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Author: Daryl B. O'Connor PhD Jessica A. Green MSc Eamonn Ferguson PhD Ronan E. O'Carroll PhD Rory C. O'Connor PhD

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Cortisol reactivity and suicidal behavior: investigating the role of hypothalamic-pituitary-adrenal axis responses to stress in suicide attempters and ideators

Daryl B. O'Connor, PhD[1*], Jessica A. Green, MSc[1], Eamonn Ferguson, PhD[2,], Ronan E. O'Carroll, PhD[3], & Rory C. O'Connor, PhD[4]

[1]School of Psychology, University of Leeds, Leeds UK

[2]School of Psychology, University of Nottingham, Nottingham, UK

[3]Division of Psychology, University of Stirling, Stirling, UK

[4]Suicidal Behavior Research Laboratory, Institute of Health & Wellbeing, University of Glasgow, Glasgow, UK

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Correspondence to:

Daryl B. O'Connor

School of Psychology

University of Leeds,

Leeds, UK

e: d.b.oconnor@leeds.ac.uk

t: ++44 113 3435727

Highlights

Participants who had made a previous suicide attempt exhibited significantly lower cortisol response to acute stress.

Participants who made an attempt within the past year exhibited a blunted cortisol response compared to participants with a more distant history of attempt.

Participants who had made a suicide attempt and had a family history of suicide also exhibited a blunted cortisol response to stress.

Lower levels of cortisol in response to acute stress were associated with higher levels of suicidal ideation at 1-month follow-up in the suicide attempters group

Abstract

Every 40 seconds a person dies by suicide somewhere in the world. The causes of suicidal behavior are not fully understood. Dysregulated hypothalamic-pituitary-adrenal (HPA) axis activity, as measured by cortisol levels, is one potential risk factor. The current study aimed to investigate whether cortisol reactivity to a laboratory stress task differentiated individuals who had previously made a suicide attempt from those who had thought about suicide (suicide ideators) and control participants. One hundred and sixty participants were recruited to a previous attempt, a suicidal ideation or a control group. Participants completed background questionnaires before completing the Maastricht Acute Stress Test (MAST). Cortisol levels were assessed throughout the stress task. Measures of suicide behavior were measured at baseline, 1 month and 6 month follow-up. Participants who had made a previous suicide attempt exhibited significantly lower aggregate cortisol levels during the MAST compared to participants in the control group; suicide ideators were intermediate to both groups. This effect, however, was driven by participants who made an attempt within the past year, and to some degree by those with a family history of attempt. Participants who had made a suicide attempt and had a family history of suicide exhibited the lowest levels of cortisol in response to stress. Finally, lower levels of cortisol in response to the MAST were associated with higher levels of suicidal ideation at 1-month follow-up in the suicide attempter group. These results are consistent with other findings indicating that blunted HPA axis activity is associated with some forms of suicidal behavior. The challenge for researchers is to elucidate the precise causal mechanisms linking stress, cortisol and suicide risk.

Keywords: cortisol reactivity, chronic stress, HPA axis, self-harm,allostatic load

1. INTRODUCTION

Every 40 seconds a person dies by suicide somewhere in the world (WHO, 2014). Researchers have been exploring the causes of suicidal behavior for many decades with a view to identifying targets for suicide prevention. To this end, numerous models have been proposed that differ in their emphasis on the role of psychological, social, psychiatric and neurobiological factors in predicting risk of suicide (Mann *et al*., 1999; O'Connor, 2011; O'Connor and Nock, 2014; van Heeringen and Mann, 2014; van Orden *et al*., 2010). Central to many of these models is a stress-diathesis component, which states that suicidal behavior is a result of an interaction between acutely stressful life events and a susceptibility to suicide (a diathesis). Stress-diathesis explanations of behavior (and illness) typically have three aspects: a predispositional vulnerability factor, a stressful life event(s) or trigger(s) and protective factors that may shield the individual from developing the illness (or in this case, engaging in suicidal behavior). In terms of a vulnerability factor, data from post-mortem, neuroimaging and in-vivo studies are emerging that a trait diathesis is not only manifested in impairments of the serotonergic and noradrenergic neurotransmitter systems, in structural brain abnormalities and via epigenetic pathways but also in dysregulation of hypothalamic-pituitary-adrenal (HPA) axis stress response activity (Mann, 2013; Turecki *et al*., 2012; van Heeringen *et al*., 2011; van Heeringen and Mann, 2014).

Cortisol is the primary effector hormone of the HPA axis stress response system, and has received extensive empirical investigation. In the context of suicide research, the majority of previous work has focused on assessing HPA axis functioning through pharmacological manipulation of the stress system (e.g., see Coryell and Schlesser, 2001; Coryell *et al.*, 2006; Mann and Currier, 2007; Pompili *et al.*, 2010). However, recently researchers have turned their attention to investigating other aspects of the cortisol response, such as cortisol reactivity to laboratory stressors (e.g., Giletta *et al*., 2015; McGirr *et al*., 2010). McGirr and colleagues (2010) explored the extent to which dysregulation of the HPA axis to a laboratory stressor was a heritable risk factor for suicidal behavior. In this study, a small sample of first-degree relatives of suicide completers and matched controls were compared on their cortisol reactivity to a well-established psychosocial stressor known as the Trier Social Stress Test (TSST; Kirschbaum *et al*., 1993). The results showed that the first-degree relatives exhibited a

blunted cortisol (and α amylase) response to stress. In addition, this study also measured executive function and found that participants who had a first-degree relative who had completed or attempted suicide did not improve on measures of inhibition upon repeated testing after the TSST. Taken together, these authors have suggested that their findings indicate that blunted cortisol reactivity to stress may represent a trait marker (or phenotype) of suicide risk and impairment of aspects of executive function may be a consequence of dysregulation that increases vulnerability to suicide. These findings are undoubtedly important, however, as the authors acknowledge, the design utilised cannot rule out the possibility that the observed effects were accounted for by the impact of the traumatic loss of a close family member on the HPA axis (and may not represent a trait diathesis per se). Therefore, important next steps for this line of research are: i) to investigate differences in cortisol reactivity to stress in individuals who have attempted suicide with and without a family history of suicide and, ii) to draw comparisons with individuals who have thought about taking their own life (suicide ideators), but have not translated these intentions into action (cf., Dhingra *et al*., 2016; O'Connor, 2011).

