**Research objective and hypotheses**

In the current study, we seek to assess the effects and relationship between exposure to early traumatic events and acute stress disorders/responses (ASD/R) and the development of post-traumatic stress disorder (PTSD) after exposure to a traumatic event in the present. We further seek to examine whether this relationship is mediated by biological indexes such as cortisol and orexin levels measured shortly after the traumatic event.

To that end, we will clinically and biologically monitor people who arrive at an ER within six hours (on this time frame and the concept of “golden hours,” see Carmi et al., 2016) from the moment they have been exposed to a traumatic event (e.g. car accident, assault, work accident, violent robbery, etc.) and exhibit ASR symptoms. Upon their arrival at the ER and once their consent to participate in the study has been obtained, physiological measurements such as blood pressure and heart rate will be collected along with blood samples that will be tested for cortisol and orexin levels. During this visit, the subjects will receive either a placebo or a single oral dose of cortisol (180 mg) to elevate cortisol levels, which as previous studies have shown, may reduce symptoms and even prevent the development of PTSD (Carmi et al., in press; Zohar et al., 2011). In follow-up sessions, we will inquire as to which of the subjects has previously been exposed to a traumatic event and collect additional biological and psychological measurements. Finally, we will also examine whether and which of the biological measurements mediate the response to traumatic events with or without cortisol treatment administered within six hours after exposure to the traumatic event.

Research hypotheses:

1. A positive relationship will be found between previous exposure to a traumatic event and an ASD/R following a traumatic event in the present and PTSD.
2. Endogenous cortisol levels will be found to mediate the relationship between previous exposure to a traumatic event and an ASR following a traumatic event in the present, ASD, and PTSD.
3. Endogenous orexin levels will be found to mediate the relationship between previous exposure to a traumatic event and an ASR following a traumatic event in the present, ASD, and PTSD.
4. Finally, we will also examine whether the biological measurements mediate the response to cortisol treatment administered within six hours from exposure to the traumatic event.

**Research importance and scientific background**

A traumatic event is one in which a person senses that they or others are in mortal danger or may be significantly harmed. After undergoing a traumatic event, many people experience an ASR, which can include symptoms of stress, repeated thoughts about the event, dreams, and the feeling that the event is recurring. At the same time, and in some cases after a while, other symptoms can appear, such as bad moods, a sense of being disconnected from reality (dissociation), avoiding certain thoughts, places, or people who bring to mind the event, feelings of anxiety or unrest, sleep disorders, concentration problems, and a hypersensitivity to noise (APA, 2013; WHO, 1992). On average, about twenty percent of people who have undergone a traumatic event suffer from these symptoms, and this condition is known as ASD or ASR. For most people, these symptoms last between three to thirty days after the traumatic event has occurred, and they subside over time. However, for some (about 15%), these symptoms persist for over a month after the traumatic event has occurred and thus become PTSD (Kessler, 2000; Kessler et al., 2005; Breslau, 2012; Brewin et al., 1999).

PTSD is a mental disorder that develops after a person has been exposed to an event or events in which they perceived their physical or psychological integrity to be in danger. According to the current phenomenological definition (APA, 2013) the disorder comprises several types of symptoms. These include invasive thoughts and reconstruction of the traumatic experience, refraining from mentioning the event, hyper physiological arousal, and mood swings, which cause distress and impair the person’s functioning in social, occupational, and family contexts. The prevalence of the disorder is high, affecting 1.3%–7.8% of the civilian population (Breslau et al., 1997; Kessler et al., 1995) and up to about 30% of combat soldiers or victims of sexual assault (Kulka et al., 1990; Resnick et al., 1993).

The literature describes various risk factors for the development of the disorder, such as gender, the type of traumatic event, IQ, a history of early exposure to a traumatic event, and a lack of cortisol secretion in response to the stressful event (Brewin et al., 2000; Nemeroff et al., 2006). In the current study, we chose to focus on two of these and their relationship to ASD/R and PTSD.

As mentioned, a known risk factor in relation to various psychopathologies such as depression and anxiety (Green et al., 2010; McLaughlin et al., 2010; Nemeroff et al., 2004) and one of the most consistent predictors of PTSD, is a history of early exposure to a traumatic event in childhood (Koenen et al., 2007; Van Voorhees, 2012). A review of the literature (Brown, 2003; Kendall-Tackett, 1993) and meta-analyses (Brewin et al., 2000; Chen et al., 2010), alongside epidemiological studies (Kilpatrick et al., 2003) and cross-cultural studies (Kessler et al., 2010) point to a direct relationship between early traumatic events and PTSD.

