SUMMARY OF THE FINDINGS UP TO DATE

The specific aims were to study cardiac vagal control and mood repair in the context of juvenile onset depression (JOD) risk in 200 probands, 200 never depressed (high-risk) siblings of probands, and 100 controls. We proposed that bio-behavioral inflexibility, evidenced in impaired cardiac vagal control indexed by respiratory sinus arrhythmia (RSA) /mood repair (MR), will distinguish the groups and predict depression. We recruited 224 probands, 279 (2siblings of probands, and 199 controls (from which 181 were eligible, free of mental disorder). The overall sample of 702 adolescents were aged 11-19 yrs; M=16.5 yrs. The rates of subjects in early-, mid- and late-adolescence (ages 11-13, 14-16, 17-19, respectively) were 13%, 39%, and 48%, respectively. With the mean age of 17 yrs (SD:1.4), probands were about 1 year older than siblings (M=16 yrs, SD:2.2) and controls (M=16 yrs; SD:2.1). Altogether 58% lived in intact families (both biological parents). Ethnic breakdown (5.4% minorities, including Roma) was typical of the Hungarian population (102). The 3 research sites contributed similarly to the sample (Budapest region: 32.7%, Szeged region: 39.8%, Pecs region: 27.5%). Probands were 9 years old, on average, at their first major depressive disorder (MDD) episode (102), as determined during their participation in a prior study funded by the NIH. At entry into this study, 43% already had 2 or more MDD episodes but most were in remission from depression (31 were in an MDD episode). Altogether 7.6% had some anxiety disorder, 21% had externalizing disorder, and 3.1% were on psychotropic medication. As typical of depression-prone cohorts, 39% had histories of comorbid anxiety disorders and 37% had past externalizing disorders. Among never-depressed (high risk) sibs (n=214), 7% had anxiety and 6% had externalizing disorder histories; another 65 sibs already had an MDD. Controls were free of major psychiatric disorders.

Findings on cardiovascular risk factors: However, our study was not designed to focus on cardiovascular disorders (CVD), we collected data on some variables relevant to CVD risk including: weight, smoking history, and physical activity. We examined rates of these variables in 205 JOD proband (M age= 17 years; 35% females), 190 never-depressed biological siblings of probands (*M* age=16 years; 56% females), and 160 biologically un-related sex-matched controls (M age= 16 years; 42% females). We computed body mass index (BMI; age and sex corrected and converted into CDC-based weight status categories); physical activity scores were reduced to a main Physical Activity factor (extent of aerobic exercise) and 2 sedentary items (e.g., hours spent watching TV); smoking history was coded as regular smoker vs. non-smoker. We also mailed participants a questionnaire on Family/Parental History of Cardiovascular Disease.

The behavioral risk factors were most prevalent among proband and least prevalent among normal controls, while never-depressed siblings were intermediate. More probands and never depressed siblings were regular smokers (33% & 13%, respectively) than were controls (3%) (*Figure 2*). Probands were more likely to be obese than controls (OR = 3.61); proband and siblings also spent more hours viewing TV and using the computer during school nights than did controls (*d = .38 - .65 & .32 - .53,* respectively) (all *ps* $\leq$ .01). Further, probands were over 1 standard deviation lower in physical activity levels than controls (*d* = 1.26), and about a quarter standard deviation lower than siblings (*d* =.28) (*Figure 3*). These results were

maintained after controlling for age, sex, parental education, and familial heart disease history (130). The plausible "causal" role of depression in these findings is suggested by the fact that, of the probands who were smokers at age 17 (after lengthy exposure to depression), 99% were non-smokers 7 years earlier, when they entered the archival study. Altogether 316 parents (141 controls) reported on their own and their spouse's medical histories. Compared to control families, more proband parents had histories of high blood pressure (51% vs. 39%, OR =1.62), angina or chest pain (21% vs. 8%, OR = 3.06), blood pressure medication use (47% vs. 33%, OR = 1.70), and medication for heart- or CV-disease (34% vs.17%, OR = 2.48) (all ps <.05). Parents in proband families also were more likely than were control parents to have experienced heart attacks (12% vs. 4%, OR = 3.13) or to have been hospitalized with cardiac or circulatory problems (22% vs. 11%, OR = 2.36) (all ps < .01). Notably, proband parents were still young and in their 40's (and did not differ in age from control parents). These surprising findings suggest that probands and their siblings are at increased risk of eventual CVD by virtue of familial CVD.