More recently, two studies have been published that have used the TSST to examine HPA axis responses to stress in vulnerable, at risk groups (Giletta *et al*., 2015; Melhem *et al*., 2016). Giletta and colleagues explored the extent to which cortisol reactivity to stress was associated with lifetime history of suicide ideation (i.e., developing suicidal thoughts) and whether reactivity predicted future suicidal ideation in at-risk adolescent females. The results of this study found that adolescents who exhibited heightened cortisol responses to stress were more likely to report a lifetime history of suicide ideation and they were approximately 16 times more likely to report suicide ideation 3 months later. This study also found a subsample of adolescents who exhibited a blunted response to stress in which low cortisol reactivity also predicted future suicide ideation. However, when compared to those who exhibited a heightened response, the likelihood of suicide ideation was substantially lower. This research has numerous strengths including the relatively large sample size and the prospective design. Nonetheless, its focus on female adolescents and suicide *ideation* only limits the extent to which the findings can be generalised to actual suicide attempts and to vulnerable populations more generally.

Melhem *et al.* (2016) conducted the second of the recent studies utilising the TSST and this study examined cortisol responses to stress in a large sample of adult offspring of parents with mood disorder. The results of this research found that an offspring suicide attempter group exhibited the lowest levels of total cortisol output during the stressor compared to an offspring with suicide-related behavior but never attempted suicide group, a non-suicidal offspring group and a healthy control group. Moreover, the suicide attempter group also showed the lowest baseline cortisol levels pre-TSST, but, surprisingly, there were no significant differences between groups on their measure of cortisol reactivity to stress. Taken together, these results suggest that blunted HPA axis activity may increase risk for suicide attempt among vulnerable individuals.

The conflicting results of these three key cortisol reactivity studies highlight the complexity of attempting to understand the causes of suicidal behavior and the interplay between a myriad of different influences. A number of factors may account for these mixed findings including deviations in the measurement of cortisol levels, differences in the nature of the samples recruited (e.g., first-degree relatives of suicide completers versus at-risk adolescents), variations in cumulative exposure to stress and the age of participants. In terms of the latter, a recent meta-analysis of naturally fluctuating cortisol levels and suicidal behavior showed that cortisol was associated with suicide attempts in an age-dependent fashion (O'Connor *et al*., 2016). Relatedly, variations in the cumulative exposure to chronic stress over a life course may account for differences in observations of enhanced secretion compared to blunted secretion in vulnerable individuals (cf., McEwen's notion of allostatic load, McEwen, 1998; 2000). Moreover, in line with a stress-diathesis approach, it is also likely that the time elapsed since any acutely stressful event(s) or trigger(s) will influence cortisol reactivity. In their influential review, Miller *et al*. (2007) highlighted the importance of the temporal features of stressors and showed that time of onset of stress was negatively associated with HPA axis activity. More specifically, they found that the greater the amount of time that had elapsed since the stressor was initially encountered, the lower participants' morning cortisol and total daily cortisol output (which will also include cortisol reactivity to stress). These authors argued that the HPA axis exhibits initial activation in the form of elevated cortisol release and following prolonged exposure to the stressors, they theorized that, this activity reduces and cortisol secretion rebounds to less than normal.

Therefore, in the current study, we were also interested in exploring whether the time since suicide attempt (i.e., within the last 12 months versus more distant history of suicide attempts) was related to cortisol reactivity to stress in the laboratory.

To summarise, the primary aim of the current study was to determine whether heightened or blunted cortisol reactivity to stress was associated with a history of suicide ideation and/or suicide attempt in comparison to healthy controls. Secondary aims were: i) to explore whether family history of suicidal behavior and the time since suicide attempt (i.e., within the last 12 months versus more distant history of suicide attempts) were related to cortisol reactivity to stress and ii) to investigate whether cortisol reactivity to stress predicted later suicide ideation or attempt at 1 month and 6 month follow-up.

2. METHOD

2.1. Design and Participants

One hundred and sixty participants (100 females) were recruited to a previous attempt (n=49), a suicidal ideation but no attempt (n=55) and a control group (n=48) based upon established measures of suicidal behavior (see below). Participants were aged between 18 and 62 years (M = 26.84 years, SD = 9.32) with 73.8% identified as Caucasian. Participants were enrolled to the study in response to a local advertising campaign on websites (e.g., Gumtree, Twitter), via poster, flyers and emails. Eligible participants were required to be at least 18 years old and to understand English. Suicide ideation and attempt were assessed using the Self-Injurious Thoughts and Behaviors Interview (SITBI; Nock *et al*., 1997) and the Beck Scale for Suicide Ideation (Beck *et al*., 1979; Beck *et al*., 1988). Participants were allocated to the previous attempt group if they reported attempting to take their own life in the past or to the ideation group if they reported having thoughts of killing their self in the past 12 months. Participants were recruited to a control condition who reported no history of suicide attempt or ideation (and did not report any current psychiatric or psychological conditions). Of the 160 participants recruited to the study, 6 participants withdrew due to having a negative reaction to the Maastricht Acute Stress Test (e.g., felt faint, or did not want to take part in all of the stress test), 8 other participants were unable to be clearly allocated to any of the conditions (e.g., reporting an

inconsistent suicide history or changing their suicide history between screening and commencing the study) and 1 participant who had extreme cortisol values (e.g., exhibiting values of 45.80 nmol/L and 52.42 nmol/L which remained outside the distribution after log transformation). Following removal of these participants, the statistical analysis was conducted on 145 participants (control group = 45, ideator group = 53, attempter group = 47; see Table 1 and Table 2 for baseline characteristics and descriptive statistics for the main study variables). In the attempter group, 14 reported an attempt within the previous 12 months and 33 reported an historical attempt. The range of methods used in the most recent attempt is shown in Table 1. Preliminary analyses revealed there was no association between type of suicide method (using the Traskman *et al*., 1981 classification of violent [e.g., hanging, drowning, gas poisoning] vs non-violent methods [e.g., drug overdoses by ingestion, alcohol]) and cortisol reactivity to stress, $F(1, 40)=0.85$, $p=0.36$. In terms of family history of suicide, 25 participants reported they had a first degree relative who had attempted or completed suicide (control group = 3 [6.7%], ideator group = 6 [11.3%], attempter group = 16 [34%]). Moreover, it is important to note that 5 of the 14 participants in the recent attempter group had a family history of suicide. At baseline, 26.2% (n=38) of participants reported using prescribed medication (control group = 5 [11.1%], ideator group = 16 [30.2%], attempter group = 17 [36.2%]).

