.

Materials and methods

Animals

Male Sprague-Dawley rats (n=36) (obtained from Charles River) weighing 150 g at arrival, were housed in groups of three (with ad libitum food and water) at the VA Ann Arbor animal facility. Animals were acclimated to colony rooms for 7 days and then handled for 6 days prior to behavioral testing. During the 6 days of handling, animals were habituated daily to sweetened condensed milk (Eagle Brand) administered orally via a 1 ml syringe. The milk later served as the vehicle for oral administration of topiramate.

Procedure

The experiment was conducted using a 2×3 design, with two groups: single prolonged stress (SPS) and control, in one of three treatment conditions: vehicle-treated, topiramate low dose (10 mg/kg) and topiramate high dose (30 mg/kg) (n=6/group). Pretreatment ASR data was collected for all animals, after which one-half underwent SPS (see below). Following SPS, both control and SPS animals received two daily administrations of topiramate or vehicle over 7 days. ASR data was then collected for all animals at least 24 h after the last drug administration (see Table 1).

Acoustic startle response

On the test day, animals were placed in a sound-proof chamber (37×39×49 cm), containing an electro-voltage platform, connected to a piezoelectric accelerometer (San Diego Instruments). After 5 min of accommodation, startle stimuli was delivered every 30 s for 15 min, consisting of a 50 ms burst of 98 dB or 108 dB white noise. Background noise was set at 55 dB. All animals were tested in both light and dark environments (counterbalanced) with a 5 min break in between. Voltage data was filtered, converted, sampled and transferred to a PC computer using an automated hardware/software package (San Diego Instruments).

Single prolonged stress (SPS)

Rats were restrained for 2 h, followed immediately by a 20-min forced swim in 24°C water. Forced swim occurred with six rats at a time in an 18 gallon plastic tub (55.6 cm diameter, 45.4 cm H), filled two-thirds from the bottom. Following 15 min recuperation, animals were exposed to ether until the loss of consciousness, and then left undisturbed in their home cage for 7 days. The undisturbed period is necessary to replicate conditions that produced PTSD-type symptoms in previous studies. Prior research demonstrated that SPS-induced neuroendocrine effects, such as enhanced HPA negative feedback and changes in the expression of glucocorticoid receptor mRNA, were only evident if a 7-day undisturbed period followed SPS (Liberzon et al. 1997, 1999a).

Drug

Vehicle, 10 or 30 mg/kg doses of topiramate (Johnson & Johnson: Pharmaceutical R & D; Raritan, N.J., USA) were administered twice daily (at approximately 9:00 a.m. and 5:00 p.m.) for 7 days. The drug was mixed in sweetened condensed milk (Eagle Brand, pH 6.3) and 10% 0.1 M KOH solution and administered orally through a 1 ml syringe. Prior research established that orally administered topiramate in male rats has a half-life of 2.5 h (Johnson & Johnson Research Institute: topiramate data sheet), thus necessitating a twice-daily dosing regimen. Prior research has also estimated that, on a relative scale, 7 days in the life span of a rat is roughly equivalent to 6 months in the human life span (Adamec 1997). Thus 7 days of drug administration was believed to reflect the dosing regimen for chronic drug treatment in human populations. Following the final drug administration, at least 8 half-lives elapsed before startle responses were assessed, making it highly unlikely that the acute effects of topiramate were still influencing behavioral measures.

Statistics

A repeated measures ANOVA was used to analyze the effects and interactions of Time (Pre versus Post test), Light (Dark versus Light), Group (control versus SPS), Sound (98 dB versus 108 dB) and Drug (vehicle, 10 mg/kg or 30 mg/kg topiramate) on mean startle amplitude. Where applicable Bonferroni post-hoc analysis (adjusting for multiple comparisons) was conducted for individual comparisons.