During our analyses we considered several factors may have moderator effect on the association of JOD and vagal control and mood repair which were our main interest.

Covariates that may affect vagal control/mood repair.

At RSA assessment, smoking was reported by 9.5% of controls, 47.3% of probands, and 19.6% of never depressed sibs (p <.001); psychotropic medication (newer antidepressants; neuroleptics; stimulants) was reported by 0% of controls, 3% of probands, and 2% of never depressed sibs (p=.035). In a mixed linear model that controlled for group, sex, and family effect, neither of these variables affected baseline RSA (Smoking: $F_{2,610}$=0.71; Meds: $F_{1,605}$=0.01, both p>.40).

Although prior studies found medication effects on RSA, those were mostly due to tricyclic antidepressants, now rarely used (reported only for 1 youth). While probands and sibs tended to be from families of middle class socioeconomic status (SES; mean Hollingshead index = 3), controls have higher SES (M=3.96, SD=1.09) than probands (M=3.10, SD=1.24; t_{421}=7.57 p<.001).SES was unrelated to RSA (r=.04, p=.30), but was related to adaptive mood repair by self- (r=.09, p=.017) and parent- (r=.13, p=.001) report. However, these significant associations mirror very small effect sizes after adjusting for age/sex/ family effects (partial R^2_{β}≤.01).

Comorbid anxiety/externalizing disorders Current anxiety/externalizing disorders (described earlier and in Human Subjects) was unrelated to resting RSA (r's from -.03 to .01, p>.60). But current anxiety disorder signaled more extensive maladaptive mood repair repertoires by self- and parent-report (r's: .25 to .26, p's<.001), while externalizing disorder was associated only with parent-reported (r=.19, p=.004) but not self-reported maladaptive mood repair responses (r=.00). After adjusting for age and sex, presence of these comorbidities was associated with small effect sizes (partial η^2 from .03 to .07).

Mental health treatment since their prior research contact was reported by 37% of probands: 29% had outpatient therapy,7% had inpatient care, and 1% had both. Having received

treatment was related to higher negative affect (PANAS: r=.17, p=.013) and depression symptoms (CDI: r=.15, p=.029) and more extensive maladaptive mood repair by proband self-report (r=.22, p=.001) and parental report (r=.32, p<.001), which, after adjusting for age and sex mirrored small/ medium effects (partial η^2 from .03 to .10). However, treatment was unrelated to positive affect or adaptive mood repair by youth or parent informant, did not affect resting RSA (all |r|<.10, all p>.17), and did not predict recurrent MDD episodes in probands (χ^2=2.42, p=.12).

As contextual moderators, we examined social support, life stress, and parental depression (BDI as contextual moderators. Robust regressions controlled for age, sex, and family clustering, with variable results. For example, youth rated maladaptive mood repair predicted youth depression, regardless of contextual factors. But parent-rated maladaptive mood repair predicted greater depression only in the presence of increased life stress ($b_{Stress\text{-}by\text{-}MMR}$=0.04; $F_{1,364}$=5.44, p=.020). As another example, while RSA patterns predicted clinicians' ratings of offspring's depression regardless of maternal depression, this was not the case for child-rated depression (CDI scores; $F_{1,470}$=6.40, p=.012): a normative RSA pattern (high baseline+ withdrawal reaction) was more closely associated with low self-rated depression in the presence of maternal depression (b=-0.07), suggesting a possible protective effect. History of childhood abuse was reported for 19% of probands, 8% of controls, and 8% of sibs ($F_{2,631}$=7.87, p<.001). Regardless of group, abuse history signaled lower self-reported adaptive mood repair ($F_{1,631}$=5.86*, p<.05) and higher parent reported maladaptive mood repair $F_{1,620}$=5.01, p<.05) but did not affect the relations of depression to mood repair. Variable results as a function of information source has led us to use latent variable modeling of key constructs, whenever possible.