2.2. Maastricht Acute Stress Test (MAST)

The MAST is a recently developed stress protocol designed to be both physiologically and psychologically challenging by combining an uncontrollable physical stressor (i.e., a cold pressor challenge) with a social-evaluative (i.e., mental arithmetic) component (Smeets *et al*., 2012). In addition, it has been shown to yield similar subjective and cortisol stress responses to the Trier Social Stress Test (TSST), however, it does not require the presence of a panel (see Kirschbaum *et al*., 1993).

2.3. State-Trait Anxiety Inventory–6 item short form (STAI-6)

The STAI is a 6-item measure which is sensitive to fluctuations in state anxiety and has been found to demonstrate good reliability and validity (Marteau & Bekker, 1992). Participants were asked to complete the STAI at baseline, immediately following the MAST and during recovery. Respondents have to rate how they feel right now (e.g., I feel calm) on a 4-point Likert-type scale

ranging from 1 (not at all) to 4 (very much). Cronbach's alphas for the scale ranged from α = 0.84 to 0.85.

2.4. Scale for Suicidal Ideation (SSI)

The SSI is a 21-item scale to assess current intensity of suicidal thinking and planning over the previous 7 days (Beck *et al*., 1979). The first 5 items are screening items. The additional items are only completed if the respondent reports an active or passive desire to engage in suicidal behaviour. Each item consists of three response options (e.g., 'I have no wish to die', 'I have a weak wish to die', 'I have a moderate to strong wish to die'). The SSI was administered at baseline, 1 month and 6 months follow-up. In the current paper, we combined the 'wish to die' item (#2) with the 'desire to kill myself' item (#4) to provide a separate, clear and unambiguous measure of suicidal ideation. Cronbach's alpha for the summed scale ranged from α = 0.81-0.91. Scores were log transformed due to skewness before statistical analyses were conducted.

2.5. Cortisol measurement

Cortisol levels were assessed five times during the MAST: at the beginning of the stress task (T00) and at +10, +20, +30 and +40 minutes post-task. Cortisol was collected from saliva, using Salivettes (Sarstedt, Germany). Participants were instructed to refrain from drinking alcohol, doing excessive exercise or taking any pain medication on the day of the test session. They were also instructed to not eat food, brush your teeth or have any drinks (except water) in the hour before the testing session. Note that all laboratory visits were scheduled after 11am in the morning (with 97% taking place between 11am and 3pm) in order to ensure the sampling was not influenced by the cortisol awakening response. Moreover, testing time schedules were very similar across groups with the most frequent testing times at 12pm or 14:30pm. In the control group, 48.9% and 33.3% of participants were tested at 12pm and 14:30pm, respectively; in the attempter group, 46.8% and 31.9% of participants were tested at 12pm and 14:30pm respectively and in the ideator group, 39.6% and 37.7% of participants were tested at 12pm and 14:30pm, respectively. Cortisol samples were stored at -20°C or lower until assay. Cortisol levels were determined by using a competitive enzyme-linked immunosorbent assay kit (ELISA) designed for analysing saliva. Intra-assay and inter-assay coefficients of variation of this assay were 4.26% and 4.91%, respectively. Before conducting the

statistical analyses the cortisol levels were log transformed due to skewness, however, non-transformed values are presented in Table 2 for ease of interpretation. In addition to the cortisol levels at each time point, two measures of area under the curve were calculated. Using the five sampling time points, the two AUC measures were determined following established procedures (Gartland *et al*., 2014; Pruessner *et al*., 2003). Area under the curve with respect to ground (AUCg) is a measure of total cortisol output throughout the stressor and area under the curve with respect to increase (AUCi) captures the sensitivity of the HPA system and is a measure of its ability to change in response to the MAST.

2.6. Procedure

After a short preparation and anticipation phase (5 min), participants were asked to complete five socially evaluated cold pressor trials where participants immersed their hands in cold water for varying durations (60 to 90s) over a 10-minute time span. In between trials, participants were instructed to perform a mental arithmetic task as fast and as accurately as possible and received negative feedback on their performance when mistakes were made. To increase unpredictability and uncontrollability participants were told the duration and the order of the hand immersion trials and mental arithmetic task were randomly chosen by the computer, whereas in reality the order and duration of trials was fixed for all participants. As outlined above, the state anxiety measure (STAI) was completed at baseline, immediately after the MAST (at +10 mins) and again at the end of the recovery period (at +40 mins). To measure cortisol, saliva samples were taken using Salivettes at the beginning of the stress task (T00), at +10, +20, +30 and +40 minutes post-task. At 1-month and 6-month follow-up, participants were invited to complete a brief telephone interview, where suicide ideation and attempt were assessed using the Self-Injurious Thoughts and Behaviors Interview (SITBI; Nock *et al*., 1997) and the Beck Scale for Suicide Ideation (Beck *et al*., 1979; Beck *et al*., 1988).

2.7. Statistical analysis

Repeated measures analysis of covariance (ANCOVA) for a mixed design was utilised to examine the main effects of study group (controls, ideators & attempters) and time (baseline, T10, T20, T30, T40) on cortisol reactivity to stress, together with the group by time interaction effects.

Next, ANCOVA was used to investigate the same main and interactive effects on cortisol output as assessed by AUCg and AUCi. Age, BMI, medication usage, time of day and smoking status were controlled for and entered as covariates. We tested the assumption of sphericity and where the results were significant, the Greenhouse-Geisser correction was applied. As outlined earlier, all statistical analysis was performed on the log transformed data. Preliminary analyses including gender as a factor revealed there was a significant effect on cortisol levels during the MAST, $F(1, 136)=4.40$, $p =0.04$, indicating that female participants secreted lower levels throughout the stress task compared to their male counterparts. However, there was no significant gender x suicide group interaction, $F(2, 136)=0.34$, $p=0.71$. Nevertheless, in order to reduce the variability in our outcome measure, gender was included as a covariate in all analyses. As indicated above, smoking status was also entered as a covariate because there were more smokers in the attempter (n=16; 34%) and ideator (n=16; 30%) groups compared to the control (n=6; 13%) group, $\chi^2 = 5.78$, $p=0.055$. Hierarchical linear regression was utilised to test the final hypothesis following the procedures outlined by Kenny *et al*. (1998). First, in order to control for age, gender, BMI, medication usage, time of day and smoking status, adjusted cortisol values were calculated (in the form of residuals) by regressing the control variables against AUCg and then AUCi. Second, for each outcome variable (suicide ideation at 1 month, suicide ideation at 6 months), study group (suicide attempt vs suicide ideation) was entered at step 1, baseline suicide ideation (and 1 month suicide ideation when predicting 6 month ideation) at step 2, adjusted AUCg or adjusted AUCi at step 3, and finally the study group by adjusted AUC multiplicative interaction term entered in step 4.