Results

As indicated in the introduction, it was expected that all animals would show a higher acoustic startle response (ASR) in the light condition compared to the dark. Furthermore, it was expected that this light potentiation would be enhanced in animals that underwent SPS. A main effect of Light was observed for all animals in response to both the 98 dB [$F(1,30)=5.017$, $P<0.05$] and the 108 dB sound burst [$F(1,30)=12.019$, $P<0.01$] showing that, indeed, the ASR was higher in the light

Table 1 Procedure

Group	Day 1 pre-test	Day 2	Days 3–9	Days 10–16	Day 17 post-test
Control Single prolonged stress (SPS)	Acoustic startle response (ASR)	– SPS	7-day recovery period	Pharmacotherapy (topiramate or vehicle twice daily)	ASR

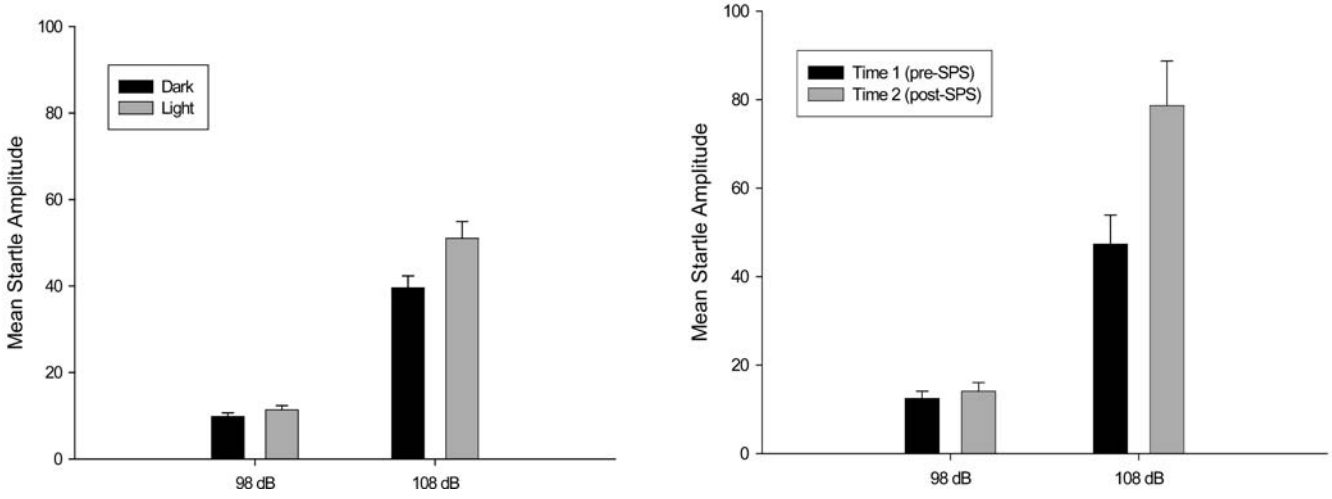

Fig. 1 Comparison of the effects of light (*left panel*) and SPS (*right panel*) on mean startle amplitude in response to 98 dB or 108 dB sound bursts. The *left panel* shows the effect of light on all groups, whereas the *right panel* shows the effect of SPS on vehicle-treated animals

environment. However, this potentiation did not differentially effect the SPS group more than the controls as there was no Light×Group interaction at either sound level [98 dB: $F(1,34)=1.119$, $P=0.282$; 108 dB: $F(1,34)=1.237$, $P=0.274$].

In the current study, two pulse intensities, 98 dB and 108 dB, were used in the LPAS paradigm. However, the lower pulse intensity appeared to produce a very modest ASR. Thus, the mean startle amplitude in response to 98dB (10.5±0.64) was less than 25% of that produced at 108 dB (45.2±2.4). Even though light did potentiate ASR in response to both sound levels, at 98 dB this potentiation was minimal (dark: 9.8±.83, light: 11.4±0.97) in comparison to 108 dB (dark: 39.57±2.76, light: 51.02±3.87; see Fig. 1). Furthermore, 98 dB was ineffective in eliciting significant changes in ASR in animals exposed to SPS [$F(1,5)=0.600$ $P=0.474$) (see Fig. 1). Indeed, a significant Sound×Time×Group interaction [$F(1,30)=9.114$, $P<0.01$] revealed the effect of SPS on the ASR was at the 108 dB sound only. Therefore, further analysis of the effect of topiramate on SPS-enhanced ASR could only be assessed in the 108 dB condition.