Another factor found to affect the development of PTSD is the level of endogenous cortisol at the time of the traumatic event. Cortisol, also known as “the stress hormone,” operates on many target areas. It allows the person to cope with stressful situations efficiently by affecting their metabolism, encouraging a rise in blood glucose levels, helping to break down glycogen in the liver, raising blood pressure, and reducing the immune response. Cortisol secreted during a traumatic event affects how the brain functions by increasing activity in the amygdala and decreasing activity in the hippocampus. Among other things, this hormone participates in reducing the memory’s intensity following the traumatic event. The activation of the stress axis and the various effects of the hormones it secretes have a clear purpose, which is to prepare the body for coping with a stressful event, be it by fighting or fleeing (the “fight or flight” response) (Chu et al., 2021; Cohen et al., 2006).

**Cortisol levels following exposure to a stressful event:** The general consensus is that following exposure to a stressful event high levels of cortisol will be found in the blood and urine to help the person cope with the stress. However, neuroendocrine studies demonstrate that the risk of developing PTSD is higher among people who experience relatively high sympathetic arousal or who have relatively low levels of cortisol soon after being exposed to the trauma (Delahanty et al., 2005; Galatzer-Levy et al., 2017; McFarlane et al., 2011; Resnick et al., 1995; Shalev et al., 1998; Videlock et al., 2008; Yehuda et al., 1998a; Yehuda et al., 1998b). These studies and others have contributed to the development of a model that suggests that hyperactivity of the sympathetic system and impaired functioning of the hypothalamic-pituitary-adrenal (HPA) axis jointly contribute to the development of PTSD and the failure to regain balance and achieve physiological homeostasis after experiencing a trauma (Cohen et al., 2006; Yehuda, 2002). As mentioned, one of the important roles of cortisol secretion in times of stress is to help contain physiological responses such as the ones mediated by the sympathetic nervous system. This model suggests that if cortisol levels in the body are lower than their optimum when adrenaline levels are very high (such as when being exposed to an immediate threat), this inhibits biological recovery and amplifies phenomena in the sympathetic nervous system, which leads to hyperarousal and invasive symptoms.

An expanded model, which was developed in an aim to answer the question of why low cortisol levels were found in certain people after being exposed to a traumatic event, suggests that increased sensitivity of the glucocorticoid receptor (GR) contributes to lower base levels of cortisol among people who are at risk of suffering from PTSD (Yehuda, 2002). Increased GR sensitivity may stem from a history of being exposed to traumatic events, from genetic or epigenetic factors, or a combination of them (Turecki, 2016; Tyrka, 2012).

There has been a longstanding debate in the clinical literature regarding cortisol levels in those suffering from PTSD, with reports of a decreased, increased, or unchanged concentration of cortisol in their blood or urine samples compared to those of healthy subjects (Delahanty et al., 2000; Mason et al., 1988; Pitman & Orr, 1990; Yehuda et al., 1995). As cortisol secretion changes throughout the circadian cycle, with levels rising in the morning upon waking and lowering in the evening before bedtime and when sleep begins, some attribute PTSD to impaired cortisol secretion only during a specific part of the circadian cycle (Inslicht et al,. 2011).

Another biological system found to be linked to PTSD that has been researched in recent years in the hopes of shedding light on the biological mechanisms underlying the disorder and perhaps influencing the way it is treated, is the **orexinergic system** (Prajapati, & Krishnamurthy, 2021). Orexin is an endogenous neuropeptide that regulates arousal in both the central and peripheral nervous system (Tsunematsu & Yamanaka, 2012; Winrow et al., 2012). When a person is exposed to a traumatic event, the orexinergic system is activated, leading to hyperarousal, sleep disorders and a sense of anxiety (Klenowski et al., 2016). In addition, it appears that the orexin-A neuropeptide can activate the HPA axis by encouraging the activation of the corticotropin-releasing hormone (CRH) in the hypothalamus (So et al., 2018; Srinivasan et al., 2013).

CRH secretion alongside impaired functioning of the HPA axis were found to be a pathological factor in the development of PTSD (Lu et al., 2008; Yehuda et al., 1993). Studies show that CRH antagonists are capable of weakening the behavioral implications of stress, highlighting the role of endogenous CHR in mediating stress-related behaviors (Lu et al., 2008; Zobel et al., 2000). The selective CRH type 1 receptor (CRH-R1) activates a glucocorticoid response, which as mentioned, has been found to be related to previous exposure to a traumatic event. CRH-R1 has also been found to be related to fear response acquisition in times of stress (Krishnamurthy et al., 2013).