3. RESULTS

Descriptive statistics for the main study variables are shown in Table 1. Inspection of these data show that the mean levels of cortisol at pre-test were similar across the groups and that the levels were within acceptable normal ranges (Aardal and Holm, 1995; Newman *et al*., 2007).

3.1. Effects of study group on cortisol reactivity to stress

In terms of the cortisol levels at each of the time points, a significant main effect of group was observed, F (2, 136)=3.31, p=0.04, but not for time[1], F(2.32, 315.50)=0.18, p=0.86, or for the group x time interaction, F=(4.64, 315.50)=1.67, p=0.10. Bonferroni post-hoc comparisons were used to decompose the main effect of group and showed that overall (across all time points) the attempter group exhibited significantly lower cortisol in response to the MAST compared to control participants (p=0.01). The ideator group did not differ from the control group (p=0.56) or the attempter group (p=0.48; see Figure 1) and were intermediate to both groups. Next we explored effects of group on the AUC measures. Using ANCOVA, a significant main effect of group was found for AUCg, F(2, 136)=3.44, p=0.035, and a marginal effect for AUCi, F(2, 136)=2.61, p=0.08. Post-hoc comparisons for AUCg showed that the attempter group released significantly lower total cortisol in response to the MAST compared to control participants (p=0.03), and the ideator group did not differ from the control group (p=0.55) or the attempter group (p=0.45).

Given the absence of an overall main effect for time or a time by group interaction, the subsequent analyses focused on the AUC measures. Next in order to explore the extent to which family history contributed to the main effects of study group, we re-ran the analyses excluding participants who reported a family history of suicide (i.e., they had a first degree relative who had attempted or completed suicide). The results showed that the main effects of study group became non-significant for AUCg, F(2, 111)=2.05, p=0.13, and remained non-significant for AUCi, F(2, 111)=0.72, p=0.49. However, importantly, when the ANCOVAs were run including the control and attempter groups only (given this is where the main effects of group were observed), the main effects of study group were statistically significant for AUCg, F(1, 65)=4.28, p=0.04, but not for AUCi, F(1, 65)=1.35, p=0.25.

Next, we re-ran the main analyses excluding participants who had a recent attempt (n=14) in order to explore the extent to which recent history of suicide attempt (i.e., less than 1 year ago) contributed to the main effects of study group. The results showed that the main effects of study group became non-significant for AUCg, F(2, 122)=2.05, p=0.24, and remained non-significant for AUCi, F(2, 122)=0.80, p=0.45. In addition, when the ANCOVAs were run including the control and attempter groups only, the main effects of study group also remained non-significant for AUCg, F(1,

70)=1.67, p=0.20, and for AUCi, F(1, 70)=1.02, p=0.32. Therefore, taken together, these sensitivity analyses indicate that familial history does influence the main effects of study group on AUC, but does not account for the observed difference between participants in the attempter and control groups. However, recent history of suicide attempt appears to have a stronger effect and accounts for the significant differences between the attempter and control groups.

3.2. Effects of study group on psychological reactivity to stress

In terms of state anxiety levels at baseline, post-stress and during recovery, a significant main effect of group was observed, F (2, 137)=9.91, p<0.001, but not for time, F(1.68, 229.78)=0.51, p=0.57, or for the group x time interaction, F=(3.35, 229.78)=0.81, p=0.52. With respect to the main effect of group, Bonferroni post-hoc comparisons showed that the attempter and ideator groups reported significantly higher state anxiety levels in response to the MAST (means = 12.65, 12.50, respectively) compared to control (mean = 10.18) participants (p<0.001). The attempter and ideator groups did not differ from each other.

3.3. Effects of recent attempt (less than 1 year ago) versus historical attempt (greater than 1 year ago) on cortisol reactivity to stress

Next in the suicide attempt group only, we explored the extent to which length of time since suicide attempt was associated with AUC measures (see Figure 2). The results showed there was a significant main effect of attempt history group on AUCi, F(1, 39)=4.74, p=0.03, such that individuals who had attempted to take their own life within the past 12 months exhibited a lower AUCi compared to those individuals with a lifetime history of suicide attempt (greater than 1 year ago). The main effect of attempt history group on AUCg was not statistically significant but was marginal, F(1, 39)=3.00, p=0.09.

3.4. Effects of family history of suicide and study group on cortisol reactivity to stress

As outlined in the Method section, only 3 participants in the control group reported a family history of suicide. Therefore, given the associated small cell sizes, this analysis focused on the ideator and attempter groups only. The results of two-way ANCOVAs revealed a significant family history x study group interaction for AUCi, F(1, 90)=8.59, p=0.004. Post hoc ANCOVA revealed that the effect

of family history on AUCi was only significant in the attempter group, F(1, 39)=10.18, p=0.003, such that significantly lower levels of cortisol were secreted by the participants who had a family history compared to those participants who did not have a family history (see Figure 2). The interaction between family history and study group was not significant for AUCg, F(1, 91)=1.07, p=0.30.

3.5. Predictive effects of cortisol reactivity to stress on suicide ideation at 1 month and 6 months follow-up in combined suicide attempt and ideator group

3.5.1. 1-month follow-up

As outlined earlier, the predictive effects of cortisol reactivity to stress (as measured by AUCg and AUCi) on suicide ideation at follow-up were examined using hierarchical regression. For suicide ideation at 1-month follow-up, study group (at step 1) did not significantly enter the equation. However, at step 2, baseline suicide ideation significantly explained an additional 41% of the variance, F(1, 91)=66.30, p <0.001, such that higher levels of suicide ideation at baseline were associated with higher levels of ideation at 1 month follow-up. Next, adjusted AUCg was entered at step 3 and did not significantly contribute to the equation. However, at step 4, when the study group x adjusted AUCg interaction term entered the equation, it explained an additional 5% of the variance in suicide ideation at 1 month follow-up, F(1, 89)=9.52, p =0.003. The two-way interaction is depicted in Figure 4 following procedures outlined by Aiken and West (1991) and Dawson (2014). The results show that participants in the attempter group who secreted lower levels of cortisol in response to the MAST were significantly more likely to report higher levels of suicide ideation one month later (whilst controlling for baseline suicide ideation and the other covariates).