With regards to the 108 dB sound burst, subsequent analysis assessed the effects of Time and Group on ASR. Thus, in animals that received vehicle only, a significant Time×Group interaction was observed [$F(1,10)=14.49$, $P<0.01$]. Bonferonni post-hoc analysis revealed that animals exposed to SPS had a significantly higher ASR post-SPS (time 2) compared to pre-SPS (time 1) ($P<0.01$). Furthermore, at time 2, SPS-exposed animals had a significantly higher ASR than control animals ($P<0.05$) (see Fig. 2). The effect of SPS on startle was evident in both the dark [Time×Group: $F(1,10)=12.68$, $P<0.01$] and light environments [$F(1,10)=5.54$, $P<0.01$].

In response to 108 dB, analysis also examined the effect of topiramate on the ASR of SPS and control animals, at the second time point (day 17). In this case, a

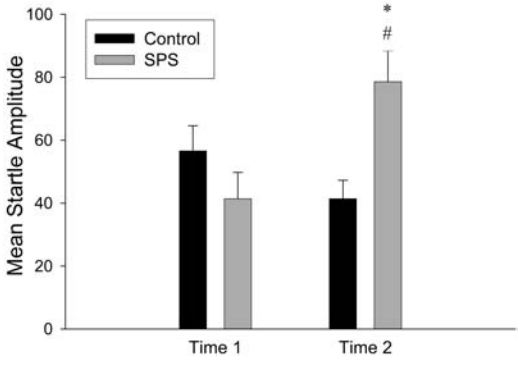

Fig. 2 Mean startle amplitude, in response to 108 dB, for control and SPS rats at time 1 (day 1) and time 2 (day 17). SPS-exposed rats show a heightened startle response at time 2, whereas for control animals the startle response remains unchanged or slightly decreased over time. *Indicates significantly higher startle response compared to SPS (time 1) ($P<0.01$). #Indicates significantly higher startle response compared to control (time 2) ($P<0.05$)

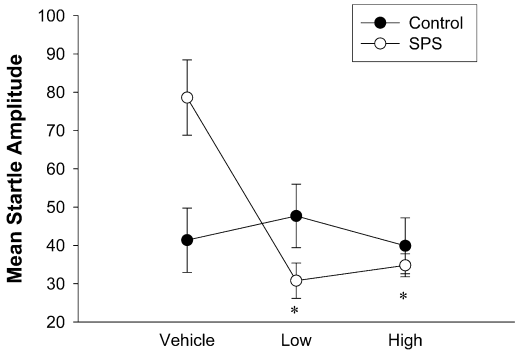

Fig. 3 Mean startle amplitude for control and SPS rats, in response to 108 dB, following chronic treatment with vehicle, low (10 mg/kg) or high (30 mg/kg) topiramate, twice daily. *Indicates significantly different startle amplitude between topiramate and vehicle-treated SPS animals ($P<0.01$)

two-way ANOVA revealed a significant Group×Drug interaction [$F(2,30)=4.835$, $P<0.05$] (see Fig. 3). Bonferroni post-hoc analysis further revealed that, in animals exposed to SPS, a significantly lower ASR was observed for topiramate-treated animals (10 mg/kg and 30 mg/kg) as compared to vehicle-treated animals ($P<0.01$). The effect of topiramate on ASR was observed in both the dark [Group×Drug: $F(2,30)=3.441$, $P<0.05$] and light environments [$F(2,29)=3.436$, $P<0.05$].