**Expected innovations of the research**

Previous exposure to traumatic events and biological measurements of cortisol and orexin levels have been studied in the past in the context of the development of PTSD (Xie et al., 2010). However, and to the best of our knowledge, the relationship between these variables and ASD and ASR following exposure to traumatic events have not been sufficiently researched. As mentioned above, ASR has been found to be linked to and even a risk factor for PTSD (APA, 2013; Breslau, 2012). Focusing on ASR and ASD and their relationship to various biological measurements can contribute to future thinking in regards to the early detection, treatment, and prevention of PTSD.

At the same time, and in-depth examination of: 1) the effects of cortisol; 2) its use as a means for intervention following exposure to a traumatic event; and 3) the orexinergic system that participates in arousal responses during stress and the acquisition of fear responses, may shed light on the systems underlying stress disorders and perhaps even change their course.

In recent years, two pilot studies were conducted at the Sheba Medical Center dealing with secondary prevention of PTSD. Their goal was to examine whether a single dose of hydrocortisone (HCORT), a hormone that imitates the activity of cortisol in the body, can prevent the development of PTSD. These studies recruited subjects who had been exposed to a traumatic event, arrived at the ER, and exhibited symptoms of an ASR or subthreshold PTSD.

In the first study, 24 subjects were recruited and randomly divided into two groups: one that received 110–140 mg of HCORT intravenously (14 subjects) and one that received placebo (10 subjects). Of these, 19 subjects completed a two-week follow-up process and 12 completed a three-month follow-up process. After two weeks, 77.7% of subjects from the placebo group met the criteria for an ASD diagnosis, while only 12.5% of the HCORT group met the criteria for the diagnosis. Following-up after one month and three months, 60% of the placebo group met the criteria for an ASD diagnosis as opposed to 16% of the HCORT group. In terms of the severity of the PTSD, members of the HCORT group scored 75% lower on the Clinician-Administered PTSD Scale (the CAPS-5 questionnaire), which is used to assess PTSD, compared to the placebo group (Zohar et al., 2011).

The second study, financed by the United States’ National Institute for Mental Health (NIMH), investigated the involvement of various hormones, including cortisol, as mediators in the development of PTSD. Here too, subjects were randomized into two groups: a study group receiving 100–140 mg of HCORT and a placebo group. Following-up after 13 months, data from 96 subjects were entered into the final analysis (HCORT=51, placebo=45). While a decrease was observed in the HCORT group’s CAPS-5 scores compared to the placebo group, it was not statistically significant. However, the study found that the prevalence of PTSD in both groups was higher among those who had arrived in the ER at night (between 6 p.m. and 6 a.m.). Moreover, the results indicated that administering cortisol at night was related to a greater reduction in the severity of PTSD symptoms with an effect size of 0.41 for the HCORT group, compared to an effect size of 0.02 for the placebo group. In this study, none of the subjects who had received HCORT at night developed PTSD (Carmi et al., in press).

These studies suggest that ASR, PTSD, and cortisol secretion are related. At the same time, no significant relationship has been found between endogenous cortisol levels and the development of PTSD, nor between previous exposure to a significant traumatic event and the disorder. While it is possible that these relationships do not exist, it is also possible that these findings are the result of a small sample size, inaccurate sampling, or missing definitions of various variables.

Pursuantly, in a literature review of 199 articles from the past decade, researchers investigated studies whose goal was to gain a better understanding the role biology plays in the way previous exposure to traumatic events effects life later on (Cooke et al., 2021). The results point to a range of genetic, physiological, and neurological factors involved in the response of those who had previously been exposed to a significant traumatic event. Studies have pointed to a relationship between the cortical system and previous exposure to a significant traumatic event with an emphasis on childhood experiences. Apparently those who had gone through such experiences showed a decrease in the volume of their hippocampus (the area of the brain related to long-term memory, Debiec et al., 2002) and lower levels of cortisol activity. In addition, higher functionality of the cortical areas appeared to be related to a smaller adverse effect of the previous traumatic event. The same review also found a relationship between exposure to a previous traumatic event and orexin. In a study that compared orexin levels among 54 women, half of whom suffered from a psychopathology (depression and/or anxiety) and half of whom were healthy, serving as a control group, a positive relationship was found between orexin levels and high scores on the CTQ-28 questionnaire, which deals with childhood trauma (Bernstein et al., 1994; Bernstein et al., 2003). This relationship was found among the study group as well as the control group, which led the researchers to consider that orexin levels may be related to the experience of distress in childhood, unrelated and independently of the development of a psychopathology (Ozsoy et al., 2017). Additional studies also found an indirect relationship between the two, such as a relationship mediated by aggressiveness (Harro et al., 2019).

In light of the above, the current study seeks to add another layer to the knowledge base that links previous traumatic events to ASD/R and PTSD, while investigating what role the biological cortical and orexinergic systems play in this relationship.