The above analyses were repeated for AUCi, however, the study group x AUCi interaction term (entered at step 4) did not significantly explain additional variance in suicide ideation at 1-month follow-up.

3.5.2. 6-month follow-up

For suicide ideation at 6-month follow-up, neither the study group x AUCg nor the study group x AUCi interaction terms significantly explained any additional variability in suicide ideation at 6-month follow-up (results not shown).

3.5.3. Suicide attempt at 1-month and 6-month follow-up

A single suicide attempt was reported at 1-month follow-up in the attempter group only (and not at 6 month follow-up). Therefore, no further analyses were conducted. However, it is worth noting that this participant's cortisol levels were in the lowest quintile for the entire sample.

4. DISCUSSION

The results of the current study confirm that HPA axis activity, as measured by total cortisol output in response to an acute laboratory stressor, is markedly lower in suicide attempters compared to controls, but not ideators. The ideator group appear to be an intermediate group between the suicide attempter and control groups. The observed effects were not accounted for by smoking status, medication usage, age, gender, BMI or time of testing. Moreover, our sensitivity analyses showed that family history influences the observed main effects of study group on cortisol secretion, but does not account for the observed differences between participants in the attempter and control groups. In addition, recent history of suicide attempt appears to have a stronger effect than family history and accounts for a large amount of the differences between the attempter and control groups. These findings are consistent with Melhem *et al*.'s (2016) study that also utilized a stress induction procedure (the TSST) and investigated cortisol responses to stress in a large sample of adult offspring of parents with mood disorder. This research also found the lowest levels of total cortisol output in the offspring suicide attempter group (compared to the offspring with suicide-related behavior but never attempted suicide group, a non-suicidal offspring group and a healthy control group.) The current findings are also in keeping with another recent study that found further evidence of low baseline cortisol levels in suicide attempters compared to non-attempters (Keilp *et al*., 2016) and also with an earlier investigation also showing that low cortisol activity is associated with suicidal behavior (Lindqvist *et al*., 2008). Therefore, based upon the current findings and the existing literature reviewed, the weight of evidence suggests that blunted cortisol responsiveness to stress is associated with suicide attempt in adults.

The finding that participants who attempted suicide within the last 12 months appear to exhibit a clearly defined, blunted cortisol response to the laboratory stressor, compared to those with a lifetime history of suicide attempt, is a very important observation (see Figure 3). In particular, it is noteworthy that AUCi was the only cortisol measure to demonstrate a significant difference between

the attempter groups. As outlined earlier, the two AUC assessments capture different aspects of the cortisol response. AUCg is a measure of total output and AUCi provides a measure of the sensitivity of the HPA axis system. Therefore, this finding indicates that the sensitivity of the HPA stress response system may be particularly compromised in individuals who have made a recent suicide attempt. Inspection of Figure 3 clearly shows that participants with a recent history of suicide attempt do not exhibit a marked increase in cortisol following the stress. Indeed these participants, on average, do not appear to mount much of a stress response at all. The markedly different cortisol profiles in those who recently attempted suicide compared to those with a history of suicide is also important because it suggests that the cortisol response to stress may have returned to close to normal in the lifetime history group, albeit, their levels remain lower than in the control and ideator groups. The current design does not allow us to infer a causal, temporal relationship between the timing of a suicide attempt and changes in HPA axis functioning. However, this finding does indicate that psychological and pharmacological intervention may yield benefits over time and help facilitate (partial) recovery of the HPA axis stress response system (hence the higher cortisol levels in the distance history group). It is incumbent on researchers to utilise longitudinal designs to explore whether dysregulation of cortisol reactivity to stress is restored over time. Moreover, if the HPA axis has the potential to return to normal following psychological and/or pharmacological intervention, then this points to the urgent need for researchers to test the effectiveness of relevant stress management interventions.

The current study also found that having a family history of suicide was associated with making a suicide attempt and with exhibiting the lowest cortisol response to stress in the laboratory. This finding is in line with McGirr *et al.* (2010) who found that first degree relatives of suicide completers showed a blunted cortisol (and α amylase) response to an acute laboratory stressor. These findings are also consistent with the conjecture that dysregulation of the stress response system may be a heritable risk factor for suicidal behavior. In the context of stress-diathesis explanations of suicidal behavior, cortisol reactivity to stress may be considered a trait diathesis that confers vulnerability to suicidal behavior. The McGirr *et al* study was unable to rule out the possibility that their findings were accounted for by the deleterious impact of losing a close family friend on the HPA

axis (instead of indicating the existence of a trait diathesis increasing vulnerability to actual suicidal behavior). However, the current study design, confirms that suicide attempters with and without a family history of suicide had lower levels of cortisol reactivity compared to ideators and controls, but that the lowest cortisol reactivity to stress was observed in suicide attempters *with* a family history. The observation suggests that family history of suicide confers additional vulnerability to suicide behaviour.

We found that lower cortisol reactivity to stress predicted increased levels of suicide ideation at one-month follow-up in the suicide attempter group but not in suicide ideator group (after controlling for baseline levels of suicide ideation and a full range of covariates). This finding is contrary to Giletta *et al.* (2015) who found that *heightened* cortisol reactivity to stress was the strongest predictor of suicide ideation at three-month follow-up in at-risk adolescent females. This study also found a subsample of adolescents who exhibited a blunted response to stress and high levels of suicide ideation 3-months later (though, this effect was only a trend). It is difficult to reconcile these inconsistent findings without further work and replication. However, one possibility is that there is a non-linear, inverted U relationship between cortisol reactivity and suicide ideation, such that high and low levels of cortisol are deleterious and are associated with suicide ideation. Similar relationships have been demonstrated for other hormones and important aspects of behavior (cf., O'Connor *et al*., 2001). Alternatively, the Giletta *et al*. findings may simply reflect that there are different 'dominant' predictors of suicide ideation in adolescents and adults and/or between individuals who have and have not attempted suicide. It may be that the adolescent brain is more sensitive to the effects of high levels of cortisol and/or the adult brain is more responsive to the effects of low levels of cortisol (the latter in relation to impaired executive control function and capacity to adapt to stressors). The different findings may also reflect complex interactions between traits such as impulsivity (measured in the Giletta *et al*. study), cortisol and suicide ideation. Therefore, an important next step is to understand the precise causal mechanisms linking stress, changes in cortisol reactivity to stress and suicide attempt. Changes in aspects of executive function processes and coping and adapting to stressors are likely to prove to be fruitful avenues of future research. McGirr *et al*. (2010) have shown that, in participants who had a first-degree relative with a suicide history, exposure

to an acute stressor led to a failure to improve on measures of inhibition following repeat testing. These authors argue that their findings suggest that stress may lead to cognitive inflexibility and decreased ability to inhibit inappropriate actions that may increase vulnerability to suicidal behavior.