Discussion

The current study demonstrates that SPS produces a sustained exaggeration of the ASR. In addition, chronic treatment with topiramate, following SPS, eliminated this exaggeration. This reduction in ASR occurred at both doses, and in both the light and dark environment. Topiramate produced no noticeable effects on startle response in control rats. To our knowledge, this represents the first demonstration of an anti-convulsant agent successfully attenuating stress-induced exaggerations of ASR, without affecting the ASR of non-stressed controls. Ebert and Koch (1996) had found that carbamazepine could reduce the ASR in control and partially kindled rats, although it had a limited effect in fully kindled rats. Research with other established anticonvulsants, such as phenytoin or valproic acid have been limited with respect to ASR.

At this point, the precise mechanism behind the effect of topiramate on stress-induced enhancement of ASR is unknown. Since kindling has been linked to ASR potentiation in certain conditions (Ménard et al. 2002), the anti-kindling and/or anticonvulsant properties of topiramate may be involved. Its effects as an AMPA antagonist might also be relevant. Walker and Davis (1997) showed that AMPA antagonists infused into the bed nucleus of stria terminalis (BNST) blocked light-potentiated ASR, though not fear-potentiated startle. Infusions into the basolateral amygdala disrupted both phenomena. Topiramate may also act at the locus coeruleus (LC), which has a well-established role in mediating arousal. Forced swim sensitizes the electrical discharge activity of LC neurons in response to local CRH administration (Curtis et al. 1999), suggesting such stressors may lower the threshold of activation. Sensory input to the LC is modulated to a considerable degree by glutamate and GABA neurotransmission (Aston-Jones et al. 1990), both of which are targets of topiramate activity. Future studies would benefit by elucidating the precise mechanisms mediating the effects of topiramate on ASR and by further characterizing the full range of therapeutic doses. For instance, additional experiments may seek to independently target specific pharmacological effects of topiramate (e.g. AMPA antagonism, $GABA_A$ stimulation) and/or attempt to identify the threshold dose or minimum dosing regimen at which topiramate begins to demonstrate its startle-reducing effects.

A second intention of the current study was to validate further the ability of the SPS paradigm to produce exaggerations in ASR. In this regard, the current results provide one of the few demonstrations of a single stress episode producing long-term ASR increases. Single episodes of inescapable tailshock also produced exaggerated ASR, though this exaggeration appeared and disappeared at 7 days post-stress (Servatius et al. 1995). Similarly, predator exposure, involving single 5-min non-injurious cat exposure, produced an exaggerated ASR in rats 8 days post-stress (Adamec 1993). In addition to behavioral effects, SPS was previously shown to reproduce PTSD-related neuroendocrine changes, including an upregulation in glucocorticoid receptor mRNA in the hippocampus and enhanced glucocorticoid negative feedback (Liberzon et al. 1997, 1999a). Kato et al. (2002) further demonstrated SPS-induced increases in low-frequency stimulated long-term depressions (LTD) in hippocampal neurons. The physiological converse, inhibition of long-term potentiation (LTP), reliably occurs in hippocampal neurons following a number of stressors including restraint, inescapable tail-shock (Shors et al. 1989, 1994; Kim et al. 1996) and predator exposure (Mesches et al. 1999). Taken together, SPS may thus prove a highly valid animal model of PTSD, capable of reproducing behavioural, neuroendocrine and neurophysiological symptoms.

The current results therefore demonstrate that SPS is capable of producing a sustained enhancement of the ASR, which is attenuated by chronic treatment with topiramate. At this point, a number of potential mechanisms are possible for the startle-reducing effects of topiramate, although further investigation is required. The SPS model may be of significant value in delineating the neurobiological basis of trauma-enhanced ASR, and topiramate itself appears to show considerable promise in treating exaggerated startle symptoms in PTSD and other stress-related disorders.

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