Findings on our currently depressed probands were fully consistent with the well documented adverse effects of clinical depression. Compared to controls, depressed probands had worse mood repair, dampened responses to positive experimental probes, remained more depressed after attempting to repair sad mood in the laboratory, had higher levels of trait negative affect, lower levels of trait positive affect (PANAS scores), more conflict with parents, less social support (ps<.001), and attenuated physiological flexibility. However, the most exciting findings concern remitted probands and high-risk siblings. Adolescent probands remitted from depression display impaired affect processing. Compared to controls, remitted probands had: much higher levels of trait negative and depressed affect, lower social support, more conflict with parents (ps<.001), larger repertoires of maladaptive mood repair responses and fewer adaptive responses, and were less successful at attenuating experimentally induced sadness in the laboratory (ts = 2.10-3.40, ps <.05, Cohen's ds change in sad/blue ratings: control vs. remitted = .53; 102). They also had lower trait positive affect (t=6.05, p<.001), dampened reactivity to sad mood induction (102) and positively valenced probes, and were less likely to report habitual mood repair responses involving positive affect (t=5.78, p<.001). When using positive personal memories for mood repair, they needed more prompts and had less positively toned memories. Furthermore, high-risk, never depressed, adolescent siblings display impaired affect processing. Compared to controls, high-risk sibs have lower levels of positive and higher levels of negative and depressed affect (PANAS & CDI scores; t=3.19,

p<.01), more conflict with their fathers, and less social support (ps <.001). There also are multiple indicators of lower positive affectivity: siblings' affective responses to hedonic probes were consistently lower than controls' (Cohen's ds across tasks: .66 – .77), although not as low as probands'; they needed more prompts and had less positive memories while using positive autobiographical memories for mood repair; and reported less use of mood repair responses involving positive affect, such as engaging in play activity to attenuate sadness (t=2.23, p<.05). Overall, siblings' maladaptive mood repair repertoires were comparable to that of probands' and more extensive than controls' (t=3.92, p<.001).

Remitted probands evidence multiple atypical indices of Autonomic Nervous System functioning, including a cardiac index of attention. The orienting response and subsequent attention to a novel stimulus is accompanied by a characteristic vagal activation that slows the heart rate. We therefore looked at the trajectories of the first 12 heart beats, when the cardiac orienting response is expected to occur, within a solvable puzzle, an unsolvable puzzle (used to induce dysphoric mood), and a mood repair task involving refocusing attention by using a kaleidoscope (article submitted for publication). Controlling for several variables, the 3 groups evidenced the expected orienting at initiation of the two puzzles. During mood repair (via attention refocusing), while controls and high-risk sibs showed the expected heart rate deceleration followed by acceleration, probands showed only a slight deceleration. And deceleration was related to trait rumination. This is the first study showing impaired orienting (via an unobtrusive index of attention) in the context of mood repair in depression-prone youths. We also found some impairment in Cardiac Autonomic Balance: higher CAB reflects greater parasympathetic relative to sympathetic activation. Controlling for key covariates, probands differed from controls only on unsolvable puzzles ($F_{1,387}$=6.65, *p*=.01) and the handgrip task ($F_{1,391}$=5.28, *p*=.02), suggesting that probands evidence increased sympathetic activation when some effort or active cognitive engagement is required.

Mood repair and RSA patterns are related and predict MDD recurrence. Among the 204 probands in remission at RSA assessment, 38 had a new episode across a period of 2.08 years (*SD.*,.1/2 yr), on average (*Fig. E*) (101). Via the K-M estimator, this translates to a cumulative new episode risk of 9.0% during the 1st follow up year and 20.5% by the 2nd follow up year. Co-varying age, sex, psychotropic medication, and remission length, maladaptive mood repair (but not adaptive mood repair) robustly predicted episode recurrence (β=1.11, p <.05), and maladaptive mood repair mediated the effects of RSA patterns on depression recurrence (indirect effect: -.15, 95% CI -.04- -.35). Atypical RSA patterns (e.g. high resting RSA + augmentation) predicted larger maladaptive mood repair repertoires (β=.21); normative RSA patterns (high resting RSA + withdrawal) predicted smaller maladaptive repertoires (β=-.05). NOTE: During the 2 year follow-up, incident depression among high-risk sibs was too low for an adequate test of the predictive values of atypical mood repair/RSA patterns among them.