Finally, we recognise that there are a number of limitations to the current study. As outlined above, the current design does not allow us to infer a causal, temporal relationship between the timing of a suicide attempt and changes in HPA axis functioning. In addition, although our sample size was relatively large, future research ought to endeavour recruiting a greater number of participants and following them over a longer time window. A larger sample would also facilitate a more detailed investigation of the influence of groups of medications on cortisol reactivity to stress. We are also aware that a limitation of the current study is an absence of formal diagnoses of psychiatric disorders using a standardized tool such as the Structured Clinical Interview for DSM Disorders. In addition, the study did not assess lethality of the suicide attempt. Therefore, future research ought to collect more detailed, formal information on current psychiatric diagnoses, lethality of attempt as well as lifetime history of psychiatric and psychological disorders. Finally, it is worth reiterating that we did not find a significant main effect of time on cortisol levels or on state anxiety during the MAST. This is noteworthy as it indicates that the effects of the MAST were not as strong as anticipated in terms of producing a consistent rise in stress-related variables. We utilized the MAST because it combines a physical stressor (i.e., a cold pressor challenge) with a social-evaluative (i.e., mental arithmetic) component, but does not require the presence of a panel. However, despite it previously (Smeets *et al.*, 2012) been shown to yield similar subjective and cortisol stress responses to the TSST (which includes a panel), our findings suggest that it may not always be as effective as the TSST. Future research should explore further the utility of the MAST in a range of different samples and conduct additional evaluation on whether there is a need for a stronger social-evaluative component.

In conclusion, these findings are consistent with other findings indicating that HPA axis activity is associated with some forms of suicidal behavior. Specifically, total cortisol output in response to a laboratory stressor is markedly lower in suicide attempters compared to controls, but not ideators. In addition, recent history of suicide attempt and family history of suicide are associated with lower cortisol secretion in response to stress. Finally, lower levels of cortisol in response to the MAST

were associated with higher levels of suicidal ideation at 1-month follow-up in the suicide attempters group. The challenge for researchers is to elucidate the precise causal mechanisms linking stress, cortisol and suicide risk.

Footnote:

1. The absence of a main effect of time was accounted for by group differences in time effects cancelling each other out (in particular in the attempter group) and by participants' cortisol levels recovering quickly by 30 mins post-stressor. In addition, it is worth noting that in the control and ideator groups, cortisol levels increased markedly and significantly between baseline and 10mins and between baseline and 20mins but were back to normal by 30mins. In the attempter group, cortisol levels only exhibited a modest increase between baseline and 10mins. Nevertheless, it is worth noting that there was also an absence of a significant main effect of time in the control group when examined separately indicating that the MAST may not be as an effective stress induction paradigm compared to other techniques that include a strong social evaluation component such as the Trier Social Stress Test (Kirschbaum *et al.,* 1993).

Authors' contributions

All authors contributed equally

Contributors

Daryl O'Connor, Eamonn Ferguson, Jessica Green, Ronan O'Carroll and Rory O'Connor designed and contributed to the review protocol. Daryl O'Connor wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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Conflict of interest

None

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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Table 1. Baseline characteristics for participants in each study group (n = 145)

Characteristic	**Control group** (n=45)	**Ideator group** (n=53)	**Attempter group** (n=47)
Age (SD)	24.13 (8.46)	27.87 (8.29)	28.55 (10.88)
Sex (% female)	29 (64.4)	29 (54.7)	30 (63.8)
Current psychiatric/psychological diagnosis*			
Depression	0	16	8
Anxiety	0	9	3
Bipolar disorder	0	0	4
Personality disorder	0	0	3
Number of lifetime attempts+			1 attempt = 21 2 attempts = 6 3 attempts = 5 4 attempts = 2 ≥ 5 attempts = 13
Method in most recent attempt+			
Own prescription drugs			13
Over-counter drugs			8
Poison			2
Immolation			1
Hanging			5
Sharp object			5
Auto exhaust			1
Suffocation			1
Alcohol			1
Salt water			1
Multiple methods			9
Family history of suicide (%)	3 (6.7)	6 (11.3)	16 (34.0)
Prescribed medications (%)	5 (11.1)	16 (30.2)	17 (36.2)

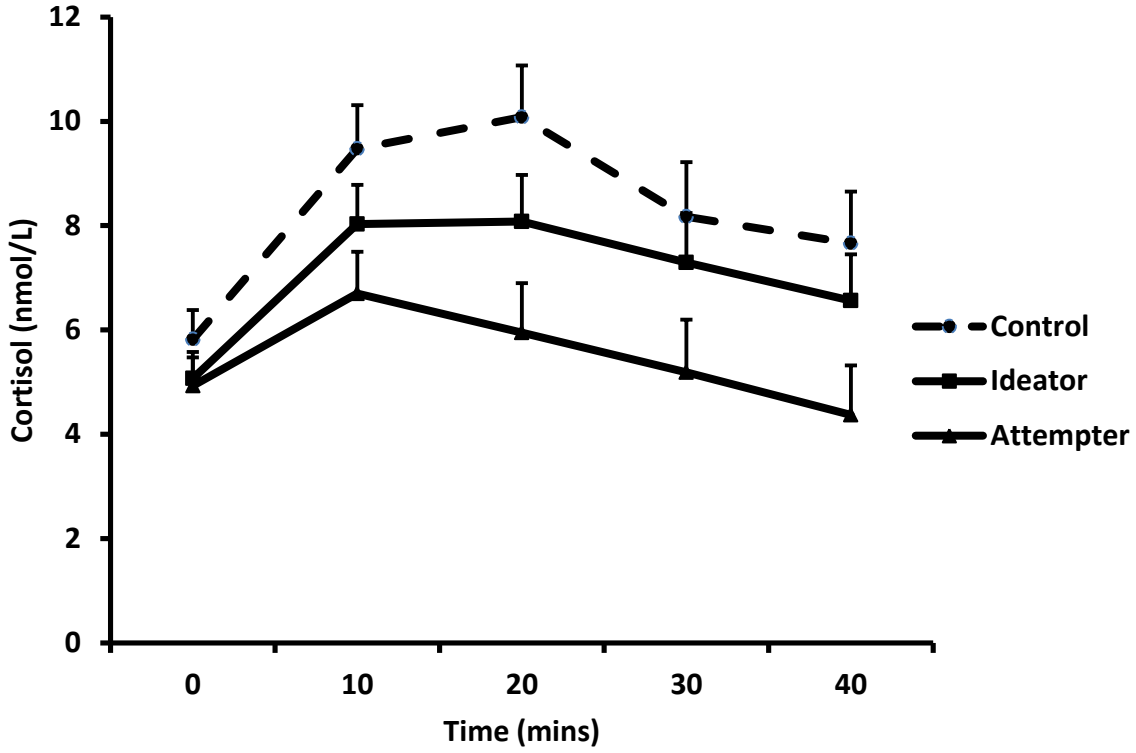
* = Participants were asked to provide details of any current diagnosed medical conditions; physical and/or psychiatric/psychological; + = From Self-Injurious Thoughts and Behaviors Interview

Table 2. Descriptive statistics (means and standard deviations) for main study variables across experimental groups (n = 145)

	Control group (n=45)		Ideator group (n=53)		Attempter group (n=47)	
	M	***SD***	***M***	***SD***	***M***	***SD***
Cortisol (nmol/L)						
Pre-MAST (00 min)	5.61	4.15	5.16	3.43	5.03	6.65
10 min post-MAST	8.87	5.76	8.29	5.87	6.98	5.28
20 min post-MAST	9.36	7.47	8.36	7.01	6.31	5.14
30 min post-MAST	7.65	7.52	7.48	7.83	5.47	4.74
40 min post-MAST	7.60	8.72	6.51	6.16	4.47	3.18
AUCg	32.49	23.07	29.97	22.93	23.52	17.30
AUCi	10.05	20.04	9.34	16.65	3.40	10.60
State anxiety-baseline	8.38	2.43	13.89	4.19	8.24	2.66
State anxiety-post test	11.32	3.66	15.77	3.97	10.27	3.37
State anxiety-recovery	11.41	3.70	15.90	4.06	10.85	3.67
Suicide ideation-baseline	1.00	0.00	1.60	0.91	1.94	1.13
Suicide ideation-1 month	1.00	0.00	1.54	1.09	1.89	1.26
Suicide ideation-6 months	1.03	0.16	1.57	0.93	1.74	1.08

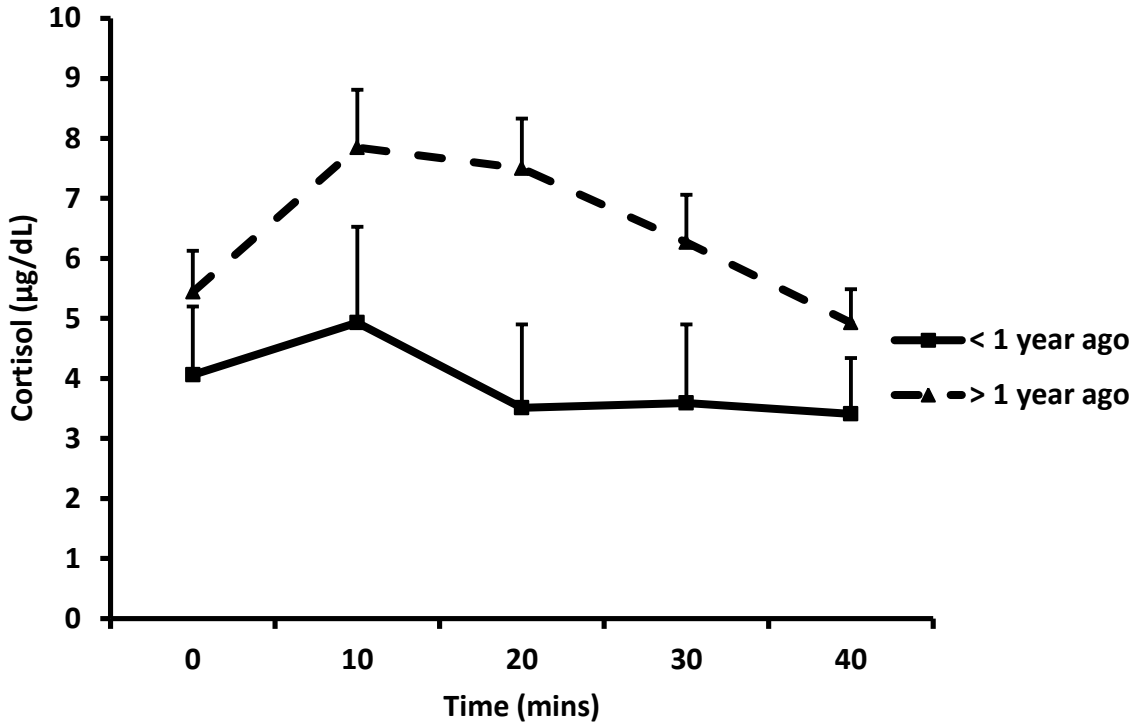
Note: MAST = Maastricht Acute Stress Test; AUCg = area under the curve with respect to ground; AUCi = area under the curve with respect to increase.