Two sets of our analyses focused on development of RSA. We used data from our probands' participation in our earlier original NIH study, named: Program Project to examine childhood precursors of RSA (article submitted for publication). Parental reports of negative life events

during probands' childhood, assessed when probands were10 yrs old, on average, predicted 7 years later youths' RSA patterns at rest, during negative mood induction, and during mood repair, evidenced by a significant interaction between childhood adverse event counts and the three RSA values ($F_{2,181}$= 7.03, p = .001, partial η^2 = .072). Compared to youths with fewer childhood negative life events, youths with high rates of early adversities exhibited less RSA withdrawal during mood induction and less RSA augmentation during mood repair. Thus, early stress events signal impaired flexibility of ANS functioning during affect-processing tasks. Findings were not affected by age, sex, time elapsed between life-event and RSA assessment, type of mood induction, or comorbid anxiety disorder (article submitted for publication). We also examined whether liability factors during development contributed to maladaptive mood repair among remitted probands and controls, and if putative protective factors were of value (article submitted for publication). We found that maladaptive mood repair repertoires were partially mediated by 3 risk factors and 2 protective factors ($F_{5,319}$=13.02, p<.001, Adj. R^2=.28). Namely, the adverse effect of proband status on maladaptive mood repair was worse (21% mediated) for youths with non-intact families prior to age 7, with currently depressed mothers, and mothers with histories of diagnosable depression. Conversely, the adverse effects of proband status were partially counteracted (40% mediated) among youths with larger adaptive mood repair repertoires and perceived availability of social support. These findings were not affected by sex and suggest that maladaptive mood repair repertoires are shaped by negative (risk) and positive (resilience) social factors.

Grant related publications are in press or submitted for publication

Kovacs, M., Bylsma, L.M., Yaroslavsky, I., Rottenberg, J., Kiss, E., Halas, K., Benak, I., Baji, I., Vetro, A., & Kapornai, K. (in press). Positive affectivity is dampened in youths with histories of major depression and their never depressed adolescent siblings. Clinical Psychological Science.

Rottenberg, J, Kovacs, M., & Yaroslavsky, I.,. Who are the non-responders?: Issues regarding sad mood induction in adolescents with histories of major depression. Submitted for publication.

Kovacs, M., Yaroslavsky, I., Rottenberg, J., George, C. J., Kiss, E., Halas, K., Dochnal, R., Benak, I., Baji, I., Vetro, A., Makai, A., & Kapornai, K. Maladaptive mood repair, respiratory sinus arrhythmia, and risk of a recurrent major depressive episode among adolescents with prior major depression. Submitted for publication.

Yaroslavsky, I. Rottenberg, J., Bylsma, L.M., Jennings, J.R., George, C., Baji, I., Benak, I., Dochnal, R., Halas, K., Kapornai, K., Kiss, E., Makai, A.,, Varga, H., Vetro, A., & Kovacs, M. Parasympathetic nervous system activity predicts mood repair use and effectiveness among adolescents. Submitted for publication.

Yaroslavsky, I., Jennings, J.R., Kiss, E., Kapornai, K., Halas, K., Dochnal, R., Lefkovics, E., Baji, I., Vetro, A., & Kovacs, M. Cardiac indices of attention during mood repair among adolescents remitted from major depression: Evidence of impairment. Submitted for publication.

Begovic, E., Panaite, V., Bylsma, L., George, C., Kovacs, M., Yarosloavsky, I., Baji, I., Dochnal, R., Halas, K., Kiss, E., Vetro, A., Kapornai, K., & Rottenberg, J. Positive autobiographical memory deficits among youth with depression history and their never depressed siblings. Submitted for publication.

Daches, S., Kovacs, M., George, C., Yaroslavsky, I., Kiss, E., Vetro, A., Dochnal, R., Benak, I., Baji, I., Halas, K., Makai, A., & Kapornai, K., Rottenberg, J. Negative life events during childhood and their relations to respiratory sinus arrhythmia seven years later among adolescents with histories of depression. Submitted for publication.

Daches, S., Kovacs, M., George, C., Baji, I., Benak, I., Dochnal, R., Halas, K., Kapornai, K., Kiss, E., Makai, A., Vetro, A., Rottenberg, J. Risk and resilience factors mediate maladaptive mood repair response repertoires of adolescents with and without histories of major depression. Submitted for publication.