Figure 1. Effects of experimental group on cortisol reactivity to stress (n=145)



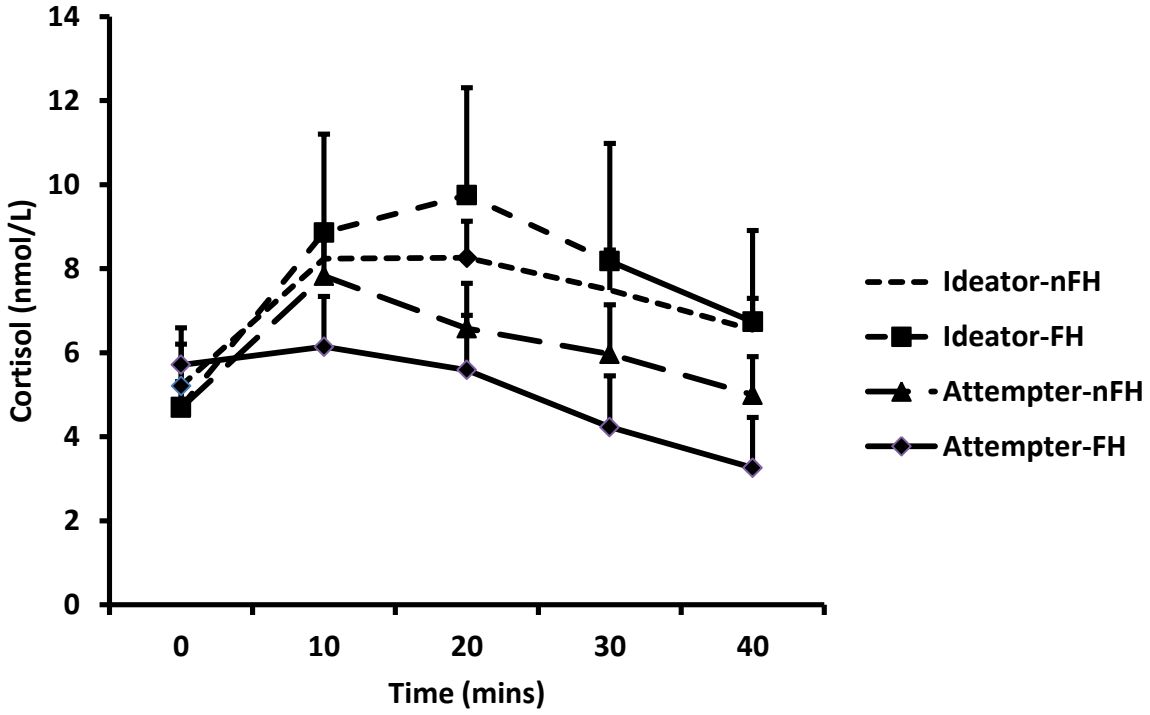
Note: Error bars represent the standard error of the mean

Figure 2. Effects of recent attempt (less than 1 year ago; n = 14) versus historical attempt (greater than 1 year ago, n =33) on cortisol reactivity to stress



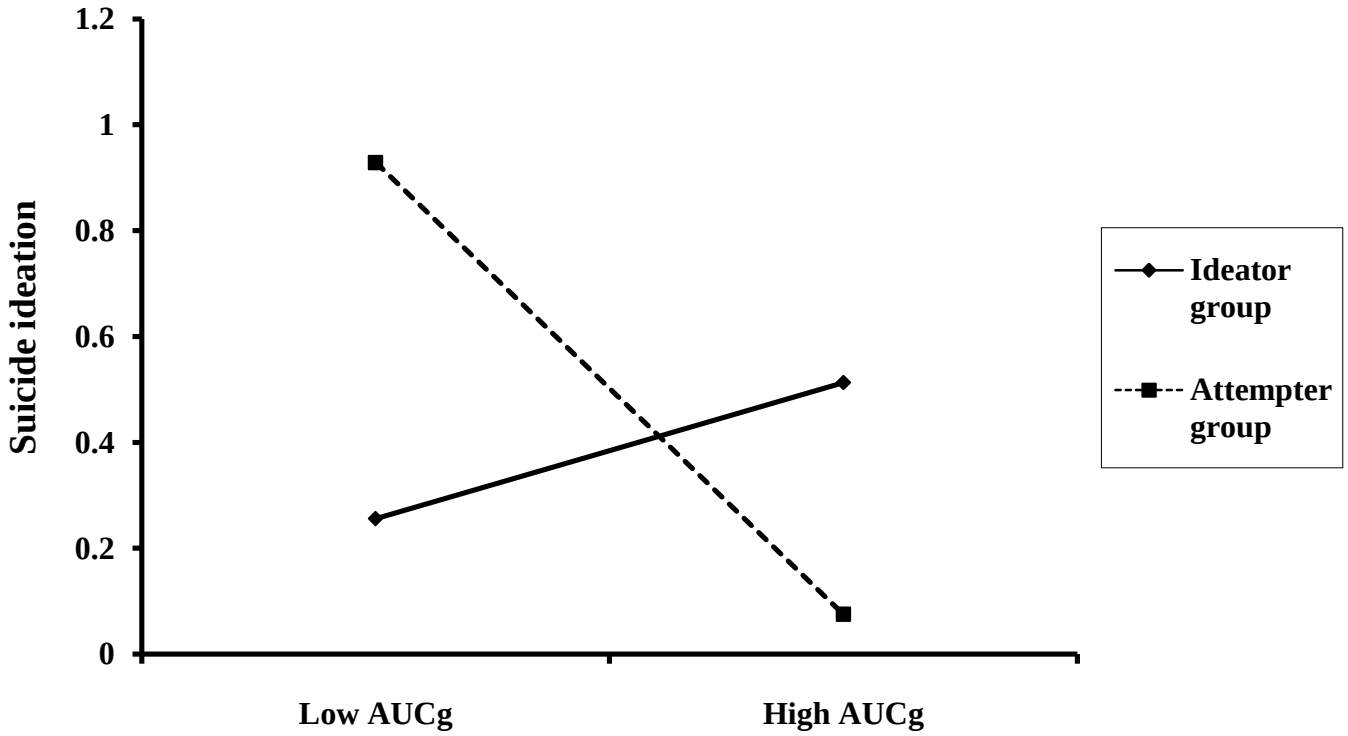
Note: Error bars represent the standard error of the mean

Figure 3. Effects of family history of suicide and study group on cortisol reactivity to stress showing lowest levels of cortisol in attempter group with family history



Note: Error bars represent the standard error of the mean

Figure 4. Predictive effects of cortisol reactivity to stress on suicide ideation at 1-month follow-up (controlling for baseline ideation)



Note: Participants in the attempter group who secreted lower levels of cortisol in response to the MAST were significantly more likely to report higher levels of suicide ideation one month later (whilst controlling for baseline suicide ideation and the other covariates).